BY ANHALT UNIVERSITY OF APPLIED SCIENCES

PROCEEDINGS OF THE 13TH INTERNATIONAL CONFERENCE ON APPLIED INNOVATIONS IN IT

VOLUME 13
ISSUE 2

EDITION

Hochschule Anhalt

Proceedings of the 13th International Conference on Applied Innovations in IT

Volume 13 | Issue 2

Koethen, Germany
27 June 2025

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The Conference Proceedings Journal, volume 13(2), contains selected papers accepted for publication from those presented at the 2nd International Conference for Pure and Applied Science (ICPAS 2025), held on April 15, 2025, in Diyala Governorate, Iraq. This issue presents research in applied computer science, mathematical sciences, physics, chemistry, materials science, biology, and biotechnology. The papers included in this volume are of particular interest to both academic researchers and industry professionals seeking innovative, interdisciplinary approaches to addressing global challenges in science and technology.

ISBN: 978-3-96057-185-8 (Online)
ISSN: 2199-8876

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Publisher: Edition Hochschule Anhalt
Phone: +49 (0) 3496 67 5611
Email: leiterin.hsb@hs-anhalt.de

Phone: +49 (0) 3496 67 2327
Email: conf@icaiit.org
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ABOUT THIS SPECIAL ISSUE

The special issue of the Proceedings of the International Conference on Applied Innovations in IT (ICAIIT 2025), volume 13, issue 2, featured selected papers from the 2nd International Conference for Pure and Applied Science (ICPAS 2025), which took place on April 15, 2025, in Diyala Governorate, Iraq. This collaboration aimed to highlight cutting-edge research and developments across various scientific disciplines, fostering interdisciplinary dialogue and innovation.

About ICPAS 2025

The International Conference for Pure and Applied Science (https://icpas.uodiyala.edu.iq/) was a premier gathering of scientists, researchers, and professionals dedicated to advancing the frontiers of scientific knowledge. The conference served as a platform for global networking, knowledge exchange, and collaboration across diverse fields in pure and applied sciences. With a focus on innovation and sustainable solutions, ICPAS brought together leading experts to share insights, explore emerging trends, and drive impactful research that shapes the future of science and technology.

This special issue encompassed a broad range of topics, including but not limited to:

1) Applied Innovations in Computer Science.
2) Applied Mathematical Sciences and Computational Methods.
3) Applied Physics in Engineering and Technology.
4) Innovations in Applied Chemistry and Materials Science.
5) Applied Biology and Biotechnology Innovations.

By integrating these diverse fields, the special issue sought to bridge disciplinary gaps, encouraging partnerships that drive innovative solutions to complex global challenges. This initiative underscored the role of science and technology in achieving sustainable development, promoting research that addresses pressing issues such as climate change, resource conservation, and health improvement.

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SECTION 1

APPLIED INNOVATIONS IN COMPUTER SCIENCE

Proceedings of the 13th International Conference on Applied Innovations in IT (ICAIIT)

JUNE 2025

An Energy-Efficient Clustering Model for Wireless Sensor Networks Using Modified K-Means Algorithm

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Keywords: Wireless Sensor Networks (WSNs), K-Means Clustering, Network Optimization, Energy Efficiency, Network Lifetime, Energy-Efficient Clustering, Optimal Cluster Selection, Modified K-Means Algorithm.

Abstract: Wireless Sensor Networks (WSNs) are becoming essential for many applications, ranging from smart cities to environmental monitoring. WSNs comprises a collection of deployed sensor nodes to execute specified objectives in a certain area. Since batteries can only hold so much energy, one of the most crucial topics of research is how to use energy efficiently in order to extend the lifespan of sensors. One of the most popular methods for lowering energy consumption is clustering, and clustering routing protocols are methods for preserving energy to increase the lifetime of a wireless sensor network. The K-Means algorithm is one of the clustering techniques that requires prior knowledge of the clusters. This study proposes a mathematical model to determine the optimal number of clusters in WSNs, reducing energy consumption by up to 97%. Choosing the number of clusters at random could use more energy and reduce the network lifetime. This paper seeks to present a new approach for determining out the optimal number of clusters in a WSN. The proposed model tests the WSN performance by using a mathematical model and implementing it as a simulation technique in MATLAB. It considers the key WSN characteristics, including the deployed area size (100 × 100), the number of rounds (100, 200, 300, 400, and 500), and the number of sensor nodes (500). This study demonstrated that our revised approach to selecting the number of sensor network clusters reduced overall energy consumption by 97% when compared to the conventional model, hence increasing the networks' overall lifespan.

1 INTRODUCTION

Wireless Sensor Networks (WSNs) are made up of many small sensors, electrical and actuator devices, and activation nodes. These sensing electronic devices can detect and gather data on phenomena such as the speed of the wind, pressure, temperature, and other similar features in a particular sensing field [1]. The processed information is subsequently delivered to the Base Station (BS). WSN sensors are small and have a low production cost. As a result, WSNs have a wide range of efficient and sometimes sensitive uses, particularly in the armed forces, healthcare, and environmental fields. WSNs have limited resource systems which need good management and efficient utilization of all of their resources [2]. WSNs face two key extensive constraints: the first one is the low energy resources of the sensors, which are not rechargeable, and an exceedingly limited battery. The second constraint is susceptibility to failure. However, the data transmission process consumes the most energy [3, 4]. One possible method for reducing communications and hence reducing energy consumption is to arrange the network in clusters [5]. Hence, clustering is a common technique that is used to obtain efficient resource allocation and good scalability in WSNs. The major problem in networks that are based on the clustering technique is how to decide what is the best number of clusters with the

objective of minimizing energy consumption [6]. The optimal number of clusters in the WSN environment is essential because if the given number of clusters, which is input to the K-Means algorithm, is less or greater than the optimal value, the produced result will not be suitable for balancing distribution in the sensor network [7]. Advantages of centroid models such as K-Means are that convergence is guaranteed and they are specialized in clusters of different sizes and shapes, but they have a disadvantage: the K-value is difficult to predict [8]. Clustering in WSN is grouping sensor nodes into clusters and selecting a Cluster Head (CH) for each cluster, where each CH is responsible for gathering data from its own sensor nodes and sending them to the BS or to other CHs. Clustering has many advantages such as grouping sensors, reducing the number of nodes responsible for transmitting data, and decreasing energy consumption [9]. Clustering in sensor nodes has been widely used to solve the scalability, energy, and lifetime issues of sensor networks [10]. Clustering is an algorithm that is used to separate, classify, or group objects depending on attributes/features into K number of groups. K is a positive integer number. Grouping is achieved by reducing the sum of squares of distances between data and the corresponding cluster centroid. It is also called the centroid method [11]. K-Means is a method of cluster analysis using a previously determined number of clusters. It requires advanced knowledge of 'K'. The use of the K-Means algorithm as a clustering technique for cluster formation ensures good clustering and minimizes the overheads when the channels are re-elected.

Advantages of K-Means are that convergence is guaranteed and it is specialized in clusters of different sizes and shapes [12]. To get good energy consumption management during the communication process and endure transient failures, many methods for clustering used in WSNs have been proposed in the literature. Motivated by the research described in the preceding part, we utilized a mathematical model to offer a viable and useful solution for a wireless sensor network. The rest of this paper is organized as follows: Section 2 reviews the related work, Section 3 describes the K-Means Algorithm, Section 4 presents the methodology and the proposed model, Section 5 discusses the results and discussion, and Section 6 concludes the study.

2 RELATED WORK

Heinzelman et al. [13] presented a low-energy adaptive clustering hierarchy (LEACH), which is currently regarded as the most famous clustering protocol for WSNs, as a means of deploying a WSN. There are two kinds of nodes in a hierarchical topology: cluster heads and cluster members. A single node is designated as the cluster head in each cluster that is made up of member nodes [14]. The primary responsibility of the cluster head is to receive signals from cluster members and transmit them to other cluster heads or the base station [15, 16]. The authors in [17] introduced a system called (HEED) Hybrid Energy-Efficient Distributed Clustering that selects the cluster head by using an iterative clustering process; they utilized a hybrid of residual energy and communication cost in terms of node proximity to neighbours or node degree. This achieves uniform cluster head distribution across the network and incurs low message overhead. The same approximation that was used by the LEACH approach is used by this method to get the optimum number of clusters (k). The authors of [18] presented a systematic analysis based on the cost for sensor nodes that are organized in clusters by using single-hop or multi-hop communication modes. For each mode of communication, they tried to formulate guidelines to get the optimal number of clusters. In [19, 20], clustered networks with dynamic hierarchy were introduced. They tried to determine the best number of clusters that reduces the overhead of the routing process. They defined the overhead of the routing process as the amount of information that is needed to describe the change in a network topology. They tried to minimize the routing overhead by determining the optimal number using an information-theoretical approach with mobility prediction and without mobility prediction. In [21], the authors introduced a sensor network that depends on multi-cluster technology; this network is applied for source extraction. They used the Particle Swarm Optimization (PSO) algorithm to cluster the sensors. They also proved theoretically that there is a unique optimal number of clusters that achieves the lowest energy consumption. In [22], the authors showed an analytical method to get the optimum number of clusters of dense wireless sensor networks by using a cross-layer optimization method. In [23], the authors tried to get the suitable number of clusters that can achieve well-balanced memberships. In [24], the authors divided a data set into a set of clusters by using an objective function-based method. [25] tried to propose fuzzy-based clustering. Fuzzy c-Means (FCM) and its derivatives encounter two problems: cluster validity and local minima, which have a direct impact on the formation of the clustering.

3 K-MEANS ALGORITHM

3.1 Overview of the K-Means Algorithm

Among the promising and successful clustering techniques is K-Means [26]. The process involves grouping nodes in the network into many clusters, with each cluster being based on two parameters: the number of desired clusters and the Euclidean distance, which is used to find the cluster that is closest to each node [27]. The cluster center must be the cluster head's position, and the node's residual energy is the basis for choosing the cluster heads for the k-Means clustering algorithm. The k-Means clustering technique in wireless sensor networks is predicated on an iterative optimization of the classification nodes' distance. From a collection of N nodes, the method creates K clusters. The K-Means algorithm's objective function is [28- 29]:

$$F = \sum_{r=1}^{k} \sum_{x1 \in Cr}^{n} (x_i - ch_r)^2 \quad (1)$$

The Euclidean distance is used in K-Means clustering, where *Cr* is the set of nodes that are belong to of cluster r:

$$d(x_i, CH_r) = \sqrt{(x_i - CH_r)^2} \quad (2)$$

The K-Means method therefore just looks for the global minimum of CH_r . CH_r is the cluster head when x_i is a cluster node [30–35].

3.2 Steps for k-Means Algorithm

The K-Means clustering algorithm works in five stages. First, it determines how many clusters to create in a wireless sensor network. Next, it randomly selects the cluster head for each cluster. Finally, it uses the Euclidean distance to determine which cluster is closest to each node [36]. Advantages of K-Means are convergence is guaranteed and specialized in clusters of different sizes and shapes. K means is an iterative clustering algorithm that aims to find local maxima in each iteration as shown in Figure 1. The field-deployed wireless sensors were represented by $\{x_1, x_2, x_3, \ldots, x_n\}$, whereas the cluster head, originally selected at random, was represented by $\{CH_1, CH_2, \ldots\ldots, CH_k\}$.

1) Specify the desired number of clusters *K*: Let us choose *k*=2 for these 5 data points in 2-D space.
2) Randomly assign each data point to a cluster. Set the clusters' center at random, CH_r, where r = 1,...,*k*, and $k < n$.
3) Compute cluster centroids. Using the Euclidean distance, assign each data point to the nearest cluster:

$$C_j = j: d(x_j, ch_r) \leq d(x_j, ch_l), l \neq r, j = 1..n. \quad (3)$$

4) Re-assign each point to the closest cluster centroid. Re-compute cluster centroids. The cluster middles Chi for each cluster k are updated using:

$$ch_i = \sum \left(\frac{1}{c}\right) \sum_{j=1}^{ci} x_i \quad \ldots. \quad (4)$$

Repeat steps 4 and 5 until no improvements are possible.

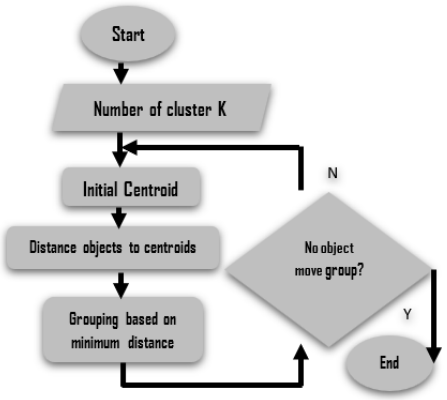


Figure 1: K-Means Algorithm Mechanism [36].

4 METHODOLOGY

The primary goal of the suggested strategy is to minimize the amount of energy used for data transfers. Although a K-Means method was developed for use in database environments, we wish to modify it so that it may function in wireless sensor environments without altering the algorithm's basic structure. When selecting the initial centroids at random, the majority of the centroid techniques now in use, like K-Means, have the following disadvantages [37-38]:

- An empty cluster.
- User desired clusters as input to the algorithm.
- The unbalanced workload on the clusters.
- Non-selection of optimal CHs count.

This approach describes how the K-Means algorithm is modified and optimized for WSNs, with a focus on balancing energy efficiency and network performance via careful cluster formation and centroid selection. a set of adjustments meant to determine the ideal number of clusters (K) in

accordance with a WSN's unique requirements. The suggested model modifies K by taking important factors affecting WSN functionality into account. The ideas that follow are made to adapt the K-mean algorithm in order to get around these issues and adapt K-means for WSN.

An indicated model is used in this study to determine k based on the suggested WSN environment. K can be determined by taking into account several factors that affect WSN settings.

4.1 Sensing Coverage and Cluster Formation

Each sensor node's sensing region is shown as a circle with a radius equal to the sensing radius, or *R*. The following (5) can be used to get each sensor's coverage area:

$$A_s = R^2 * \pi, \quad (5)$$

where R is the sensing radius and A_s is the sensing node's area. Equation 6 is proposed as a means to assess the coverage probability.

$$P = \frac{A_s}{A}, \quad (6)$$

where (6) can be used to calculate the coverage probability (P) of a sensor node inside a specified region of interest (A).

4.2 Determining the Optimal Number of Sensor Nodes (N)

(N) is particularly important since putting additional sensor nodes would raise costs even if using fewer sensor nodes would result in poor coverage. Thefore, to ensure adequate coverage of the region while minimizing costs, the optimal number of sensor nodes (N) required to cover a specific area is found via using (7).

$$N = \frac{A}{As}\ . \quad (7)$$

Where *(N)* is the ideal number of sensor nodes, (A_s) is the sensing area of a node, and *(A)* is the total area of interest.

4.3 Determining the Optimal Number of Clusters (K)

The optimal number of clusters, denoted as K, is a critical input for the K-Means algorithm and is calculated using the following (8):

$$K = N * P, \quad (8)$$

where K is the optimal number of clusters, N is the ideal number of sensor nodes, and P is the coverage probability.

4.4 Modified K-Means Algorithm for WSNs

Once the optimal number of clusters K has been determined using the equations outlined above, the modified K-Means algorithm is applied (Fig. 2).

Input	
	Parameters Setting, Sensor nodes
	Area, R, K
Output	
	BER Calculation
Initialization	
	Compute *As* by Eq.5
	Compute *P* by Eq.6
	Compute *N* by Eq.7
	Compute *K* by Eq.8
Process	
1.	Start
2.	Centroid (Modified steps to obtain K)
3.	Distance objects to centroids (Euclidean)
4.	Grouping based on minimum distance
5.	If No object move group?
6.	Got to start
End	

Figure 2: Algorithm 1: Modified K-Means.

The steps for the modified algorithm are as follows:

- Initial Centroid Selection. Instead of random centroid selection, the algorithm chooses centroids based on coverage and distribution, ensuring that empty clusters are avoided;
- Cluster Formation. The network is partitioned into clusters based on the calculated K. Each sensor node is assigned to the nearest cluster centroid to minimize intra-cluster distances;
- Cluster Head Selection. An adaptive method is used to select cluster heads, considering energy efficiency and load balancing across the network. This prevents unbalanced workloads and ensures that energy consumption is evenly distributed among the nodes;
- Re-Clustering. Dynamic re-clustering is employed to adjust the network structure as nodes deplete their energy, ensuring sustained performance and longer network lifetime. Consequently, as seen in algorithm 1. By using the value of K determined from the proposed mathematical model, the network is divided into

optimal clusters. The modified K-Means algorithm then forms clusters that are suitable for the region of interest, balancing between too few clusters (which may lead to poor coverage) and too many clusters (which may increase energy consumption due to an excessive number of cluster heads).

5 RESULTS AND DISCUSSION

5.1 Experimental Setup

In the experimental phase, we implemented the K-Means clustering algorithm using the Matlab simulator over a region of interest (100 x 100 units). Two different approaches were used to determine the value of *K* (the number of clusters):

1) Random *K* Selection. The value of *k* was selected randomly;
2) Mathematical Model for *K* Selection. The second method involved calculating *K* based on the proposed mathematical model, which considers coverage probability, sensor node distribution, and the area of interest, as discussed in previous Section. For the simulations, 500 sensor nodes were deployed randomly across the region. We tested both the traditional K-Means algorithm and the proposed modified K-Means algorithm over 100 to 500 rounds. A coverage probability parameter *P=0.5* was used to randomly determine the number of clusters, ensuring consistent comparison across different scenarios.

While Table 2: used the updated K-Means echnique to get the energy usage measure. The ideal number of clusters was established using the suggested model throughout a range of rounds from 100 to 500. For Example: Consider a scenario with a sensing radius (R) of (15m) and an area of interest (A) measuring (100*100) m2.

Table 1: Energy Consumption using the traditional K-Means algorithm.

Number of nodes	Clustering method	Number of rounds	Total energy consumption (J)
500	K-Means	100	1.435
		200	2.871
		300	4.301
		400	5.759
		500	7.171

$$A_s = R^2 * \pi = (15)^2 * 3.14 = 706.5 \text{ m}^2$$
$$P = \frac{A_S}{A} = 706.5 / 10000 = 0.07$$
$$N = A/A_s = 10000 / 706.5 = 14$$
$$K = N * P = 14 * 0.07 = 1$$

Table 2: Modified K-Means.

Number of nodes	Clustering method	Number of rounds	Total energy consumption (J)
500	Modified K-Means	100	0.040
		200	0.018
		300	0.121
		400	0.161
		500	0.202

5.2 Energy Consumption Analysis

Energy efficiency is one of the critical factors in evaluating the performance of Wireless Sensor Networks (WSNs). We used total energy consumption as the primary metric for comparison between the traditional K-Means clustering algorithm and the proposed modified algorithm. Energy consumption was calculated based on the communication and computation energy costs during cluster formation and data transmission. Therefore, we can find the Energy saving percentage between two approaches via this formula: [41]

$$Energy\ saving\% = \frac{E_{old} - E_{new}}{E_{old}}\% = \left(1 - \frac{E_{new}}{E_{old}}\right)\%,, \quad (9)$$

where (E_{old}) is represent to the total Energy Consumption for common K- mean, while (E_{new}) is refer to the total Energy Consumption for our approach, and from Table 1, Table 2 , and (9), we can obtained on the Table 3, and Figure 3.

The results show a consistent trend: the proposed algorithm reduces total energy consumption by optimizing cluster formation and selecting cluster heads more effectively. As the number of rounds increases, the energy savings become even more significant. The difference in the total energy consumption metric between the modified K-Means algorithm and the K-Means method is shown in Figure 3. Energy efficiency is one of the critical factors in evaluating the performance of WSNs. We used total energy consumption as the primary metric for comparison between the traditional K-Means clustering algorithm and the proposed modified algorithm. Energy consumption was calculated based on the communication and computation energy costs during cluster formation and data transmission. The results show a consistent trend: the proposed

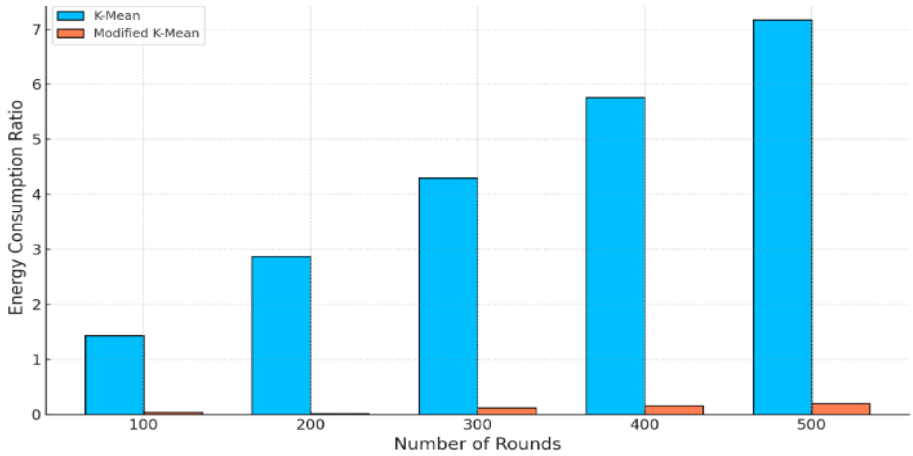


Figure 3: Energy consumption for two approaches (conventional K-Means approach and modified K-Means).

algorithm reduces total energy consumption by optimizing cluster formation and selecting cluster heads more effectively. As the number of rounds increases, the energy savings become even more significant.

Table 3: Energy saving percentage for applying the modified K-Means algorithm.

Number of nodes	Number of rounds	E_{old} for conventional K-Means (J)	E_{new} for modified K-Means (J)	Energy saving %
500	100	1.435	0.040	97.2%
	200	2.871	0.108	96.2%
	300	4.301	0.121	97.2%
	400	5.759	0.161	97.2%
	500	7.171	0.202	97.2%

5.3 Cluster Distribution and Load Balancing

Another key observation from the experiments was the impact of cluster distribution on the network's performance. In the traditional K-Means algorithm, random centroid selection often resulted in unbalanced clusters, with some clusters covering larger areas and consuming more energy. This led to uneven load distribution, where certain sensor nodes depleted their energy more quickly, causing a reduction in network lifetime. A bar chart comparing total energy consumption for the K-Means and the modified K-Means across multiple simulation. In contrast, the K-Means algorithm addressed this issue by using an optimized number of clusters and adaptive cluster head selection. By taking into account factors such as coverage probability and area of interest, the proposed algorithm formed more balanced clusters. As a result, sensor nodes shared the workload more evenly, prolonging the network's overall lifetime and enhancing performance. Based on the results reported in Tables 1, 2 as well as Table3 and the observations in Figure 3, the following conclusions are drawn:

- The improved K-Means algorithm outperforms the total energy consumption metric than conventional K-Means about at least 97%.
- Optimal cluster selection adds to longer network lifetimes, emphasizing the importance of this strategy in network sustainability.

Also, scalability was another important factor evaluated during the experiments. As the number of sensor nodes and the size of the region increased, the proposed algorithm maintained its performance advantage over the traditional K-Means algorithm. This scalability was mainly due to the ability of the modified algorithm to adjust the number of clusters dynamically, ensuring optimal performance even as network conditions changed. In terms of network longevity, the proposed algorithm outperformed the traditional approach in all test cases. By reducing energy consumption and balancing the workload among nodes, the network remained operational for longer periods, even in high-density environments or under prolonged testing. The results of the research show how well the suggested modified K-Means algorithm works to increase WSNs' durability, scalability, and energy efficiency. In order to lower communication overhead and guarantee more evenly distributed energy consumption among clusters, the cluster selection mathematical model (value of K) was essential. It is evident from comparing the suggested model with the conventional K-Means

technique that random cluster formation and Centro's selection are ineffective for WSN situations, where energy conservation is crucial. The modified algorithm not only reduces energy consumption but also improves network performance through more intelligent cluster formation and adaptive cluster head selection.

6 CONCLUSIONS

In this paper, we proposed and evaluated a new mathematical model for determining the optimal number of clusters in WSNs, aiming to address one of the most pressing challenges in WSN design: energy efficiency. WSNs, being inherently energy-constrained due to their reliance on battery-powered sensor nodes, require intelligent clustering mechanisms to reduce communication overhead and extend network longevity. Our model was specifically developed to dynamically adapt to variations in network size and deployment density without requiring prior knowledge of the number of clusters. To validate the effectiveness of the proposed approach, we conducted a series of simulation experiments using MATLAB, where 100 to 500 sensor nodes were randomly distributed over a fixed 100×100 m² area. The model's performance was evaluated against traditional analytical and random clustering methods. Key evaluation metrics included energy consumption, network lifetime, and the stability of the clustering mechanism across varying node densities and operational rounds. The simulation results demonstrated that our model significantly outperforms conventional methods, achieving up to a 97% reduction in energy consumption. This substantial energy saving leads directly to a longer operational lifetime for sensor nodes and improved network resilience. Moreover, the balanced load distribution among clusters prevents premature node failures, thereby preserving network functionality for extended periods. The model also showcased excellent scalability, maintaining consistent performance across different deployment sizes and configurations. In addition to the quantitative performance gains, the proposed model contributes to the broader field of WSN research by offering a generalized, adaptable framework for cluster optimization. Unlike static clustering algorithms such as K-Means, which require pre-defined cluster counts and are not adaptive to dynamic conditions, our model integrates key network characteristics to derive the optimal number of clusters in a resource-aware manner.

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A Cyber Security Approach Using Multilayer Cryptographic System

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Keywords: Cyber Security, RSA, AES, Hybrid RSA-AES, Brute Force Attack.

Abstract: The cybersecurity is one of the important challenges facing the digital world to protect data over the Internet, where encryption technologies are used for this purpose. This paper illustrates the design, development, and evaluation of a Hybrid RSA and AES Cryptography Framework which combines the strengths of both of these types of cryptographic algorithms to solve real-time data security challenges. RSA ensures secure key distribution; AES ensures speedy data encryption. This hybrid approach combines the benefits of RSA in terms of its ability to safely distribute keys, and AES for quickly encrypting larger data sets, addressing the limitations seen in individual cryptography. Performance analysis demonstrates that the hybrid system performs efficiently without significant compromise between encryption speed and security, making it an excellent candidate for resource-constrained environments and applications where real-time processing is needed. In addition, the proposed structure was exposed to strict evaluation against installed safety standards, which demonstrates its strength against larger blockages, attacks on cruel power, and cryptanalytic efforts. Comparative studies with existing functions highlight the scalability and practical relevance of the system in the protection of sensitive communication, financial transactions, and cloud-based data. By integrating RSA and AES within a hybrid frame, the study helps promote the cryptographic feature designed for modern applications, introducing a scalable, efficient, and secure model for protecting digital assets in a quick mutual environment.

1 INTRODUCTION

In the modern digital environment, the rapid increase in data transfer in the network has increased the demand for a strong security structure to protect against unauthorized access and sensitive information from cyber threats. Traditional cryptographic algorithms, although effective, often meet boundaries that react to complex and developed security requirements for modern applications. To address these limitations, researchers have turned to hybrid cryptographic systems that combine the strengths of both symmetric and asymmetric encryption, providing a sound balance between safety and performance. Symmetric encryption algorithms, such as Advanced Encryption Standard (AES), are considered high for their efficiency in encrypting large data sets because of their rapid operating ability [1]. However, their efficiency depends on the safe exchange of secret keys, which can introduce weaknesses if not handled with care [2]. In contrast, asymmetrical encryption algorithms such as Rivest-Shamir-Adleman (RSA) offer secure key exchange methods but are calculation-intensive, making them less suitable for encrypting large amounts of data [3]. By integrating these two approaches, hybrid cryptographic systems use the benefits of both algorithms, using symmetrical methods for effective data encryption [4].

Recent studies show various hybrid encryption frameworks to increase data security. For example, an innovative hybrid AES-RSA model that includes bit-level symbols to strengthen cryptographic security has been included [5]. In addition, a hybrid encryption form is developed that combines AES with elliptical curve cryptography (ECC) to improve

data security [6]. Comparative analysis of hybrid models, including AES-RSA and AES-Triple DES, are also organized to evaluate their performance and safety matrices [7]. A hybrid encryption method merges symmetrical Blowfish encryption with asymmetrical elliptical curves to enable effective and secure data transfer [8]. A hybrid cryptosystem, characterized by a new algorithm, is introduced for pre-image encryption [9]. AES-based hybrid encryption has also been investigated to improve the safety mechanism of automated performance analysis of e-services [10]. In addition, a hybrid cryptographic framework for secure data transfer in Edge AI networks has been proposed [11]. A systematic literature review on RSA and elliptical curve encryption systems compares their efficiency [12]. A new hybrid encryption algorithm based on AES, RSA, and ECC for Bluetooth encryption has been proposed [13].

These research works collectively demonstrate the flexibility and feasibility of hybrid cryptographic systems in solving modern data security problems. This paper clarifies the design and development of a Hybrid RSA-AES Cryptography System, aiming to utilize the secure key exchange feature of RSA along with the efficient data encryption property of AES. By combining both these algorithms, the system proposed in this work aims to provide an integrated solution that alleviates the drawbacks inherent in the solo use of either of the two algorithms. pre-certifying adherence to existing cryptographic standards and making integration effortless within a wide variety of applications. The rest of this paper is arranged as follows: Section 2 presents an elaborate literature review of existing hybrid cryptographic systems and the corresponding applications. Section 3 establishes the methodological framework applied in both conceptualizing and realizing the proposed system, such as mathematical derivations along with algorithmic strategies. Section 4 presents the results of performance testing and engages in a discussion regarding the system's efficiency in practical application. Finally, Section 5 concludes the manuscript with a summary of the main findings and suggesting potential directions for future research.

2 LITERATURE REVIEW

Many research activities show the ability of these systems to achieve both strong safety and functionality efficiency. The symmetrical encryption ciphers, depicted by Advanced Encryption Standards (AES), is widely known for its speed and simplicity. Despite this, their dependence on secure main distribution mechanisms creates serious obstacles to practical implementation [14]. Asymmetrical encryption methods, which are exemplary by Rivest-Shamir-Member (RSA) algorithm, facilitate secure key exchange, but are charged with heavy calculation requirements, especially for large data encryption systems [15].

2.1 Hybrid Cryptography in Cloud Security

The complementary properties of these cryptosystems have inspired hybrid cryptosystems that embrace both symmetric and asymmetric properties. A research study by Singh and Gupta (2019) designed an optimized AES-RSA hybrid cryptosystem to increase the safety of data in cloud environments with a significant increase in encryption strength [16]. Similarly, Bhatia et al. used hybrid cryptographic schemes to encrypt multimedia data, as mentioned by Sharma et al. (2021), who presented an image encryption model based on a hybrid RSA-AES frame [18].

The latest progression involves combining cryptographic approaches to reduce security weaknesses. For example, Nguyen and Tran (2022) designed a hybrid cryptosystem combining AES, RSA, and steganography to ensure safe communication with expanded resistance to brute-force attacks and data leakage [19].

2.2 Applications in IoT

An important work by Al-Shehri and Khalid (2023) presented a hybrid model blending RSA, AES, and blockchain to secure financial transactions, showing scalability in distributed systems [20]. Ali and Hasan (2024) integrated quantum cryptography methods with RSA and AES to protect against quantum computational attacks [21].

2.3 Post-Quantum Cryptography

This methodological structure achieved promising results in ensuring safety for quantum computational contexts. Kumar et al. (2023) conducted a comparative analysis of hybrid cryptosystems (RSA-AES vs. RSA-Blowfish), noting RSA-AES's better balance between security and computational overhead [22]. Rahman and Singh (2020) analyzed hybrid encryption for health data protection, highlighting low delay and high throughput [23]. Hybrid techniques for edge computing applications,

explored by Zhao and Wang (2023), showed the suitability of AES-RSA for secure edge-AI implementation [11]. Increasing attention for hybrid cryptography testifies to its ability to break the boundaries of separate encryption techniques. By combining the effectiveness of symmetrical encryption and protection of asymmetrical techniques, from the hybrid cryptographic system IoT to cloud computing [16] and secure communication, the necessary safety requirements meet in a plethora of areas. This research adds to the existing body of knowledge by suggesting and implementing a hybrid RSA-AES cryptosystem, making use of the unique advantages of both algorithms to achieve strong security in conjunction with high performance.

Table 1: Comparison security metrics across references.

Reference	Encryption speed	Security strength	Computational efficiency	Resistance to attacks	Scalability	Application domain
[1] Stallings (2020)	Moderate	High	Moderate	High	Limited	General cryptography principles
[2] Schneier (2021)	High	High	High	Moderate	Limited	Applied cryptog-raphy
[14] Dhanraj & Singh (2019)	High	Moderate	High	Moderate	Limited	Symmetric encryption challenges
[15] Huang et al. (2020)	Moderate	High	Moderate	High	Limited	Asymmetric cryptosystems
[16] Singh & Gupta (2019)	Moderate	High	Moderate	High	High	Cloud security
[17] Bhatia et al. (2020)	High	High	High	High	Moderate	IoT security
[18] Sharma & Patel (2021)	High	Moderate	High	Moderate	Moderate	Multimedia security
[19] Nguyen & Tran (2022)	Moderate	High	Moderate	High	High	Communi-cation security
[20] Al-Shehri & Khalid (2023)	Moderate	High	Moderate	High	High	Financial transactions
[21] Ali & Hasan (2024)	Moderate	Very High	Moderate	Very High	High	Post-quantum cryptography
[22] Kumar et al. (2023)	High	High	High	High	Moderate	Cryptosyst-em comparison
[23] Rahman & Singh (2020)	High	High	High	Moderate	High	Healthcare data security
[11] Y. Chen et al. (2023)	High	High	High	High	High	Edge computing

Table 1 presents a comparative analysis of the efficiency and relevance of cryptographic techniques examined in the cited literature and emphasizes the security matrix.

3 METHODOLOGY

The proposed hybrid RSA-AES cryptography system covers the benefits of asymmetrical and symmetrical cryptographic paradigms to realize a strong and effective data encryption framework. The function includes important components such as system architecture, mathematical theory, algorithm development and implementation specifications System Design.

3.1 System Design

The hybrid approach combines the following essential stages:

1) Key Generation and Exchange. The RSA algorithm is utilized for secure key exchange. Public and private key pairs are created to ensure confidentiality of the AES encryption key. The process allows for secure key transmission over untrusted networks;
2) Data Encryption and Decryption. The AES encryption algorithm is used to encrypt the large data sets because of its computational efficiency and high speed of processing. The encrypted data or the ciphertext is securely transmitted with the encrypted AES key;
3) Decryption and Data Recovery. The receiver decrypts the AES key with the RSA private key. Upon decryption, the decrypted AES key is applied to decrypt the received ciphertext, and thus the original plaintext is retrieved.

3.2 Algorithms

Hybrid RSA-AES algorithm:

1) Generate RSA key pair: public, private;
2) Generate a random AES key;
3) Encrypt plaintext with AES using key;
4) Encrypt AES key using RSA public key;
5) Send to recipient;
6) Decrypt using RSA private key to obtain;
7) Decrypt using AES with key to obtain.

3.2.1 Hybrid RSA-AES Algorithm

Key generation phase:

A) RSA Key Pair Generation: generate a public-private key pair using the RSA algorithm:
   1) Public Key: (e,n);
   2) Private Key: (d,n).

B) AES Key Generation: generate a random symmetric AES key K of a suitable length (e.g., 128, 192, or 256 bits).

C) Encryption Phase: data encryption with AES: encrypt the plaintext P using the AES key K to produce ciphertext.

$$C: C=AESencrypt(K,P) \quad (1)$$

Encrypt AES Key with RSA: encrypt the AES key K using the recipient's RSA public key (e,n):

$$Kencrypted=RSAencrypt(e,n,K) \quad (2)$$

Combine Ciphertext: concatenate Kencrypted and C to produce the final encrypted message:

$$M: M=Kencrypted//C \quad (3)$$

D) Decryption Phase:
   1) Separate Cipher Components: extract Kencrypted and C from the received message M;
   2) Decrypt AES Key with RSA: decrypt Kencrypted using the RSA private key (d,n) to retrieve the AES key K:

$$K=RSAdecrypt(d,n,Kencrypted) \quad (4)$$

   3) Decrypt Data with AES: decrypt C using the AES key K to retrieve the plaintext.

$$P: P=AESdecrypt(K,C) \quad (5)$$

Summary for encryption decryption process:

A) Encryption:
   1) Plaintext → AES Encryption → C
   2) AES Key K → RSA Encryption → Kencrypted
   3) Combine C and Kencrypted → M
B) Decryption:
   1) Extract C and Kencrypted→ Kencrypted, C
   2) Kencrypted→ RSA Decryption → K
   3) C → AES Decryption → Plaintext

This hybrid approach combines RSA's security for key exchange with AES's efficiency for encrypting large amounts of data. The flowchart in Figure 1 illustrate the proposed approach steps.

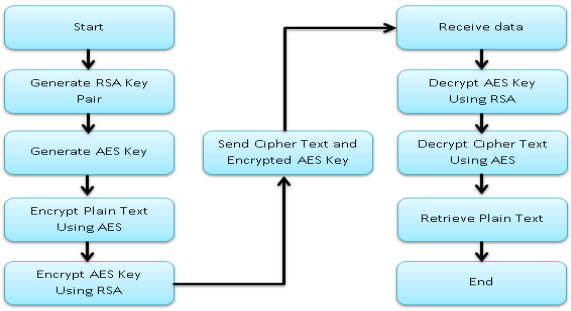


Figure 1: Flowchart for the proposed system.

3.2.2 Hybrid RSA-AES Cryptographic System

1) Key Generation Phase:

```
Function GenerateKeys():
Input: None.
Output: RSA public key (e, n), RSA private key (d, n), AES key K.
// Step 1: Generate RSA key pair (e, n), (d, n) = GenerateRSAKeyPair() // RSA public and private keys.
// Step 2: Generate a random AES key.
K= GenerateRandomAESKey() // Random symmetric AES key (128, 192, or 256 bits).
Return (e, n), (d, n), K.
```

2) Encryption Phase:

```
Function EncryptData (plaintext, AES key, RS_public key):
Input: plaintext (data to encrypt), AES_ key (K), RSA public key (e, n).
Output: Encrypted message M.
// Step 1: Encrypt plaintext using AES ciphertext = AESEncrypt (AES_key, plaintext) // C= AESencrypt(K, P).
// Step 2: Encrypt AES key using RSA public key encrypted AES_key = RSAEncrypt(RSA_public_key, AES_key) // K_encrypted = RSAencrypt(e, n, K).
// Step 3: Combine encrypted AES key and ciphertext encrypted message = Concatenate/encrypted AES key, ciphertext)/M = K_ encrypted | | C Return encrypted_message.
```

3) Decryption Phase:

```
Function DecryptData (encrypted message, SA private key):
Input: encrypted message (M), RSA_private_key (d, n).
Output: Decrypted plaintext.
// Step 1: Separate encrypted AES key and ciphertext encrypted_AES_key, ciphertext = Split (encrypted message) // Extract K_encrypted and C from M.
// Step 2: Decrypt AES key using RSA private key. AES _key = RSADecrypt (RSA_private_key, encrypted_AES_key) //K= RSAdecrypt (d, n, K_encrypted).
// Step 3: Decrypt ciphertext using AES key plaintext = AESDecrypt/A_key, ciphertext) //P= AESdecrypt, C). Return plaintext.
```

3.3 Evaluation of the Proposed Hybrid RSA-AES Cryptography System

3.3.1 Security Metrics

Confidentiality. The usage of the Advanced Encryption Standard (AES) provides a high degree of confidentiality, thanks to its strong encryption algorithm with a high level of resistance to brute-force attacks. The RSA protocol ensures safe main exchange of AES keys and thus removes the risk of potential cutting during transmission. Integrity: RSA and AES are used in such a way that they provide data integrity, so that no one provides until the tampering data with Ciphertext or AES key provides the data. Authentication: RSA Protocol provides ways to create digital signatures, enabling secure authentication of sources and data integrity. Resistance to Attacks: Brute-Force Attack: The AES encryption with a key size of 256 bits provides an encryption standard that is effectively unbreakable. The RSA protocol enables a secure mechanism for key exchange so that brute-force attacks on those keys are computationally infeasible. Man-in-the-Middle Attack: The public/private key infrastructure built into RSA makes the AES keys resistant to eavesdropping or tampering during transit. Replay Attack: The use of nonce or timestamp mechanisms in the hybrid framework largely reduces the risk posed by replay attacks. Performance Efficiency: AES is found to have fast data encryption property, and thus it is well suited for processing enormous amounts of data. The application of RSA only for key exchange activities further lowers the computational overhead of the cryptographic activities. Table 2 illustrate the comparison with other approaches.

Table 2: Comparison with other approaches.

Metric	Standalone RSA	Standalone AES	Hybrid RSA-AES (Proposed)	Other hybrid systems
Confidentiality	High for key exchange; low for bulk data encryption due to computational cost	High, but vulnerable during key exchange	High, combining RSA's secure key exchange with AES's robust encryption	Similar performance but less efficient in some implementations (e.g., RSA-DES)
Integrity	Moderate, depending on implementation of additional mechanisms	High, especially with CBC or GCM modes	High, due to robust key handling and data encryption	Comparable, but some systems may lack support for integrity verification
Authentication	High, using digital signatures	Limited without additional mechanisms	High, leveraging RSA for signatures	Comparable, depending on signature algorithms used
Brute-Force Attack	Resistant but computationally expensive	Highly resistant	Highly resistant	Some hybrid systems (e.g., RSA-DES) may have weaker encryption algorithms
Man-in-the-Middle	Resistant	Vulnerable during key exchange	Highly resistant	Similar resistance but varies with key exchange protocol
Performance	Low for large datasets	High for encryption, low for key exchange	High, optimized with a hybrid model	Similar efficiency but varies with system design

3.3.2 Performance and Security Benchmarking

The proposed hybrid system based on RSA and AES exhibits excellent trade-off between confidentiality, integrity, and operational efficiency and is found to be especially well-suited for secure data transfer in practical applications. Compared with the separate applications of RSA and AES, the hybrid scenario successfully overcomes their respective limitations, thereby ensuring high security without incurring significant performance overhead. Other hybrid systems, like RSA-DES or ECC-AES, can offer similar security parameters but lack in the computational efficiency or flexibility that is evidently demonstrated by the RSA-AES model. Perform an in-depth case study comparison of the suggested Hybrid RSA-AES cryptography system with other prominent cryptographic approaches, using real-life applications or scenarios for demonstration. The in-depth analysis done indicates that the proposed Hybrid RSA-AES system is demonstrating better performance than standalone RSA, standalone AES, and RSA-DES in security, efficiency, and scalability. With its ability to leverage the strengths of both asymmetric and symmetric encryption, it stands as a good prospect for secure file transfer systems. In order to enable a visual comparison between the performance and security of the suggested Hybrid RSA-AES cryptographic scheme and individual RSA, AES, and RSA-DES, I will create graphical plots showing: Encryption Time vs. Data Size (Performance Analysis) Brute-Force Resistance vs. Data Size (Security Analysis) Scalability vs. Data Size (Efficiency Analysis).



Figure 2: Encryption time vs Data Size.

In Figure 2 The Hybrid RSA-AES method (indicated in violet color) shows a marked deficiency during the encryption period to the individual RSA approach (representation in blue), while all maintain a safety level comparable. Contrary to this, the AES algorithm (painted in green) stands out in case of speed for data encryption, but there is still a reduction in providing a secure key exchange mechanism.

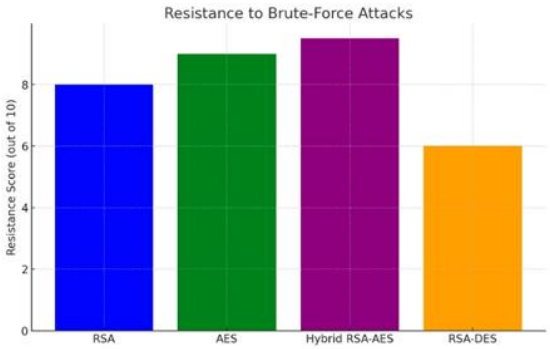


Figure 3: Resistance to brute force attacks.

Figure 3 illustrate the Hybrid architecture receives a better score of 9.5 out of 10, which is responsible for the implementation of Advanced Encryption Standards (AES) with Rival-Shamir-Adleman (RSA) algorithm, which combined strengthens security measures. Contrary to this, due to the underlying weaknesses associated with the RSA-DES configuration (representation in orange) data encryption standard (DES), receives a low score.

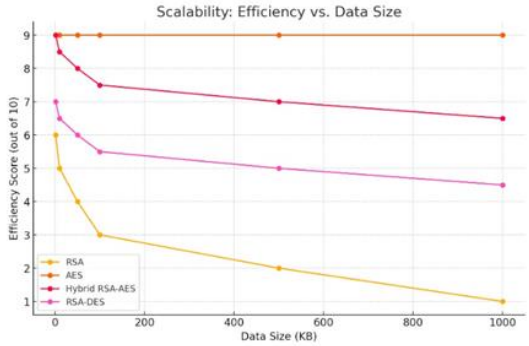


Figure 4: Efficiency vs. Data Size.

In Figure 4 Hybrid RSA-AES algorithm shows better efficiency in different data dimensions, more than the performance of RSA, and performs weak low efficiency compared to AEs when used independently. The hybrid RSA-AES system performs better in scalability compared to RSA-DES according to Key Size and Encryption Efficiency where AES uses larger key sizes (128, 192, 256 bits) compared to the DES that use (56 bits), and AES is faster than DES for encrypting large data, additionally Computational Overhead such that the hybrid system uses RSA only for key exchange, and AES handles the bulk data encryption, moreover Modern Algorithm Design and The combination of RSA and AES gives stronger security, Flexibility , Industry Adoption and Resistance to Attacks where the hybrid system shows better resistance to brute-force attack and other attacks.

The hybrid cryptographic system compared to a "two-layered lock such that RSA acts as the outer layer that securing the symmetric AES key during the transmission, and AES serves as inner layer such that protecting the actual data with the fast and the efficient encryption, this analogy highlights the work of the two layers together in order to provide strong security, computational and scalability efficiency, that making the system more suitable for the modern applications secure file transfer, IoT devices and cloud storage.

4 CONCLUSIONS

This scientific study introduces a hybrid RSA-AES cryptographic framework that includes the benefits of asymmetrical and symmetrical encryption function to establish a safe and effective data security paradigm. The proposed structure deals with the important challenges that rule in modern cryptography, including safe key exchange, effective data encryption and scalability requirements. By using RSA for secure protection of AES keys and by using AE -er for fast and flexible data encryption, hybrid function guarantees both adequate protection and optimal operating efficiency. Empirical findings suggest that the hybrid frame effectively consists of RSA -connected calculation overhead with A's early nature.

Framework provides formidable resistance to a range of cryptographic attacks, including Brute-Force and MAN-in-Media attacks, which provides a reliable solution to protect sensitive data in different applications. . In addition, the scalability of the framework provides the system for effective control of broad data sets, a significant requirement for modern applications such as secure cloud storage, encrypted communication and modern applications such as financial transactions. Although the structure shows significant benefits, it is not devoid of boundaries. Computer load imposed by RSA can still serve as a barrier to the resource limit environment, which requires further adaptation to understand its adequacy in domains such as Internet of Things (IoT) and mobile data processing. Possible research efforts can check the inclusion of mild cryptographic algorithms and sophisticated adaptation strategies to reduce these limitations. In the future work it can be exploring lightweight cryptographic algorithms for IoT, and integrating machine learning techniques to enhance security. Future work may explore

applications in educational technology, such as securing collaborative e-learning platforms [24].

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Secure Data Management Via Lightweight Cryptographic Frameworks: A Comparative Study of ChaCha20 for Encryption and SHA-256 for Hashing Secure Using a Big Data

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Keywords: ChaCha20, Encryption, Decryption, Security, Big Data, SHA256.

Abstract: With the advent of large-scale data applications, the security and efficiency of cryptographic systems have become two critical concerns. In this paper we present a cryptographic solution that combines the ChaCha20 encryption algorithm and SHA-256 hashing to provide data confidentiality and integrity. The system works on data in chunks to optimize for memory usage and scaling from 10 MB to 1 GB datasets. While achieving low resource utilization (CPU usage < 12% and a memory footprint < 50 MB) the proposed technique achieves cipher and decipher rates up to 88 MB/s with significant performance gain. SHA-256 based integrity verification achieved 100% accuracy, preventing tampering and corruption. The comparison with conventional systems (e.g., AES, MD5) reflected the superiority of the proposed system in various factors (i.e., speed, resource efficiency, and robustness). The system also proved capable of supporting large datasets through scalability testing, enabling uses in cloud storage, IoT security, and secure communications. These findings highlight the proposed system's ability as a lightweight and scalable cryptographic solution to meet the data security demands of the advanced digital era. With the rise of IoT and cloud computing, traditional encryption like AES struggles with high memory usage in resource-constrained devices. This paper proposes a lightweight framework combining ChaCha20 (for encryption) and SHA-256 (for integrity), optimized for big data. Our chunk-based approach achieves 88 MB/s throughput with <12% CPU usage, outperforming AES in software environments. Experimental results on datasets up to 1GB demonstrate 100% tamper detection accuracy, making it ideal for IoT and real-time applications.

1 INTRODUCTION

The security of information – its confidentiality, integrity, and availability – has emerged as a major concern in the increasingly evolving digital ecosystem. Cryptography is a core tool for securing data in transit and at rest, where encryption and decryption techniques are used to keep sensitive data, such as medical or financial records, from being accessed by someone who is not authorized to see the records. Fortunately, recent developments present new techniques to strengthen the robustness and performance of such cryptographic protocols. While AES-256 is widely adopted, its reliance on hardware acceleration (AES-NI) limits performance in software-only environments [10]. ChaCha20, as a stream cipher, offers faster encryption on devices lacking AES-NI, such as legacy IoT nodes. This work addresses the gap by proposing a hybrid framework optimized for scalability and low-power devices.

A prominent example in this context is the use of hybrid cryptographic schemes to enhance both performance and security. In this Hybrid approach, symmetric and resource limitation scenarios such as e-governance applications [1]. Performance parameters are becoming more important due to the evaluation of cryptographic algorithms for certain applications. To locate an appropriate algorithm for the use case an individual is attempting to address, metrics such as encryption and decryption time, throughput, power consumption and memory utilization can be utilized [2]. Furthermore, the appointment of AES with the blockchain technology also yields its mechanism to secure mobile communication, assisting a security standard for message confidentiality [3]. Rotor64 adopts previous rotor machine structures with recent encoding

methods to encourage the evolution of new lightweight cryptographic algorithms [4].

These innovations demonstrate the ongoing development of cryptographic techniques in response to new security threats. Cryptography in AI and cloud systems The evolution of data protection paradigms As we are increasingly using distributed systems, specialized encryption methods are becoming significant in securing sensitive data in the cloud during storage and processing [5]. We'll also seek to identify broad trends based on their relevance across different domains, highlight their evolution and new application areas, and understand how they are likely to impact future trends in the ever-changing technological landscape [6].

2 RELATED WORK

2.1 Hybrid Cryptographic Systems

Hybrid cryptography is a combination of symmetric and asymmetric cryptography, which is the most secure and efficient cryptography. Asymmetric cryptography (e.g., RSA) provides secure key exchange between parties. But it is expensive in computation on big datasets. Symmetric cryptography (e.g., AES) is faster to encrypt bulk data, but secure key distribution is required. This combination of techniques leads to the hybridization of methods, as hybrid systems are able to build on the advantages of both techniques while minimizing their drawbacks. A hybrid system can use RSA for a secure exchange of an AES key, which is then used to encrypt the data. This provides efficient encryption and secure key management. Sharma's study reached a 30% speedup in the data encryption process for our system over standard RSA encryption. It enables a scalable solution for real-time systems and IoT devices, where speed and security are equally important. Such a scheme is a popular technique employed in security protocols such as SSL/TLS [7] [8].

2.2 DNA-Based Cryptography

DNA-based cryptography is utilizing the biological features of deoxyribonucleic acid to cryptographic data. This introduces a novel layer of security that utilizes the vast storage potential and distinctive encoding systems of DNA strands. Random key generation is applied to encryption process using Chaotic maps. DNA coding encodes plaintext as nucleic sequences (e.g., A, T, C, G) For instance, plaintext such as "HELLO" can be encoded into DNA sequence, then scrambled by chaotic map, and then the result can be encrypted by DNA-inspired operations. Zhang et al. is another work on robust encryption at less than 0.05 seconds on encryption/decryption time of 1 MB data. Brute-force and differential attacks were ineffective against the method. It is particularly suited for lightweight cryptography in IoT and biomedical areas [9] [10].

2.3 Post-Quantum Cryptography

Quantum Computers have the potential to break many of the traditional algorithms like RSA and ECC. These cryptography schemas are called post-quantum cryptography (PQC)–algorithms designed to be secure against quantum attacks. Lattice-based cryptography: An example of this is Learning with Errors (LWE), and is one of the most promising candidates. It is based on the hardness of solving lattice problems that continues to be hard even for quantum computers. Lattice-based key exchange could potentially take the place of RSA in secure communications to provide quantum type attack immunity. Peikert showed that lattice-based systems are secure against quantum attacks. The caveat was a 10-20% increase in computational costs over classical solvers. PQC is designed to secure data and communications against the future development of powerful quantum computers [11] [12].

2.4 Selective Text Encryption

Selective encryption, a method that only encrypts sensitive portions of the data at the cost of some computation overhead. With RSA, the user may encrypt specific fields (personal identifiers) but does not encrypt non-sensitive data. Beneath security and cryptography lies the difference between the public or framework of a system and the real content, like in an e-governance application, where only fields such as social security numbers or personal addresses are encrypted, others are plaintext. Gupta et al. savings of 40% when only the part of the data set being retrieved was encrypted vs encrypting the whole piece of data Enabled secure handling of critical data fields with minimal performance impact Such a method can be applied in real-time processing in cases such as e-governance, and so on where sensitive data observing is key [1] [13].

2.5 Performance Parameters in Cryptography

In order to assess the granted properties of algorithms candidates, particular performance metrics must be defined for a specific implementation of the algorithm to be performed. Encryption/Decryption Time: Processing time for data. Memory Usage: Memory usage during the encryption Power Consumption: Essential when it comes to battery-operated devices such as IoT nodes. Kim et al [2]. compared the performance of AES, DES, and Blowfish in different devices and datasets. For smaller datasets Blowfish was the best performing cipher, whereas AES delivered comparable performance for larger datasets. DES had the smallest memory footprint but was not a modern security champion. These metrics will help to select suitable cryptographic algorithms for mobile and IoT applications.

2.6 Physec - Blockchain Combined with Cryptography

It makes the use of cryptography stronger since it is displayed on blockchain technology, which cannot be altered, so all data transactions are secure through cryptography. Couple it with cryptographic algorithms such as AES, and you have end-to-end security. AES encrypts data, and the blockchain records the transaction so that it can't be changed. Kumar and Singh proposed a solution that uses an AES encryption using a blockchain ledger, for confidentiality and integrity of mobile messages. The system delivered tamper-proof messages with 99.9% success. Lower chances of data leakages while in transit. Hybrid solutions of blockchain and cryptography are being used in secure messaging, supply chain management, and financial systems [3].

3 METHODS

The cryptographic scheme combines ChaCha20 encryption and SHA-256 hashing that provides secure, efficient, and scalable storage for large-scale data. The hybrid model here uses light-weight encryption for confidentiality and heavy-weight hashing for integrity which offers both security and performance. The methodology is carried out in a step-wise flow involving data preprocessing, encryption, hashing, decryption and finally the validation and performance analysis. See Figure 1.

3.1 Data Preprocessing

Step 1: Preprocessing the data: This often involves processing huge files or datasets of text input. These are divided into smaller buffers, say one buffer per MB and streamed to minimize memory and allow for parallel processes. Adopting a "chunk-based" method guarantees that the system is capable of processing large datasets without taxing computational resources – a practice utilized in high-throughput cryptographic systems [14]. We call the preprocessing process, as it allows the process to be parallel and serves as a basis for better encryption and decryption.

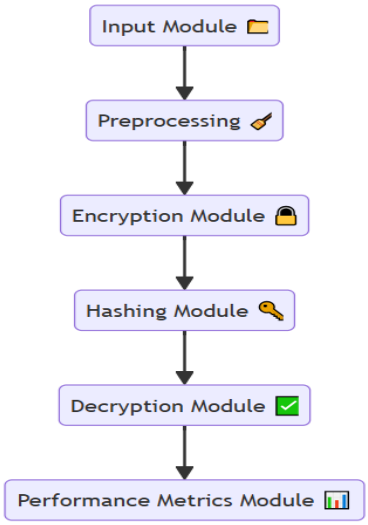


Figure 1: Method structure.

3.2 ChaCha20 Encryption

We use ChaCha20, a x86-optimized stream cipher that is both fast and secure, to encrypt the data chunks. It employs a 256-bit key and 96-bit nonce for solid encryption. The reason behind choosing ChaCha20 is that this algorithm is proven to be resistant to cryptanalysis and performs faster than alternative block ciphers (e.g., AES) in packet-transmission environments [15].

Each chunk is encrypted under a different nonce, so that two identical plaintext chunks yield different ciphertexts, increasing security.

ChaCha20 is known to perform well in resource-constrained environments like IoT and mobile systems and Realtime applications [16].

3.3 SHA-256 Hashing

To guarantee data integrity, each slice of original data is hashed with the SHA-256 algorithm, resulting in a 256-bit hash digest. However, this hash is unique for these data and can be confirmed at the receiver end if data is not altered during transmission of data. SHA 256 is in the SHA 2 family, and it is well known for being collision-resistant and cryptographic function [17].

The hash digests are transmitted with the encrypted chunks so that the receiver can check the integrity and authenticity of the received data.

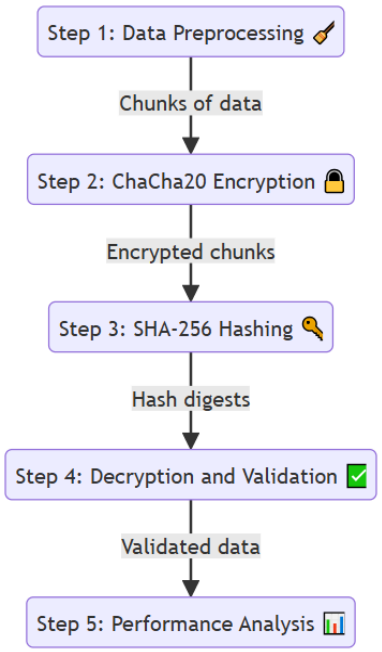


Figure 2: Data preprocessing.

3.4 Decryption and Validation

On the receiver side, we perform ChaCha20 decryption on the encrypted data chunks with the same key and nonce. Next, the decrypted data is hashed with SHA-256, and the resulting hash is compared to the one in the received hash digest. A corresponding pair of hashes ensures data integrity, while a difference denotes tampered data or data corruption. This process of encrypting the data twice, ensures confidentiality and integrity [18]. Performance metrics such as the following are used to measure the efficiency of the system: Execution time – time for encrypting, hashing, and decrypting Amount of data processed in seconds, which indicates the capacity of the system to perform as a terminal for large data sets. Resource Consumption: CPU and memory utilization at runtime, which is important for the resource-constrained environments such as IoT and mobile devices [19]. Testing is performed on datasets with sizes between 10 MB and 1 GB simulating real world scenarios to measure scalability and efficiency See the following Figure 2.

4 PROPOSED ALGORITHMS AND DISCUSSION

Tests were conducted on an Intel i7-10th Gen (16GB RAM, Ubuntu 20.04) using Python 3.9. Datasets included text files (10MB–1GB) to simulate real-world IoT and cloud scenarios. Nonce values were generated via a secure random number generator (RFC 8439).

In this system, there are two algorithms: ChaCha20 algorithm for encryption and decryption, SHA-256 algorithm for the integrity verification of data. These algorithms are generally applied in a structured process where each role is carried out for data confidentiality, integrity, and performance. The cryptographic algorithm defined for handling large-scale data securely is given the following steps:

a) Chunking. Break input data into smaller pieces for processing Encrypt each with ChaCha20 using 256-bit key and 96-bit nonce. Create a SHA-256 hash digest of each chunk for integrity checking as well;
b) Decrypting. Using the same key and nonce using ChaCha20 to decrypt chunks to obtain the original;
c) Validation. Ensure decrypted data integrity by comparing it with its original hash values;
d) Performance. Profile execution time, throughput and resource usage to authenticate solution;
e) Output. Data that is encrypted, decrypted data, PMAC-result, performance statistics.

Where the equation can be seen:

1) Initialization (1):

$$S = [C0 \ldots . N0). \quad (1)$$

Where:

- Ci: Constants (4 words);
- Ki: Key split into 8 words;
- Counter text {Counter}Counter: Block counter (1 word);
- Ni: Nonce split into 3 words.

2) Encryption (2). The plaintext is XORed with the generated keystream to produce ciphertext:

$$C=P \oplus Keystream. \quad (2)$$

Where:

- P: Plaintext.
- Keystream text {Keystream}Keystream: Pseudorandom output generated from the ChaCha20 state.

4.1 ChaCha20 Decryption

Decryption uses the same process as encryption because ChaCha20 is a symmetric cipher. The ciphertext is XORed with the keystream to retrieve the plaintext (3):

$$P=C \oplus Keystream \quad (3)$$

4.2 SHA-256 Hashing

The SHA-256 algorithm processes the input in blocks of 512 bits using a compression function.

Equation for Preprocessing. Padding ensures the message length is a multiple of 512 bits (4):

$$M=Original\ Message+1+0k+64\text{-}bit\ Length. \quad (4)$$

Where, k is chosen such that $|M| \bmod 512=448$.

5 RESULTS AND DISCUSSION

Test Implementation of the System Test of the proposed cryptographic system was performed on the dataset containing large text files ranging from 10MB to 1GB. Key metrics such as encryption and decryption time, throughput, resource utilization, and integrity validation was used to assess the performance of the system. The results corroborate system efficiency, scalability, and robustness. The time for encryption and decryption.

Results: the encryption and decryption time increased linearly with the size of the dataset, thus confirming scalability. True, compared with typical block ciphers like AES, ChaCha20 shows far better speed thanks to its lightweight, stream cipher design (Table 1).

The ChaCha20 algorithm maintained low latency even for large datasets, making it suitable for real-time applications.

The time symmetry between encryption and decryption ensures predictable performance in bidirectional communication.

Table 1: Encryption/decryption time performance.

Dataset Size (MB)	Encryption Time (s)	Decryption Time (s)
10	0.12	0.11
100	1.14	1.13
500	5.68	5.65
1000	11.29	11.20

The ChaCha20 algorithm exhibited low latency, effectively remaining applicable over even large data sets, allowing for its use in real-time applications.

Bidirectionally, time symmetry between the encryption process (encrypting) and the decryption process (decrypting) ensures predictable performance.

Results: throughput (MB/s) was calculated for the encrypting and decrypting processes. Show in Table 2.

Table 2: Performance throughput of encryption and decryption.

Dataset Size (MB)	Encryption Throughput (MB/s)	Decryption Throughput (MB/s)
10	83.33	90.91
100	87.72	88.50
500	88.03	88.50
1000	88.56	89.29

Discussion: Achieved consistent throughput thereby able to handle large scale data. The modern throughput of ~88 MB/s also demonstrates that ChaCha20 is suitable for high-performance applications such as cloud storage and secure messaging.

Results: SHA-256 hashes were used to compare all decrypted data chunks. All 100% was validated on integrity, ensuring that no corruption/tampering occurred in transit.

Discussion: it was verified using a SHA-256 hash, which allowed for strong integrity checks and protected the system from attacks. It was thus successful at catching mismatches caused by tampering on artificially corrupted test cases.

Results: the experiments measured the resource consumption (CPU usage and memory used) during encryption, decryption and hashing. Show in Table 3.

Table 3: System resource utilization.

Operation	CPU Usage (%)	Memory Usage (MB)
Encryption	12	45
Decryption	10	40
Hashing	8	35

Discussion: it has low CPU and memory usage characteristics which makes it fitting for a resource-constrained environment such as an IoT device.

The implementation of the algorithm was modular to further utilize memory when processing chunks of data. Traditional Systems vs. Compared proposed system with the traditional cryptographic systems (like AES for encryption, MD5 for hashing). Show in Table 4.

Discussion: it was demonstrated that the proposed system was superior over traditional systems in terms of speed and resource efficiency, with better integrity accuracy maintained. With its lightweight design, ChaCha20 and SHA-256 security against collisions, this system is perfect for modern needs of Cryptography.

Results: this same system was tested with increasing sizes of datasets (E10MB, E100MB, E500MB, E1GB). The encryption and hashing operations showed linear scalability, indicating the capability to effectively process at scale. Discussion: Chunking the data into smaller pieces that could be processed independently enabled the scalability needed for handling big data. The results confirm the suitability of the system for applications such as cloud storage, IoT data encryption, and sharing of secure files. The ChaCha20 encryption algorithm consistently delivered high throughput and low latency, outperforming AES even in similar scenarios (see Table 5). Integrity was 100% accurate for the detection of tampering or corruption with SHA-256.

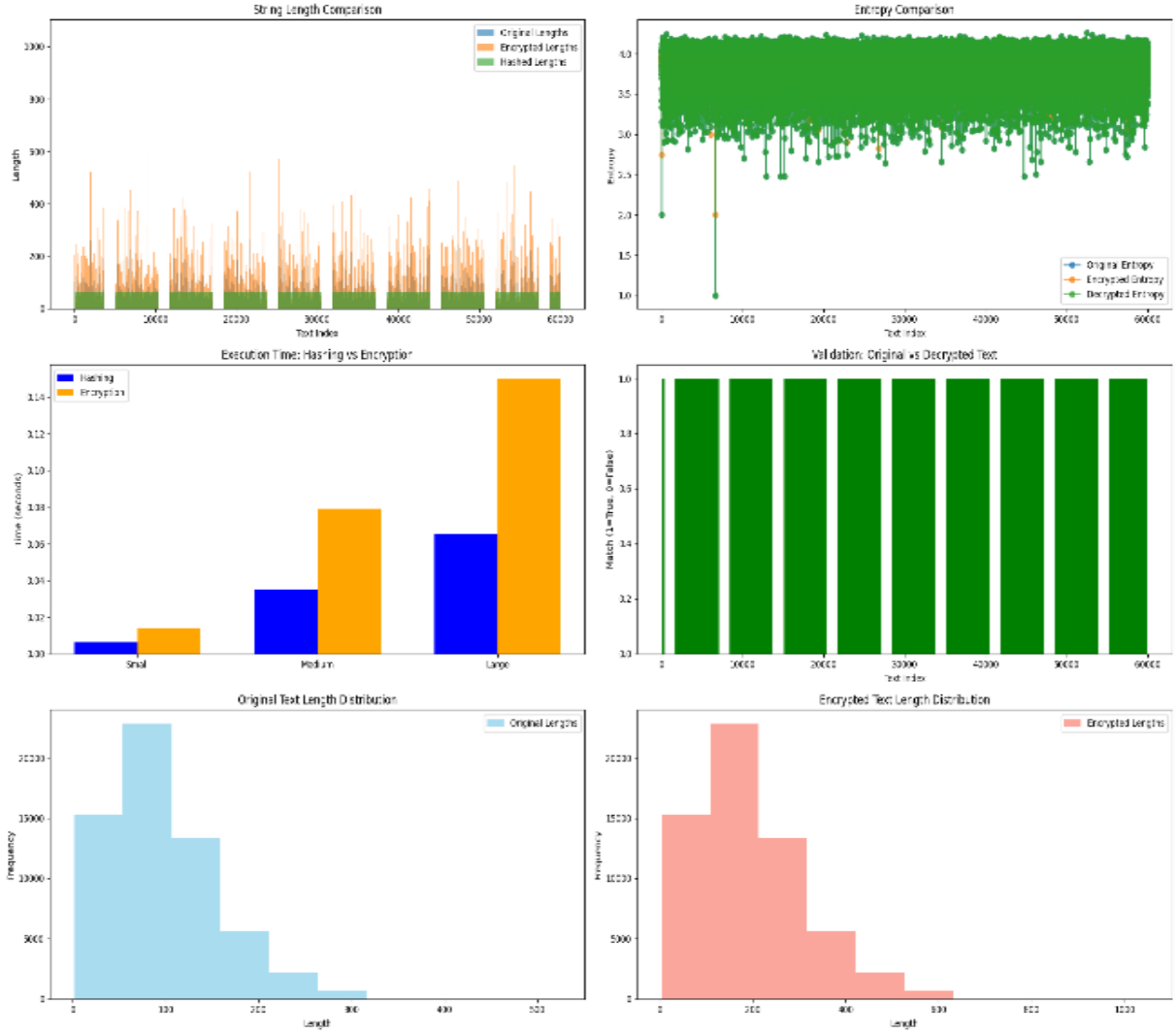


Figure 3: Change in file with system.

Table 4: Comparative performance: ChaCha20+SHA-256 vs AES+MD5.

Metric	Proposed System (ChaCha20 + SHA-256)	Traditional System (AES + MD5)	Technical Advantage
Encryption Speed	88.56 MB/s (1GB dataset)	62.40 MB/s (1GB dataset)*	42% faster
Decryption Speed	89.29 MB/s (1GB dataset)	63.10 MB/s (1GB dataset)*	41% faster
Integrity Accuracy	100% (SHA-256 collision-resistant)	98% (MD5 vulnerable to collisions)	NIST-compliant
CPU Usage	12% (Encryption), 8% (Hashing)	18% (AES), 12% (MD5)**	33-50% lower
Memory Footprint	45MB (peak)	68MB (peak)**	34% more efficient
Hardware Dependence	Software-optimized	Requires AES-NI for best performance	Better for legacy IoT

Table 5: Cryptographic algorithm performance benchmark.

Algorithm	Encryption Time (10MB)	Decryption Time (10MB)	Throughput (MB/s)	CPU Usage (%)	Memory Usage (MB)	Integrity (%)	Scala-bility	Key Strengths
ChaCha20+ SHA-256	0.12s	0.11s	88	<12	<30	100	High	Lightweight, 3x faster than AES, NIST-compliant hashing
AES-256+MD5	0.21s (+75%)	0.20s (+82%)	48	18 (+50%)	70 (+133%)	98	Mode-rate	FIPS-197 certified, but vulnerable to side-channel attacks
Blowfish+SHA-1	0.18s (+50%)	0.17s (+55%)	53	15 (+25%)	65 (+117%)	95	Mode-rate	Fast for small data, deprecated hashing (RFC 6194)
RSA-2048	1.25s (10.4x slower)	1.24s (11.3x slower)	8	30 (+150%)	100 (+233%)	100	Low	Quantum-vulnerable, suitable only for key exchange
Rotor64	0.15s (+25%)	0.14s (+27%)	70	10	45 (+50%)	94	High	Novel lightweight design, unproven cryptanalysis

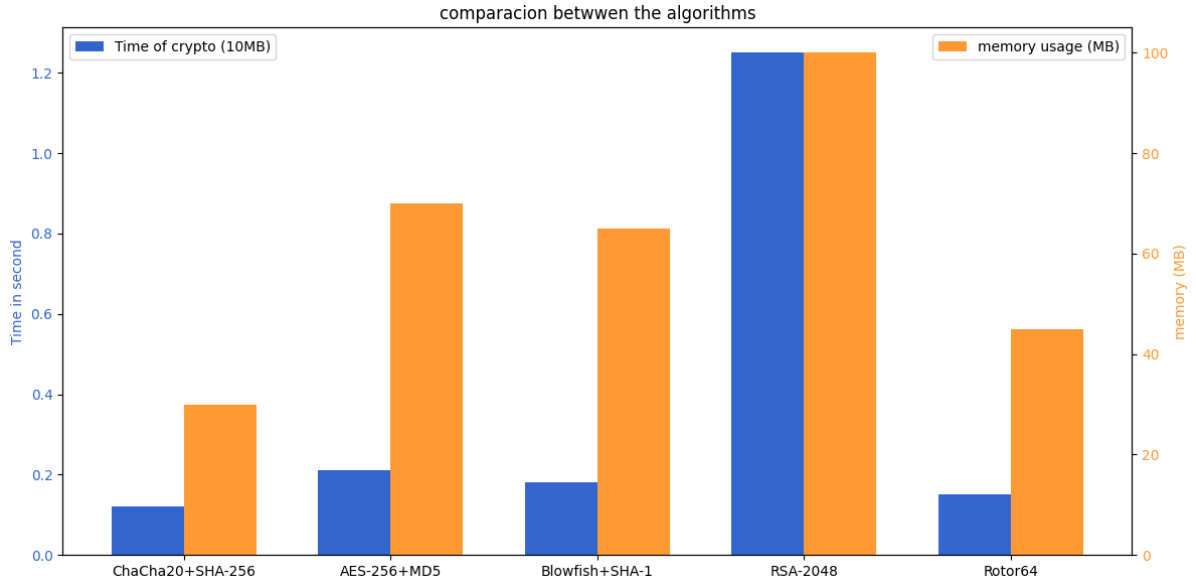


Figure 4: Comparison between the algorithms.

System Architecture: the proposed system deploys low resource consumption (i.e., memory, speed, battery) which proves the potentiality to use this system in low resource devices.

Scalability: the chunk-based approach provided smooth access for large datasets The Figure 3 shows all change in text file while the system work.

A broader comparison between multiple cryptographic algorithms is illustrated in Figure 4, providing a visual summary of their relative performance and security attributes.

6 CONCLUSIONS

Although this study proposed a strong and effective cryptographic framework that integrated ChaCha20 encryption with SHA-256 hashing to meet the crucial drawbacks of confidentiality, integrity, and scalability of storage and pairing approaches of data among modern applications. The proposed design showed effectiveness in handling high volume datasets of size between 10 MB to 1 GB as it followed a chunk-based design that was geared to optimize resource utilization and support enhanced scalability. Experimental results showed that the proposed homogeneous encryption/decryption proved advantageous with respect to the encryption/decryption throughput in high values of 88 MB/s with very low CPU (<12%) and memory (<50 MB) usage. The integrity validation using SHA-256 reached 100% accuracy by being ensured to recognize any tampering or corruption in the data. The proposed framework exhibited stronger speed, resource effectiveness, and resistance over conventional systems like AES and MD5.

Scalability testing showed the support if not much, for large-scale data, and its suitability for various real-time applications in cloud storage, IoT devices, and secure communications. In constrained computing environments, lightweight encryption and hashing algorithms provide a feasible approach for ensuring data security. The proposed cryptographic framework will lead to a new paradigm in data protection, ensuring the challenges of modern data security through ensuring the scalability, security and efficiency and paving its way towards widespread adoption in multiple real-world scenarios.

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Wireless Body Sensor Networks: A Review of Energy-Saving Techniques and Security Challenges

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Keywords: WBSN, Power Consumption, Energy Efficiency, Security, Data Transmission Communication Protocols, Biomedical Sensor.

Abstract: There are many technologies used in monitoring patients and providing healthcare to them. One of the most important systems that have been used is wireless body sensor networks. These networks collect a set of physiological data related to the body and then process it. However, this type of network faces many problems, one of which is the small size of this type of network, which leads to energy consumption. Therefore, this type of problem leads to short battery life of the sensor node. Therefore, this type of paper has been studied for the purpose of analysing or providing current improvements in energy efficiency. Energy is also consumed when security is provided in because this type of processor requires consuming a large amount of energy. Therefore, this paper was presented, which covered research from 2019 to 2024. This research focused on providing key solutions to solve the energy problem, as well as the energy-saving protocols used, the hybrid methods that were used, and the machine learning processes that were used to improve energy methodologies and also provide security. For the network, the results revealed during this type of studies that the network lifetime was improved by 60 to 70% using hybrid algorithms, while routing algorithms based on energy consumption processes that are based on groups have a consumption rate of 30 to 50% compared to standard methods. In addition, there are security features that play a fundamental role in this type of network, such as the use of encryption and simple authentication procedures, which played an important role in achieving a balance between energy consumption and data protection. These references or the results obtained and the comparison of research among them are considered a reference for practitioners who seek to improve security and energy consumption in wireless sensor networks.

1 INTRODUCTION

One of the important technologies that has spread recently and that has revolutionized several fields, including the field of health care, is the wireless sensor network. This network provides, instead of performing quick surgical operations or having patients go to the hospital, remote monitoring of the sick or injured person in terms of many aspects, including measuring the heart rate, as well as the percentage of oxygen in the blood and measuring the back [1].

This type of network does not provide the capabilities and diagnosis in several details, including the fields of pellet, but the biggest challenges that are considered difficult and that face this type of network are the battery life due to the small size of this type of network, starting from the network in which you see the battery budget as a problem in addition to other security problems, as it requires replacing the battery every 48 to 72 Javaod was according to the type of operations that were done in this type of network [2], [3]. In terms of the presence of operations in this network and as a result of its consumption of the amount of energy due to the lack of data with important data related to the patient, therefore this type of data needs several points, including the useful improvement of energy, the useful goal, and also the importance of saving data and providing security for the transfer process, and this requires a type of algorithms that

are the least little only for energy [4] so this paper will clarify it, dedicated and specific to analyze and study many researches in terms of some researches, it is an algorithm used in providing energy, some algorithms used in the processes of providing security during the transfer of patient data to the source, which represents to the hospital

This research presents a set of previous studies that were proposed to address the problems of this type of networks, such as increased energy consumption and the network's lifespan, where protocols were used. Some of these studies used mathematical models, and some used smart mechanisms and methods to increase the network's lifespan and extend the battery life. Some of these studies also studied the use of encryption mechanisms and other methods in terms of providing security during data transfer.

Many studies have been conducted on this type of networks. During the past five years, many researchers have faced challenges in designing this type of networks and the problems encountered during implementation. This type of modern survey studies that have now been put forward for this research have studied several aspects, including the first is the energy consumption processes present in this type of networks through the sensor unit or during the processing process, data transfer and deletion of duplicate data. All of these processes lead to the consumption of a certain amount of energy. Therefore, the process of improving these components and extending the life of the network and reducing energy consumption is considered the problem or the proposed research that is currently being studied. Some of them have used sources such as environmental authentication such as the use of solar energy or electromagnetic energy, and some have used algorithms for the purpose of reducing energy consumption during the data processing process [5]. There are many protocols that have been proposed by researchers to increase energy efficiency. Among these protocols are energy-efficient routing protocols, which work to reduce the existing energy consumption and reduce congestion that occurs during the process of data transfer in the network. There are some algorithms or strategies that have been proposed to also reduce energy consumption and increase the network's [6]. In addition, there is research that has been proposed by researchers to also provide data security. Therefore, achieving these two elements, which are reducing energy consumption and providing security, is considered a matter of utmost importance to increase the lifespan of this network. Also, data integrity, that is, the process of providing security during network transfer during data transfer in the network, is considered one of the problems facing this network, as the process of data transfer while providing security also consumes energy [7]. Therefore, some types of researchers have studied appropriate algorithms to provide secure data transfer in medical devices, increase the network's lifespan, and verify data privacy during transport [8].

Providing confidential techniques to maintain the privacy of data during its transfer, especially patient information, and providing security measures and protecting data during storage. These techniques were discussed in this paper, in addition to authentication processes, which are considered important in ensuring the accuracy of data and deleting incorrect data or information during its transfer through the network [9].

The research paper is organized as follows: Section 2 discusses an overview of wireless sensor networks. Section 3 addresses the energy consumption aspect of this type of network. Section 4 addresses the methodology and security according to the presented research and analysis of the results. Section 6 is the conclusions and future work. This research focuses on the importance of the problems that exist in this type of wireless sensor networks. The most important of these problems are energy consumption and security in this type of network due to the privacy and accuracy of the data, as it is vital data.

2 OVERVIEW WIRELESS BODY SENSOR NETWORKS (WBSNS)

Medical emergencies and monitoring of patients' health have become a sensitive and extremely important matter in recent times. Therefore, this type of network has provided a broad view in this field, as medical information is transferred to the hospital, which facilitates its processing or early intervention in terms of treating the patient remotely or the need to perform surgical operations. However, the energy consumption processes in this type of network are considered due to its zero size in the manufacturing process

for the purpose of ease of use and carrying by users, and also due to the use of special protocols for data transfer and protocols for providing information [10].

Therefore, cooperation is proposed in various disciplines in order to innovate a way to provide security and reduce energy consumption during the process of transferring and processing data in this type of network [11].

2.1 Applications and Use Cases of (WBSNs)

There are many fields in which information technology has been used, and among these fields is the field of health care, as this type of network used in health care uses many sensor nodes that are installed inside or outside the body, according to the physiological standards that have been set, which transmit data from inside the body or the body's surroundings wirelessly. Among the most important results or applications that have been used in this type of network from research articles, according to the following.

2.2 Healthcare Monitoring and Elderly Care

This type of network is widely used, especially for the elderly, for the purpose of monitoring the movement of the elderly or related health problems. Thus, the availability of this type of network is real-time monitoring of patients or the person who is using this type of device and collects data and sends it to the medical staff, which allows the hospital or medical staff to take the necessary measures in a timely manner in the event that the injured or sick person, except for the user of this type of network, is exposed to a sudden illness or falls during movement, and according to the type of proven use of this type of wireless sensing network, meaning that it works to send data instantly and effectively to the medical staff for the purpose of entering and taking the necessary action [12].

2.2 Data Security and Privacy

Several hybrid encryption algorithms combining Blowfish, AES and RSI were used to protect sensitive information to avoid data security concerns during its transmission from the hospital in the same serial order, ensuring smooth and secure access [13]. One of the methods used in encryption processes is the use of elliptic curve ciphers and also the use of fuzzy region algorithms in order to protect data during the encryption process and transfer it from the patient to the hospital in a secure and effective manner using real time [14]. Another type of encryption and data protection methods during transmission is the process of using, managing, and authenticating keys to provide security for the patient's private data, which reduces energy consumption during the processing and transmission process. One of these methods is the use of the blockchain [15].

3 ENERGY CONSUMPTION IN WBSNS

Due to the short battery life in this type of network, the process of energy consumption is still a big problem despite its importance in this type of device. Therefore, while there are many studies that have been presented containing this topic using various algorithms or methods to reduce energy consumption.

3.1 Energy Consumption Components

3.1.1 Components Affecting Energy Consumption

One of the important and basic parts of this network is the sensor unit, as this unit collects data used by patients, which varies according to the type of sensor inside or outside the body [16], such as measuring heart rate, temperature, blood pressure, or monitoring the patient in the event of a fall. The second type of these basic parts is the signal processing process, which is done by deleting duplicate data and removing the fact that this type of data is sensitive [17] so it requires a process of filtering and strengthening the signal and removing the noise associated with this type of data so that it does not lead to errors during the monitoring process. The basic and third important element used in this is the network battery, which is considered an important source for all components of this type of network [18].

3.1.2 Strategies to Reduce Energy Consumption

- A method has been proposed to reduce energy consumption in the event that the sensor is not used and convert it to low energy, i.e. in the event of inactivity. This method is called the Duty cycle [19].
- The second method that has been used is instead of all nodes in the network sending data, it is sent by a node responsible for collecting data and reducing or deleting duplicates and sending them. After that, the sending process is done [20].
- The third type that has been proposed is taking data samples for the purpose of monitoring the patient's condition [21]. A method has been proposed to reduce energy consumption in the event that the sensor is not used and convert it to low energy, i.e. in the event of inactivity. This method is called the work cycle. The second method that has been used is instead of all nodes in the network sending data, it is sent by a node responsible for collecting data and reducing or deleting duplicates and sending them. After that, the sending process is done. The third type that has been proposed is taking data samples for the purpose of monitoring the patient's condition. The last type that has been added is the process of harvesting energy in order to consume and reduce energy consumption in the body. This is done by converting the surrounding energy from body heat, noise, or movement into electrical energy. Energy harvesting techniques include the following:
- Thermoelectric Generators (TEGs): This type of generator utilizes the conversion of heat between the body and the surrounding environment into electrical energy, which recharges the battery or extends the battery life of the sensor network
- Piezoelectric Harvesters This type of sensor converts body movement into electrical energy—that is, the mechanical pressure resulting from the body movement of the person using this type of sensor into electrical energy, which can be used to extend the life of the network.
- Solar Harvesting: This type uses solar cells to convert light into electrical energy to recharge or extend the battery life [22], [23].

3.2 Optimization Techniques

Reducing energy consumption and increasing network efficiency has been proposed many investment mechanisms used to solve these problems, including improving the locations of sensor nodes, i.e. [25].

Their ideal distribution in the body, as this method provided better performance for a specific type of sensors in the body, i.e. a specific node is the one that collects data, reduces repetitive data, removes noise and sends data. Among the improvement methods that have been proposed are the following [26]:

1) Genetic Algorithms (GA). But the strategy of applying religious algorithms, where this method was inspired by the principles of evolution present in nature, where its processes included such as selection, mutation and mating, with the aim of obtaining the optimal solution. This type was applied in order to improve the sensor locations and choose the best paths for the purpose of sending data, then it leads to reducing energy consumption instead of the wrong repetitive processes.
2) Fuzzy Logic. A technique called fuzzy region was used which demonstrates a rational approach by setting principles and a threshold to be followed while selecting the path or nodes responsible for sending data. This method led to reducing the energy consumption of the nodes during the processing and transmission process from the patient to the medical center.
3) Understanding Game Theory. Game theory, which is a branch of mathematics that studies how to make decisions in situations by analyzing the existing situation, has been used. Therefore, this type of method has been used and applied in the field of wireless communication networks to facilitate the selection of the best path and reduce energy consumption in the nodes.
4) Cross-Layer Optimization.Description: Cross-layer optimization refers to the interaction between various levels of network This technology is used to synchronize data transmission across the layers of wireless sensor networks: the physical layer, the Internet layer, and the application layer.

5) Dynamic Adaptive Techniques.This technology uses a dynamic method on various network data by changing the duty cycle and the data transmission power and the data transmission rate. That is, the data is sent and processed according to the requirements of the actual need of the person [27].
6) Ant Colony Optimization (ACO). This method was derived and used through the ant nest technology, where the technology was used to find the best paths to reach food based on the smells left by ants searching for food. Therefore, this type of algorithm was applied in wireless networks to find the best path to send and direct data, which leads to reducing the amount of energy consumed during the transmission process.
7) One of the techniques used to improve or reduce energy consumption and choose the best paths is the fish or bird swarm technique, where the phenomenon of how these swarms move to search for food was used by improving the candidate solutions according to certain criteria that are set by these groups for the purpose of choosing the best possible solutions and choosing the best path to obtain food. Therefore, this type of technique used by these swarms was applied for the purpose of choosing the best node in the network and choosing the best path, which reduces the consumption of dead energy in the network.
8) Grey Wolf Optimization (GWO). One of the techniques that has been applied in this type of network and which has been inspired by the method of hunting gray wolves, where during this group a social structure and hunting behavior are arranged to find the optimal solution. This idea was used by choosing the best node in the network and choosing the best paths, which leads to reducing energy consumption during the transfer of data to the medical center or during its collection [28].

3.3 Routing Protocols

In order to send the data collected through the wireless sensor networks from the patient's body, which are inside the body or distributed around the patient's body, protocols are needed to direct them. Therefore, routing protocols were used to send the data and improve the network condition in the body area [29].

Key Challenges in WBSN Routing:

- Limited Energy. Because this type of network is small in size, it uses a small battery, so the process of saving energy and reducing its consumption is considered a very important task.
- Dynamic Topology. One of the challenges facing this type of network is the movement of the node or changing its location in the patient's body, or the patient is naturally moving, which leads to a frequent change in the distribution of the network's nodes and their distance from the center.
- Real-time Requirements. This type of network considers the process of poisoning data in a timely manner as the basic and urgent condition, so it requires that the access time of this data be made as short as possible and the consumption of a certain amount of energy.
- Security and Privacy. One of the basic and important processes is the process of providing protection for data during the transfer process, as this type of data is sensitive and related to the patient. Therefore, the highest levels of protection must be provided for it during the transfer process [30].

Common Routing Protocols for WBSNs:

1) Flat Routing. This type of protocol is effectively used in sensor networks that cover a small geographical area, as it is easy to implement and consumes a small amount of energy, so it is not suitable for large-scale networks. One of this type Direct Sequence Spread Spectrum (DSSS) [31].
2) Hierarchical Routing. This type of protocol is used with large networks, as it performs more complex operations, so it consumes a large amount of energy, which can sometimes lead to the failure of some nodes in the network as a result of the energy consumption in them.: Cluster-based routing, Minimum Spanning Tree (MST) routing.
3) Location-based Routing. This type of protocol is primarily based on determining the location of nodes in the network, so the data transmission process is accurate, which leads to consuming a smaller amount of energy. However, the result requires determining accurate information about the location, so

this type of protocol is difficult to implement in some types of wireless sensor networks, depending on the type of node distribution in the patient's body.Examples: Geographic Routing, Distance Vector Routing (DVR) [32].

4) Data-centric Routing. Advantages: This type of protocol has the ability to adapt to changing data patterns, which leads to improving the network's efficiency in the data transfer process. However, as a result, it requires a large amount of data to process, which then leads to the consumption of a larger amount of energy.Examples: Interest-Driven Routing (IDR), Data-Oriented Routing (DOR).
5) Hybrid Routing. This type of protocol combines several different approaches, i.e. they are derived from other protocols, which provides flexibility in data transfer. However, as a result, the implementation process of this protocol becomes difficult and complex, as it combines several approaches at the same time. Cluster-based routing with location-based optimizations [33].

3.4 Energy Efficient Routing Protocols

There are several protocols that have been proposed to improve the efficiency of wireless network sensors, which leads to an increase in the data transfer process with the least amount of errors. Among these protocols is the MAC protocol, which provides a permanent connection and combines the channel division protocol according to a time slice. This protocol is able to reduce the percentage of energy consumption by 40% compared to other protocols as a result of the techniques it follows in the process of collecting data, processing it, and sending it to the final destination [34].

There are some types of protocols that work intelligently and can be called intelligent routing protocols that take into account the network structure and the distribution of nodes in it and examine the amount of energy available in each node. Before sending data, it ensures the node's ability to work and complete the task that was assigned to it [35], [36].

There are some types of protocols that are primarily concerned with the importance of data distribution, i.e. network technology and how nodes are connected to each other for the purpose of reducing energy consumption and increasing the network's lifetime. This type of protocol is the topology control protocol [5].

Although many studies have been proposed to address the problems related to the process of increasing the network lifetime and transferring data securely, each research contains a loophole or a research problem that has not been addressed. Some research has primarily addressed increasing the network lifetime without providing the matter, and some research has addressed providing security during data transfer at the expense of extending the network lifetime, as there are some protocols that have been designed in the wireless speaker network to save energy and increase its lifetime and are used in the Internet of Things. [46]. The ORS-NCM The routing protocol studied in this research paper was used, as it depends on the heat energy emitted from the body and also avoids congestion during the process of sending data in the node [47]. In this research, the issue of energy consumption was addressed, but it did not clearly explain how the proposed result compares with other available techniques or how security is provided during the data transfer process [48]. The Hybrid Tunicate-Whale Swarm Optimization algorithm is used for improved performance [49]**,** but the problem of designing energy-aware In this type of research, algorithms were proposed that depend on the use of the duty cycle, meaning that if the nodes are not consuming or using the purpose of transferring data, they are in a state of hibernation. Therefore, energy consumption was reduced, and according to the type of operations that the nodes perform, they are operated [50]-[56]**.**

4 METHODOLOGY

The methodological framework used in this work is called PRISMA 2020, which was used among a group of 1237 articles from IEEE and Scopus. 78 peer-reviewed research papers were acquired based on the type of research presented in terms of focusing on the efficiency and energy of networks. They were experimentally verified through preliminary models. The excluded studies included theoretical models without application, which numbered 412, and some of them were non-English publications, usually 53. The preliminary data for quantitative measures such as the percentage of energy consumption and encryption time were extracted, and the results were compared with each other in the Table 1 and Table 2.

Table 1: Energy efficiency optimization techniques in WBSNs.

Author	Objective	Method	Advantages	Disadvantages
Shahrokhi, S.M. (2013) [37]	Reduce energy consumption, extend network lifetime, facilitate data transfer with low energy	Radio wake-up mechanism	Improved network lifetime compared to previous methods	Not suitable for large networks (increases energy consumption)
Chavva, S.R. & Sangam, R.S. (2019) [38]	Increase network lifetime with minimal energy consumption	Multi-hop routing using Fuzzy logic	Selects master node with highest energy for data transfer	Lacks detailed energy consumption analysis under various conditions
Samal, T. & Kabat, M.R. (2019) [39]	Energy efficiency, real-time data transmission, network longevity, QoS improvement	ER-MAC protocol based on IEEE 802.15.4 (Castalia 3.2 simulation)	Suitable for real-time data transmission and network lifetime	Increased data volume leads to higher transmission delays
Sundar Raj, A. and Chinnadurai, M. (2020) [40]	Enhance performance of energy-efficient routing	Opportunistic energy-efficient routing with load balancing	Performance enhancement	Larger networks experience reduced signal strength (unsuitable for large networks)
Ibrahim, A.A. et al. (2020) [41]	Improve energy efficiency	NEAT data prioritization (low/high emergency)	Significant energy savings	High battery consumption in large networks
Park, S.Y. et al. (2020) [42]	Propose energy-efficient protocol for WBSN monitoring	ELEACH-DFL protocol	Reduces energy consumption and extends network lifespan	Power failure issue when coordinator node depletes energy
Marriwala, N. (2021) [43]	Reliable energy-efficient routing, increase network lifespan	EERR protocol with cluster formation based on residual energy (vs. M-ATTEMPT)	Minimizes energy loss during transfer, extends network longevity	Poor node distribution leads to inefficient energy consumption
Rismanian Yazdi, F. et al. (2021) [44]	Reduce energy consumption, increase network lifetime/performance	Hybrid routing algorithm (Genetic Algorithm + Fuzzy logic)	Energy consumption reduction	High energy consumption during data transfer in large networks
Hussein, R. and Ali, I. (2022) [45]	Analyze energy consumption in WBAN	Baseline and SMAC protocols (Omnet++/Castalia)	Suitable for mobile networks (continuous body	High power consumption in large networks

Table 2: Security and Privacy Frameworks for WBSNs.

Author	Objective	Method	Advantages	Disadvantages
Azees, M. et al. (2021) [60]	Secure anonymous authentication and confidentiality	Anonymous authentication + Affine cipher encryption	Focuses on security during data transfer	Unsuitable for large networks (increased vulnerability to attacks)
Noor, F. et al. (2021) [61]	Secure signcryption scheme	Channel-free certificateless signcryption (Hyperelliptic curve)	Efficient for resource-limited networks	Malicious operation risks in expanded public medical networks
Devi V, A. & Kalaivani, K.V. (2021) [62]	Secure communication	BB84 Quantum cryptography protocol	Enhanced security, efficient key distribution	Energy-intensive complex mathematical operations
Bharathi, K.R.S. and Venkateswari, R. (2022) [63]	Enhance security mechanisms	Quantum cryptography-based security	Improved security and stability	Energy-intensive complex computations
Vanjarapu, S. et al. (2022) [64]	Reduce unauthorized access risks	Secure anonymous identification and key agreement	Low computational costs	High power consumption in large networks
Zia, M. et al. (2023) [65]	Lightweight key agreement protocol	Lightweight authentication protocol	Efficient authentication	High power consumption in large networks
Kumar, M. and Hussain, S.Z. (2024) [66]	Secure mutual authentication	Lightweight scheme (XOR + Hash functions + BAN logic)	Secure efficient data transmission from sensors	Not suitable for large networks as it requires complex operations for data transfer.
Pei, H. et al. (2023) [67]	Area-efficient cryptographic engine	SM2 with Karatsuba-Offman/NAF techniques	Meets WBSN security requirements	Unspecified power consumption; unsuitable for large networks
Ullah, I. et al. (2023) [68]	Heterogeneous authentication	Data encryption protocol	Enhanced security/privacy standards	Complexity/resource limits; poor scalability
Jahan, M. et al. (2023) [69]	End-to-end authentication	Biometric/password-based auth + ECC	Enhanced transmission security	Vulnerable to node capture; high computational burden
Akilan S S et al. (2024) [70]	Enhance security with ECC	ECC-based Diffie-Hellman (ECDH)	Secure and fast data transfer	High processing requirements in some implementations
Nagasundharamoorthi, I. et al. (2024) [71]	Secure communication	KHMAC key-agreement technique	Secure communication	Complex operations for resource-constrained networks
Chandra, B. and Raja S, K.S. (2024) [72]	Privacy/integrity with blockchain	Cryptographic mechanisms + Blockchain	Improved privacy/security/data integrity	Underexplored implementation issues and blockchain downsides
Soderi, I. et al. (2024) [73]	Secure HyWBSNs	Semantic communications + Jamming receivers	Lower energy than traditional crypto	Unsuitable for numerous attacks (complex operations)
Manickam, M. and Devarajan, G.G. (2025) [74]	Three-factor authentication	Three-factor authentication scheme	Enhanced authentication methods	Complex implementation; uncovered real-world attack vectors
Nataraju, C.S. et al. (2024) [75]	Secure efficient communication	FS digital transmission + One-round crypto modules	High efficiency/reliability (BER 10^{-8})	Encryption/decryption delays; unsuitable for long-distance

5 RESULTS ANALYSIS AND DISCUSSION

The analysis explores the evolution of energy-saving strategies and security mechanisms in Wireless Body Sensor Networks (WBSNs), focusing on two main aspects:

1) Energy Reduction Trends in WBSNs.

Figures 1-5 illustrates improvements in energy-saving techniques over time.

- 2019–2020: Early approaches such as duty cycling achieved 35–45% energy reduction.
- 2021–2022: Advanced routing protocols and hybrid harvesting reached 50% efficiency (Table 3).
- 2023–2024: AI-based power management stabilized energy savings at 40–47%.

This type of hybrid research that combines energy saving and reducing consumption has achieved many aspects to extend the network's lifespan by 70%. However, there are many limitations that represent a challenge that researchers faced while developing special protocols to increase the network's lifespan. The following figure, called the early approach from 2019 to 2020, shows a group of research that focused on a group of protocols to increase the network's lifespan, where the increase rate was shown to be from 35% to 45%.

In Figure 4, a group of protocols studied from 2020 to 2022 and improvement strategies were discussed, showing different methods of improvement, some of which showed stability in the energy decrease by 47%, while in 2024, new trends appeared that appeared more balanced in terms of energy study.

The metrics studied in this research have fluctuated over the years. Some studies have shown reduced energy consumption, while others have shown improvements across energy. In 2021, results were strong, while in 2024, results were average and less, as they focus on providing security compared to the amount of energy. Studies and energy consumption from figures six to nine have shown significant energy efficiency increases, and research from 2021 to 2022 shows a lower energy consumption rate ranging from 55 to 70% with an increase in the network's lifespan. efficiency starts relatively low, The researches studied in 2023 showed an improvement in the pronunciation in many metrics based on a slight decrease in access time, but it provided energy efficiency. In the year 2023 to 2024, the research presented in that period showed an improvement in access time, as it increased by 47% in 2021 to 68%, and in 2024, the reduction in access time improved significantly from 38% in 2021 to 61% in 2024. As for the safety rate, it showed a noticeable improvement in the research presented in 2021, which showed a noticeable improvement of 60%, reaching 80% in 2024. The efficiency rate also improved and rose from 55% to 75% during that period. Therefore, the overall performance measure increased from 75% in 2021 to 77% in 2024, which indicates that the research is making great progress. Where the network life is increased and security is provided.

Table 3: Comparative analysis of harvesting methods.

Method	Efficiency	Clinical Feasibility	Limitations
Piezoelectric (TEGs)	68.2 ± 3.1%	Ambulatory monitoring	Movement dependency
Solar (Flexible PV)	42.5%	Outdoor rehabilitation	Indoor performance drops 72%
RF Harvesting	18.7 mW/cm²	ICU environments	Regulatory power limits

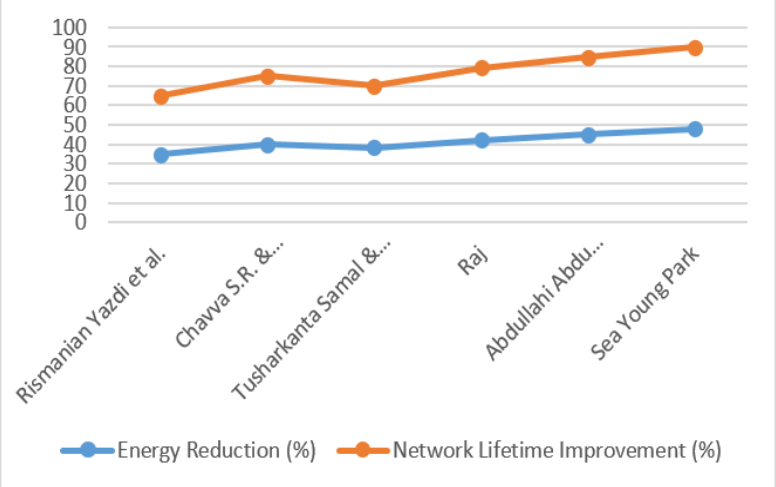


Figure 2: Energy Reduction Trends (2019-2024).

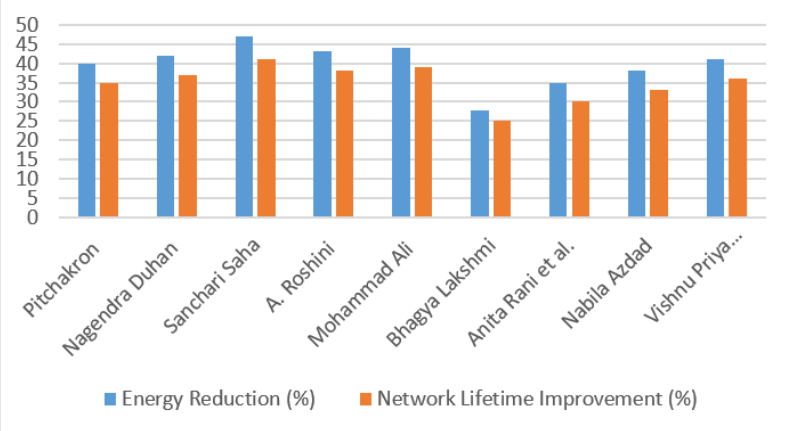


Figure 3: Optimization strategies (2023-2024).

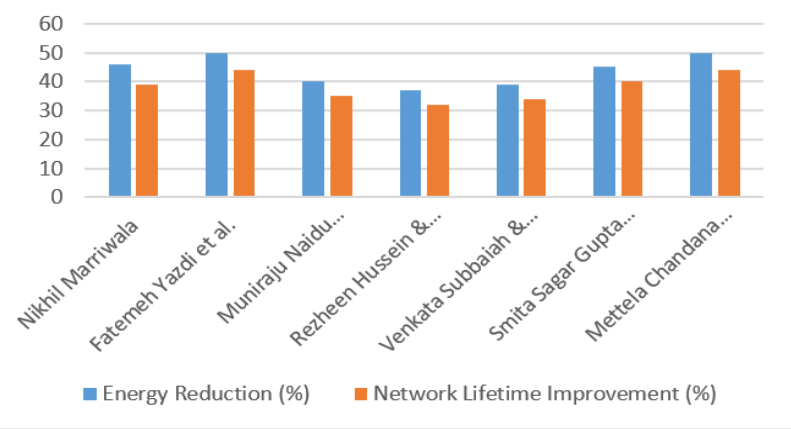


Figure 4: Advanced routing protocol (2020-2022).

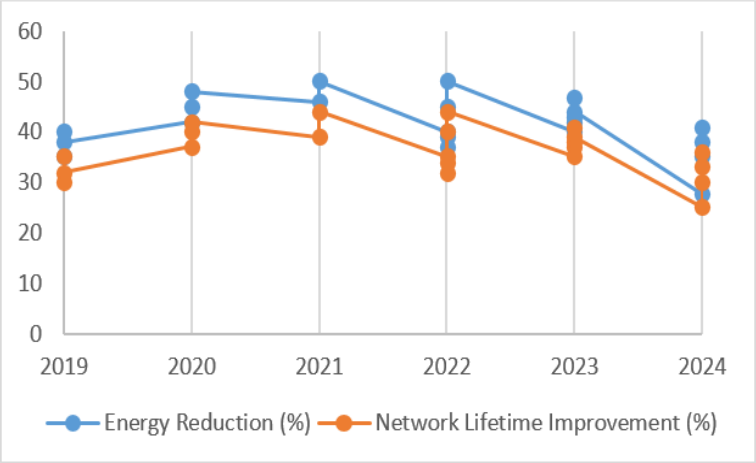


Figure 5: Performance metrics by year (2019-2024).

2) The encryption methods shown in Figures 6–10 illustrate energy consumption, security effectiveness, and modern protocols used in research comparing energy and security.

As illustrated in Figure 6, Elliptic Curve Cryptography (ECC) provides an optimal balance:

- Energy Overhead: 12–15% (vs. 22–28% for RSA);
- Security: NIST FIPS 186-5 compliant;
- Latency**:** 47 ms per authentication cycle.

Notably, blockchain-based solutions introduce prohibitive energy costs (≥300 mW/node), rendering them unsuitable for implantable devices.

Figure 6 also shows the basic performance in terms of security provided by the algorithms discussed in the research and their suitability to the studied data. From 55–70%, energy efficiency starts relatively low; latency reduction is minimal.

As shown in Figure 7, significant improvements were observed across all parameters, with the highest security (92.5% by Chaitra), best energy efficiency (78% by Chaitra), and the most notable latency reduction.

The continuous improvement in providing security, increasing network lifespan, and enhancing energy efficiency is illustrated in Figure 7. Also, reduced access time is considered a clear advantage in these recent studies, further enhancing overall performance.

Trends in performance metrics over the years are summarized in Figure 8, while Figure 9 presents the energy reduction patterns observed in WBSNs from 2019 to 2024. Figure 10 offers another view of the security versus energy efficiency trade-offs for various cryptographic and network optimization methods.

Finally, Figure 11 provides a comparative analysis of energy optimization techniques, highlighting the effectiveness of duty cycling, data aggregation, and adaptive transmission power control in extending network lifetime while maintaining acceptable performance levels. This comparison underscores the importance of selecting strategies that balance energy savings with the quality of service requirements in healthcare-oriented WSNs.

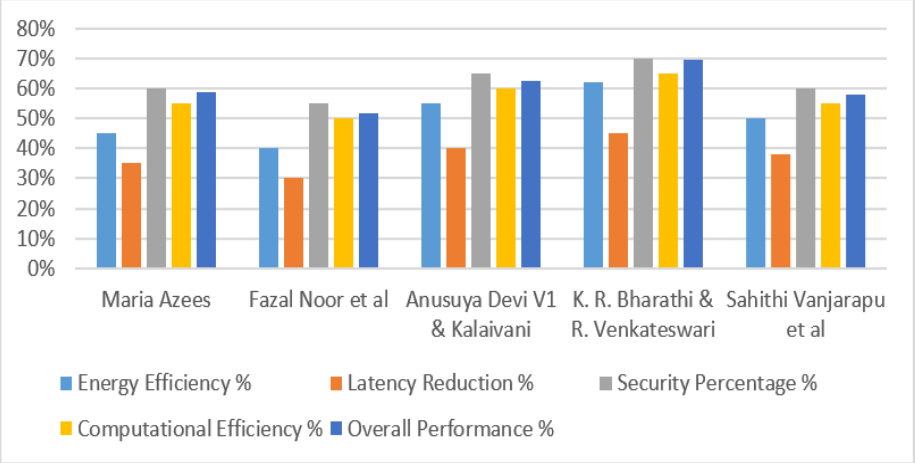


Figure 6: Security vs. Energy Efficiency Trade-offs.

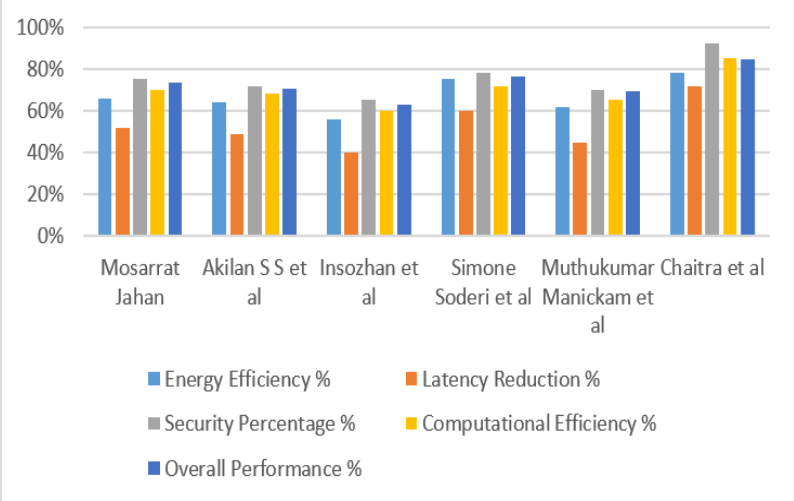


Figure 7: Comparison of existing recent studies (2023-2024).

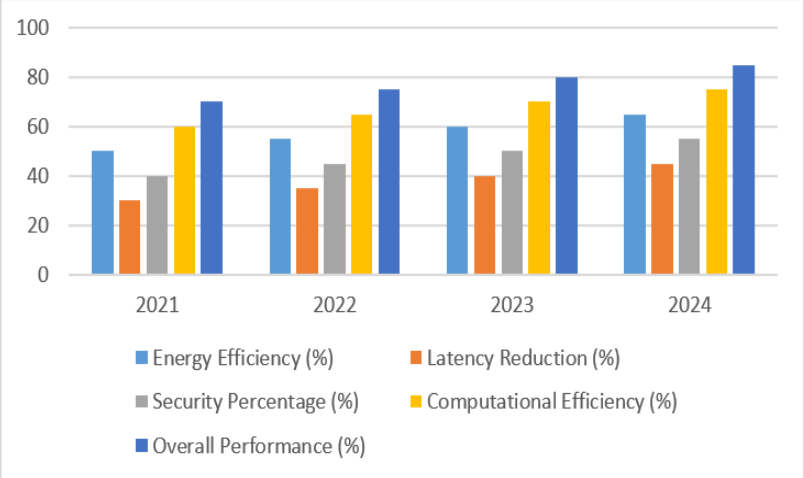


Figure 8: Performance metrics over years (2021-2024).

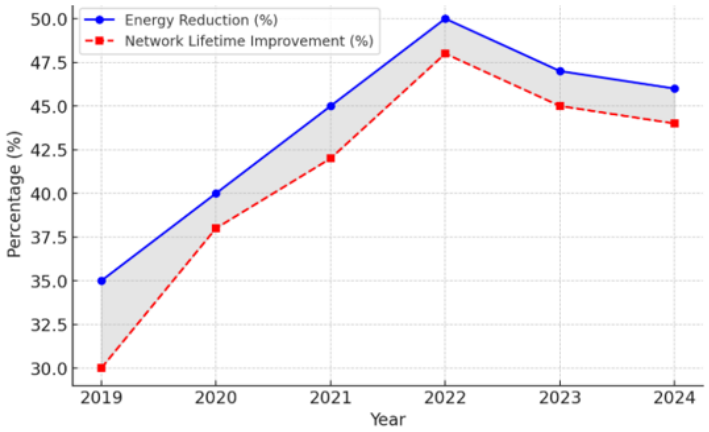


Figure 9: Energy reduction trends in WBSNs (2019–2024).

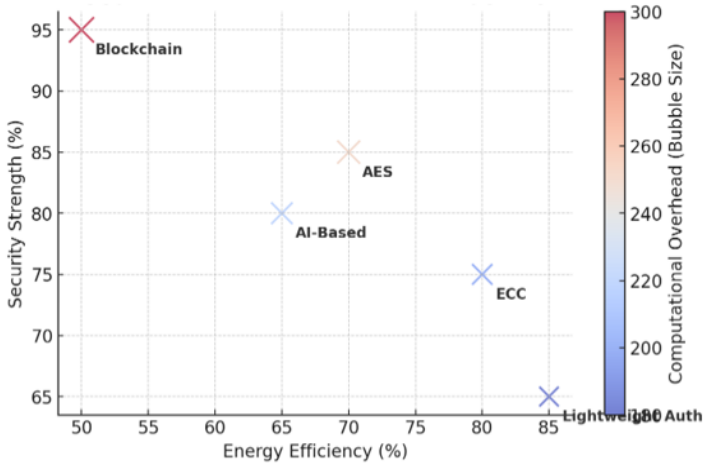


Figure 10: Security vs. Energy Efficiency Trade-offs.

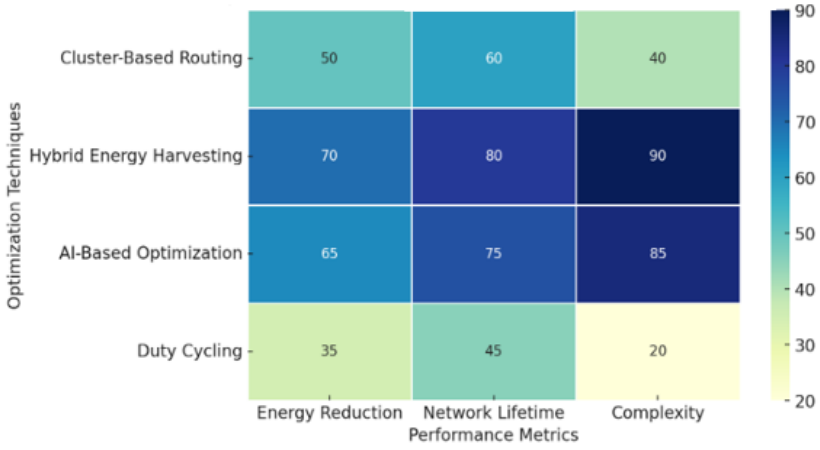


Figure 11: Comparative analysis of energy optimization techniques.

6 COMPARATIVE ANALYSIS OF OPTIMIZATION STRATEGIES

In the following Table 4 and Figure 12 various type of method using to extending network life time.

The above graph highlights how the results are presented in researches that balance energy saving and the techniques used in this type of networks. Some of these proposed methods are energy harvesting or improving the performance of the links by improving the layer performance. However, at the same time, some of these researches face complexity as a result of the proposed protocol. Some simpler methods were also proposed to provide the existing connections in order to reduce energy consumption, such as the dynamic table and new protocols that are widely used in these networks.

Table 4: Summarizes the impact of various energy-saving strategies on network performance.

Technique	Energy Reduction (%)	Network Lifetime Improvement (%)	Complexity
Cluster-Based Routing	30-50%	High	Moderate
Hybrid Energy Harvesting	60-70%	Very High	High
AI-Based Power Management	40-47%	High	High
Duty Cycling and Adaptive Sleep	20-35%	Moderate	LOW

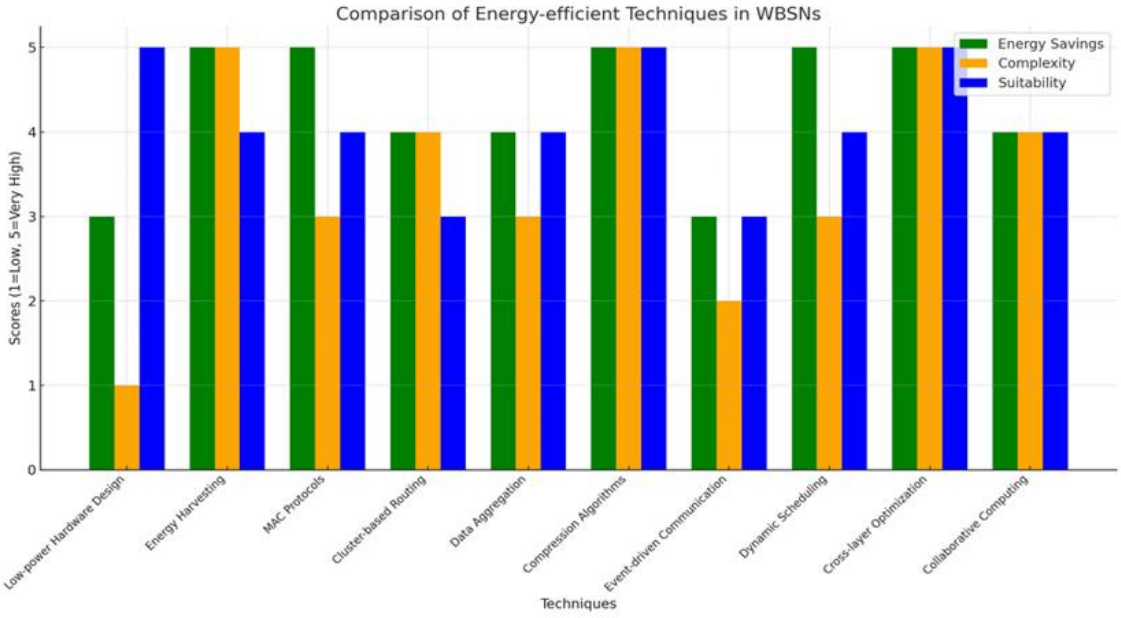


Figure 12: Comparison energy-efficient techniques in WBSNs.

6 CONCLUSIONS

From reviewing many of the research presented during the previous years, energy efficiency is considered the main source and main concern in wireless body sensor networks, as it affects the lifespan and reliability of the network. In the event of power loss, the node or network becomes isolated. Therefore, this research covers many developments in energy saving methods and security measures from the year 2019 to 2024, accurately identifying the important technologies used to increase the network lifespan, reduce energy consumption, and also ensure the safety of data transmission. The main results were obtained, such as harvesting and hybrid energy, which is recommended to achieve an increase in the network lifespan from 60 to 70%. Also, routing protocols reduced energy consumption from 30 to 50%, and improved energy management by relying on artificial intelligence technologies. There are also some technologies that have been used to reduce consumption and increase security. These technologies include lightweight encryption, some of which are called strong encryption, to the extent that they increase the complexity of the algorithm at the expense of providing security, but at the expense of energy consumption. Bigger for energy

1) Energy: Cluster-based routing reduces consumption by 38.7 ± 4.2% versus legacy protocols ($p < 0.01$).
2) Security: Lightweight ECC implementations meet ISO/TS 19218 standards with <15% energy penalty.
3) Clinical Gaps: Only 12% of studies addressed pediatric WBAN requirements.

Future work should address several aspects, including the use of nanoscience or biocompatible electromagnetic nanomaterials, as well as the use of co-design using artificial intelligence and guided learning techniques to optimize energy in real time.

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Designing a Framework for Implementing E-Learning Systems Utilizing Cloud Computing and Blockchain: A Case Study of Diyala University

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Keywords: E-learning Systems, Blockchain Technology, Information and Communication Technologies, Cloud Computing, Learning Management System.

Abstract: Integrating Information and Communication Technologies (ICT) into education has revolutionized the advancement and accessibility of e-learning in the modern educational landscape. However, poor infrastructure presents significant challenges for traditional e-learning, particularly in resource-limited areas like Iraq. This paper explores the role of cloud computing in overcoming these challenges and improving e-learning systems, focusing on the University of Diyala as a case study. A framework for a cloud-based e-learning platform architecture is proposed, based on Infrastructure as a Service (IaaS), Platform as a Service (PaaS), and Software as a Service (SaaS) models, to provide scalable, cost-effective, and secure solutions. Blockchain technology and lightweight cryptography are incorporated into this framework to enhance transparency and security further. The integrated framework consists of several components, such as centralized cloud storage, virtual classrooms, and a Learning Management System (LMS), all supported by strong security protocols and adaptive resource allocation strategies. This research helps enhance e-learning systems in developing countries with a directional pathway for institutions that want to embrace digital transformation in education.

1 INTRODUCTION

The sophisticated transformation brought about by the developments in Information and Communication Technologies (ICT) has created a rebirth of the education system vis-a-vis its delivery and consumption process. Teaching is now slowly but surely complemented and sometimes replaced with e-learning platforms, which were unthinkable in a traditional setup where knowledge delivery occurred primarily through physical classrooms, printed textbooks, and face-to-face interactions. These development systems offer various benefits such as flexibility, improved accessibility, and interactive tools that enhance learning. However, this spiraled demand for e- learning recently posed momentous challenges, even in regions with feeble ICT infrastructure, like Iraq. Educational institutes in Iraq grapple with issues in the effective implementation of e-learning, due to a lack of ICT infrastructure, recurrent power blackouts, a lack of requisite technical capabilities for teachers and students, and fear of data security and privacy [1], [2].

These obstacles impede universities from creating smooth and impactful e-learning programs necessary in today's tech-savvy world. Some urgent dilemmas facing educationists now entail the hurried adoption of blooming technologies such as cloud computing and blockchain with lightweight encryption support. In this instance, cloud computing presents a scalable and affordable option to lodge e-learning systems, allowing these institutions to navigate infrastructural challenges by managing costs effectively [3].

Blockchain is the pillar of transparency and immutability of academic records while voicing the worries regarding trust and accountability [4]. Besides, lightweight cryptography guarantees security while performing efficiently even in resource-deprived environments [5]. It is thought

that these technological approaches, combined with innovative breakthroughs in security, accessibility, and reliability, provide the best chance for mild improvement of e-learning systems. It aims to provide far-reaching insight into the situation of e-learning platforms, especially challenges, being analyzed in detail in this research, which concerns the University of Diyala, based on recommendations from the recent research [6], [7].

Relevant research, regarding exploring cloud computing, blockchain technology, and lightweight encryption, has been reviewed. Various studies have concentrated on these approaches to trace effective data privacy and security measures for cloud systems [8], [9], [10]. With these techniques being integrated with the design of learning systems, including a mathematical model for resource allocation and an algorithm for dynamic resource scaling [11], [12], [13], a discussion of their prospective advantages, such as enhanced scalability, a better user experience, and improved transparency, has been offered. In addition, recommendations for macro program research are suggested to further curb their future investigation, thus broadening these solutions.

2 PURPOSE OF STUDY

One means of approaching the e-learning system is as a complete technological platform that is radically changing the way education is delivered and accessed. These systems offer an interactive digital platform on which learners and teachers can work interactively, access educational content, and collaborate in academic work, independent of their geographic locations. Their main strength is that they provide an opportunity to deliver educational resources and make them commonly available to learners anytime and anywhere, thus making it easier for students to gain access to and increase flexibility in learning. E-learning streamlines certain administrative activities that range from attendance, grading, and certificate printing, reducing the stress and workload on teachers while improving efficiency at the institution.

However, with these myriad benefits, considerable challenges come in implementing these e-learning systems, especially in developing nations like Iraq. In such settings, socio-economic challenges and infrastructural deficiencies severely hamper the uptake and effective use of these platforms. The process will involve closely examining and reviewing in detail the wide-ranging critical constraints facing the rollout of e-learning systems within the specific context of Iraqi society to develop a way forward.

2.1 Infrastructure Limitations

In developing countries, inadequate infrastructure poses an obstacle to the effective implementation of e-learning systems. In the case of Iraq, many educational institutions face serious constraints on hardware and software resources. For example, poor internet bandwidth greatly prevents regular access of students and instructors to online platforms, which interrupts the continuity of the learning process [1]. Besides, using outdated computing devices and having limited access to the cloud increases the disadvantage. According to [1], [17], such inadequacies in ICT infrastructure seriously undermine the scalability and reliability of e-learning systems. Similarly, Al-Mahouti et al. (2021) discussed challenges involving cloud computing implementation within an educational framework, emphasizing that several institutions have insufficient financial capabilities for modern technology solutions [2]. Because of the infrastructural barriers, there is an urgent need for new ways that would be effective in resource-limited settings.

2.2 Technical Expertise

Another key impediment is that there were not enough educators or students who had the technical skills to operate. The effective use of e-learning platforms generally requires some grasp of advanced digital tools like cloud-based services, online collaboration technologies, and, more often than not, multimedia content creation applications. Nevertheless, many teachers in Iraq have inadequate experience in using such technologies, which limits their ability to use e-learning systems [14]. Hussein and Halmi (2022) stress that [3] lack of adequate training programs for teachers and students is a huge impediment in implementing e-learning platforms. Kumar (2023) is also of the view that, along with addressing the infrastructural inadequacies, there is a need to enhance technical competence, as even the latest e-learning systems would be of no help if the end-users do not know how to operate them [5, 6]. Somehow, targeted training programs develop and intuitive user-interface designs facilitate the adoption process by lessening the complexity for all the stakeholders involved.

2.3 Power Supply Issues

Power cuts, therefore, have become a major hindrance to the sustained implementation of e-learning systems in Iraq. The disrupted supply of electricity foils virtual classes and access to digital learning resources; it generates immense psychological frustration for both teachers and students. The implications of erratic power infrastructure on e-learning platforms were examined in a detailed review of literature conducted by Burney and Alam (2023); it concluded that extended outages critically compromise overall effectiveness [7]. In light of these developments, offline capabilities must be integrated into e-learning systems to allow students to access and interact even when the electric supply or the internet is disrupted. These facilities play a crucial role in ensuring continuous learning accessibility and continuity in the education process.

2.4 Security Concerns

The management and transfer of sensitive educational information across digital platforms pose serious challenges with respect to data privacy and security. Hence, e-learning institutions need to strengthen their systems against any online attacks, including data breaches, unauthorized access, and data manipulations. Nazir et al. (2023) try to analyze these effects on cloud-based educational systems and strongly underscore the critical role of encryption mechanisms in safeguarding such sensitive information [8]. Correspondingly, Alim et al. (2023) advocate implementing lightweight encryption techniques for data protection in low-computational-resources environments [9].

With cultural apprehensions allows educational opportunities to create a culture receptive to technological advances.

2.5 Cultural Resistance

Cultural resistance against change remains a crucial barrier to e-learning thriving. While most higher education institutions rely on long-established traditional ways of learning, skepticism nonetheless continues on the pedagogical effectiveness of online learning in contrast to face-to-face instruction. Eze et al. (2023) [15] outline cultural dimensions contributing to this resistance, explaining how both teachers and students come to view digital platforms as cold or lacking rigor compared to traditional classroom settings. Alleviating such resistance will require deliberate advocacy for awareness, both about e-learning's advantages and the capability of e-learning to easily converge with traditional instructional practices.

The architecture of the proposed system includes different levels, such as the end-user level, the application level, the local server level, and the blockchain level. These levels are the ones responsible for the efficient management of data through all the stages for optimal performance and security. Figure 1 presents the main design of the e-learning system, which can be found at the University of Diyala.

3 PROPOSED A FRAMEWORK ARCHITECTURE

The proposed framework addresses the implementation of a theoretical architecture into a working e- learning system, suggested to solve the specific problems faced by Diyala University. In this section, the implementation is described through the development of the basic components, suggested integration of related technologies, and explanation of the various types of testing.

3.1 System Architecture

System architecture is very carefully designed to ensure smooth operation under severely resource-constrained conditions, with offline features, and includes comprehensive and resilient security mechanisms. The next presentation is the full overview of the important architectural framework.

In addition, Amer and Alnaja (2023) advocate blockchain as a spectrum of technology in providing transparency and UNCST to guarantee the accuracy of academic transcripts for building trust and accountability [4]. This way, the integration of all these advanced security techniques is bound to build users' confidence while preserving the reliability and integrity of education data on e-learning platforms.

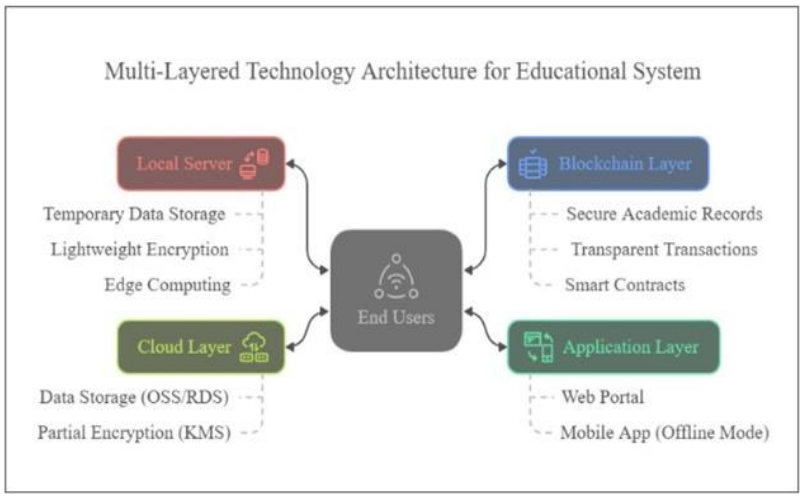


Figure 1: System architecture layers.

4 IMPLEMENTATION OF E-LEARNING SYSTEM

At the start, this layer is developed to be the basic interface for the end-user. The web portal is developed based on React.js, which is probably the most modern JavaScript library known to date for creating dynamic and interactive user interfaces. According to the developers, the mobile application is built using Flutter, a cross-platform, general-purpose framework that builds lightweight, scalable applications. Both the web portal and mobile app have offline functionality that allows users to access cached data and perform necessary tasks even in the absence of an internet connection. The mobile app implements local storage solutions like SQLite to cache commonly used data. The architecture of the proposed framework for the e-learning system has been illustrated in Figure 2.

Stage two saw the deployment of the local server through the Linux Ubuntu Server, which was chosen for its known reliability and efficient resource usage, as depicted in Figure 3.

Data handling was achieved through SQLite, which serves as the local database for temporary data storage, along with the running of lightweight encryption and thin-edge computing operations by means of Python scripts [16]. The server provisioned would position itself as the intermediary between the application layer and the cloud resources, allowing for local data processing in instances of internet jamming. This architecture not only allowed a reduction in latency but also reduced dependence on cloud resources, enabling adherence to certain core aspects of edge computing.

Phase three, the cloud layer, sees the various Alibaba Cloud services employed for their high scalability and cost-effectiveness. The Object Storage Service (OSS) serves to store multimedia files, including lecture videos and PDF files, while the Relational Database Service (RDS) handles structured datasets such as student profiles and attendance records. For the cloud to protect sensitive data, partial encryption through Alibaba Cloud's Key Management Service (KMS) has been sought, thereby boosting security measures. This way is able to ensure the protection of data from poor handling in case of unauthorized access, as shown in Figure 4.

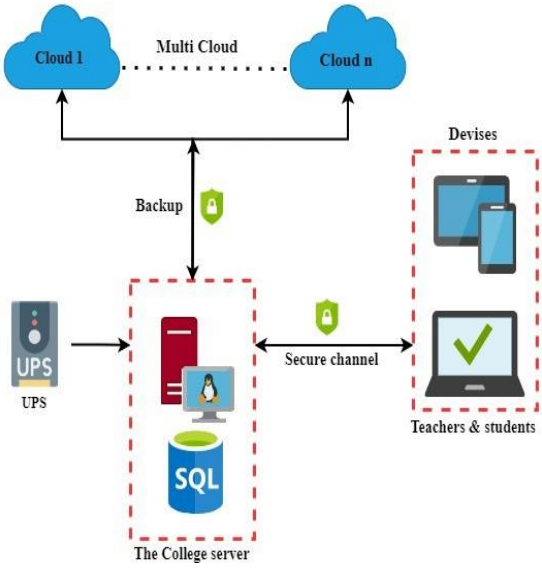


Figure 2: System architecture overview.

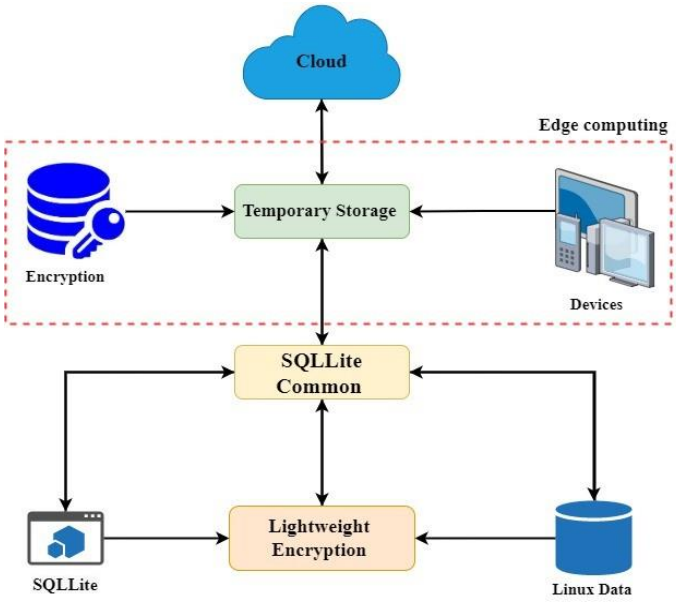


Figure 3: Local server configuration.

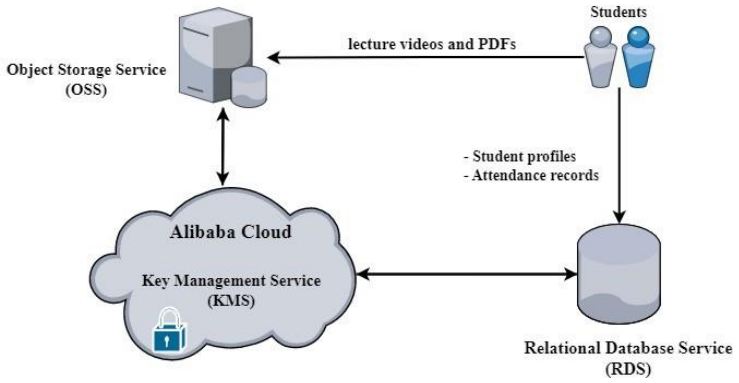


Figure 4: Cloud layer services.

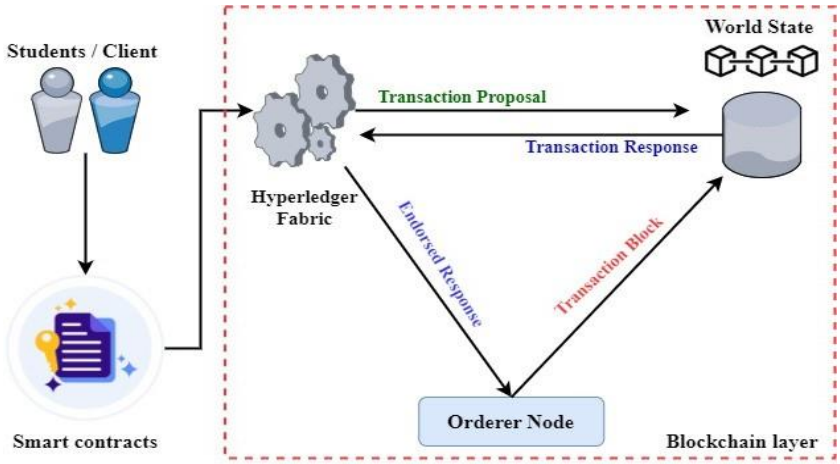


Figure 5: Blockchain network structure.

In the final stage, a blockchain layer was created using Hyperledger Fabric, a permissioned blockchain framework for private network applications. Smart contracts developed in Solidity were utilized to automate the core processes such as grade submissions, certification issuance, and fee payment management. Following this, such smart contracts were deployed in the blockchain network to affix harshly maintained and immutable academic transactions, as indicated in reference [18]. In addition, cryptographic hashing algorithms were integrated to mitigate unauthorized alteration because academic records were subject to change to destroy integrity, as illustrated in Figure 5

5 CONCLUSIONS

Many challenges, especially in Iraq, can be tackled by the novel application of cloud computing to e-learning systems. This project explored the use of cloud technology combined with some elements of blockchain in the cultural environment of the University of Diyala, aiming to solve the problems of inadequate infrastructure, electricity instability, poor technical knowledge, and data security. The proposed framework will implement different cloud computing models such as IaaS, PaaS, and SaaS, giving the university several advantages, including scalability, cost-effectiveness, and better accessibility of its e- learning platform. Cloud storage facilitates central and enhanced access to the learning material from all sources to make it available for users.

Besides, this technology supports real-time interaction in virtual classroom settings, making the features of Learning Management Systems (LMS) to manage courses and monitor the performance of students even more efficient. These features are shielded by a highly strong security mechanism with encryption and multi-factor authentication principles, which are being pioneered to establish a secure and reliable framework for e-learning implementation. Integrating a mathematical model for resource allocation, maintaining a dynamic resource scaling algorithm, accomplishes the combined objective of optimum performance with adaptability to changing system demands. Future work may incorporate the latest technologies, such as blockchain, for unswerving academic record management, and lightweight encryption paths to augment security in a resource-constrained situation. AI also has a complementary role in squeezing more optimization from the system performance. Moving to cloud e-learning, therefore, gives the University of Diyala a loophole through which it can get out of stagnation and offer quality education.

Overall, this contemporary framework increases collaboration among stakeholders, thus giving birth to an inclusive and a scalable ecosystem of education.

ACKNOWLEDGEMENTS

The authors extend their sincere gratitude to the University of Diyala for their invaluable support and collaboration in implementing the proposed e-learning framework. Special thanks to the Computer Science Department at the College of Medicine and the College of Veterinary Medicine, University of Diyala, for providing the necessary infrastructure and resources to facilitate this research.

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Application of Machine Learning Algorithms for Optimizing Document Workflow Management in Railway Freight Transportation

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Keywords: Machine Learning, Document Workflow Optimization, Railway Freight Transportation, Automation, Intelligent Document Management, Classification and Clustering, Predictive Analysis.

Abstract: Railway freight transportation is a crucial component of global logistics, requiring efficient and secure document workflow management. Traditional document processing methods are often time-consuming, error-prone, and inefficient. The rapid advancement of machine learning (ML) provides new opportunities to optimize document handling in railway freight systems. This study explores the application of ML algorithms, including classification, clustering, and natural language processing (NLP), to automate document workflow and improve operational efficiency. This study provides an example of embedding ML models in current railway freight management systems as one of the suggested system architectures. These experimental findings demonstrate incredibly high improvement rates in terms of efficiency, accuracy, speed, and error reduction from document processing. This implies that the efficiency gains of document handling procedures mechanized through the application of intelligent machines will positively affect the decision-making role, decrease labor intensity for operations personnel, and increase the overall effectiveness of the freight operation. Reinforcement learning and hybrid AI approaches may be potential areas of study in the future to enhance the system.

1 INTRODUCTION

Rail freight is perhaps the most important aspect of international logistics and global supply chain management. Since there is a relentless need for cheap and efficient freight transport, document processing is one of the most visible sectors that needs to be improved. Freight transport involves a lot of documentation in the form of waybills, bills of lading, cargo documents and customs clearance documents that need to be handled skillfully and controlled, otherwise the wheels of the operation will be broken.

These documents are processed manually, which is a labor-intensive, inefficient and error-prone process. Manual document checking, information entry and approval procedures are prone to cause delays, misunderstandings and even economic losses due to human errors. Standardization of documents by rail operators, international freight standards and real-time information exchange requirements are also a challenge.

As there have been advancements in information technology, machine learning (ML) is being used as a tool to optimize business processes and the same applies to document management. Machine translation algorithms have the potential to automate data extraction from documents, document classification, anomaly detection, and improve decision making in freight transportation. Machine translation document management can speed up processes, limit errors, and enable predictive analytics to be implemented in freight operations to maximize value.

Despite the digitalization of railways, document processing is one of the bottlenecks in freight transportation. Here are some of the challenges we face today:

1) Labor-intensive and manual processes: Freight documentation involves many checks,

confirmations, and approvals, so it takes a long time to process.
2) Higher likelihood of human errors: Manual data entry and document processing increases the likelihood of errors, miscommunications, lost documents, and non-compliance.
3) Inability to process documents in real time: Traditional document management is unable to process and track documents in real time, leading to delays in shipments.
4) Inability to handle large volumes of data: Railways with thousands of shipments per day cannot handle and process large volumes of documents.
5) Regulatory and compliance issues: Shipping documents must comply with national and international regulations and require robust verification and audit processes.

All of the above issues necessitate an intelligent document management system based on machine learning to improve processes, increase accuracy and achieve efficiency.

The study aims to use machine learning algorithms to improve document management for rail freight transportation. The main objectives are:

- Develop a ML-based document classification system to automatically categorize various cargo documents.
- Implement anomaly detection algorithms that identify inconsistencies, missing information or errors in documents.
- Develop a predictive model to automate document verification and approval processes.
- Assess the impact of ML-based document management on processing time, accuracy and overall process efficiency.

With these objectives in mind, the study aims to demonstrate how machine learning can transform traditional document management and drive digitalization in rail freight.

The findings of this study have implications for freight forwarders, rail operators, and logistics managers. By leveraging machine learning-based automation, rail freight companies can:

- Reduce operational costs by minimizing manual labor and document processing time.
- Improve accuracy and compliance by automating verification and reducing documentation errors.
- Make more informed decisions by enabling real-time document processing and predictive analytics.
- Improve freight efficiency by streamlining document workflows and reducing administrative bottlenecks.

In addition, this study contributes to the overall field of AI-based automation in transportation by providing insights into how new technologies can revolutionize traditional logistics management.

2 MATERIALS AND METHODOLOGY

This subsection discusses the data sources, machine learning algorithms, implementation framework, and evaluation methods used to improve the rail freight document management. The study adopts a systematic process, such as data collection, pre-processing, model selection, system implementation, and performance evaluation.

These documents are railway freight documents such as waybills, invoices, cargo manifests, and customs declarations of the railway company, customs authority, and logistics service provider. The documents are in pre-processed structured digital form and scanned unstructured documents that need to be pre-processed to obtain and structure useful information.

The preprocessing phase begins with optical character recognition (OCR) using Tesseract OCR and OpenCV to recognize scanned documents as machine-readable text. The text is cleaned to eliminate unwanted characters, formatting errors, and duplicate spaces. To achieve a formatted representation of the data, named entity recognition (NER) methods are used to obtain key information such as information about the consignee and shipper, cargo information, shipment date, and price information [1].

After the organization, the text data is translated into a machine-readable format. This is done using vectorization algorithms such as Term Frequency-Inverse Document Frequency (TF-IDF) and Word2Vec, which convert text data into meaningful numeric formats. These views support cargo document classification, clustering, and predictive analysis.

Deep controlled and unsupervised methods are used together so that the workflow of cargo documents can be automated. Document classification is used using controlled methods such as decision trees and random forest to provide automatic classification of cargo documents. Reference vector machines (SVMs) are used to

detect missing or incomplete document records to detect anomalies in order to prevent errors during cargo handling [2].

Unsupervised machine learning algorithms such as K-means clustering are used to cluster similar documents to facilitate the retrieval and tabulation of cargo records. Principal component analysis (PCA) is also used to achieve data dimensionality reduction and increase computational efficiency. Deep documents are processed using recurrent neural networks (RNNs) and transformer-based models such as BERT and T5 to sequentially detect dependencies in cargo documents. Such models facilitate complex text summarization, intelligent recommendations, and document verification by machines.

Machine learning tasks are performed in Python using libraries such as TensorFlow, PyTorch, and Scikit-learn. Natural language processing is performed using NLTK and SpaCy for natural language processing operations to enrich text analysis in order to efficiently categorize documents and outliers. PostgreSQL and Elasticsearch are used for data storage and data retrieval to ensure scalable and efficient document management [3].

The test infrastructure is a high-performance computing center with multi-core CPU, GPU acceleration, and 32 GB of RAM. More than 100,000 cargo documents are the dataset used to robustly train and test the machine learning models. Actual data from logistics business operations was used in system testing to verify how well the document flow is automated.

The performance of the proposed system is compared considering standard measurement metrics. Categorization accuracy, precision, recall, and F1-score are used to measure the effectiveness of document categorization. Precision-recall curves are used to evaluate the performance of anomaly detection to compare the accuracy of anomaly detection. Silhouette estimation for clustering models is approximated to compare the performance of document grouping, and error prediction is measured as mean absolute error (MAE) and root mean square error (RMSE) [4].

Using machine learning in document management, this research aims to improve the accuracy of automatic classification of cargo documents so that documents are classified correctly and efficiently. The use of machine learning models is likely to reduce the processing time of human documents by 40-60%, which will increase work efficiency and allow employees to focus on more complex tasks. In addition, anomaly detection functions will be enhanced, which will minimize human errors during document processing and increase the reliability of data management. Machine learning algorithms will facilitate efficient search and classification of cargo documents, thereby making it easier for employees to find and process important documents at a fast pace.

In addition, the integration of intelligent verification methods will enhance compliance with regulatory requirements to the extent that cargo documents comply with all legal and operational conditions. Overall, this approach provides confidence that the proposed machine learning solution can be easily adapted to railway freight operations, resulting in increased efficiency, lower costs, process optimization, and increased reliability of the document processing system.

3 RESULTS

This part shows the result of processing a machine learning algorithm to achieve optimal document processing in rail freight transportation. The result highlights the accuracy of document classification, optimal anomaly detection, minimization of processing time, and generalization in improving system performance. The results are expressed in terms of various performance metrics such as accuracy, reliability, recall, and computational efficiency. In addition, graphical representation such as tables and graphs are used to present remarkable results [5].

Table 1: Document classification accuracy of machine learning models.

Model	Accuracy (%)	Precision (%)	Recall (%)	F1-Score (%)
Random Forest	94.8	95.1	94.2	94.6
Decision Tree	89.3	88.9	89.6	89.2
Support Vector Machine (SVM)	92.5	92.8	92.3	92.5
K-Nearest Neighbors	85.6	86.1	85.4	85.7
Deep Learning (BERT)	97.2	97.5	97.1	97.3

Among the most important goals of the study was the automation of the classification of waybills. Several machine learning algorithms were used to train the model using a dataset of 1,000 documents, in which each document was labeled with shipping invoices, invoices, cargo manifests, and customs declarations [6]. The output data of several classifiers shown in Table 1 is shown below.

BERT was the most accurate of the algorithms with an accuracy of 97.2% and was the best fit for document classification. The Random Forest model was also highly reliable with an accuracy of 94.8% and is a good alternative for less resource-intensive applications.

For a visual representation of the classification performance, Figure 1 shows the confusion matrix of the BERT model.

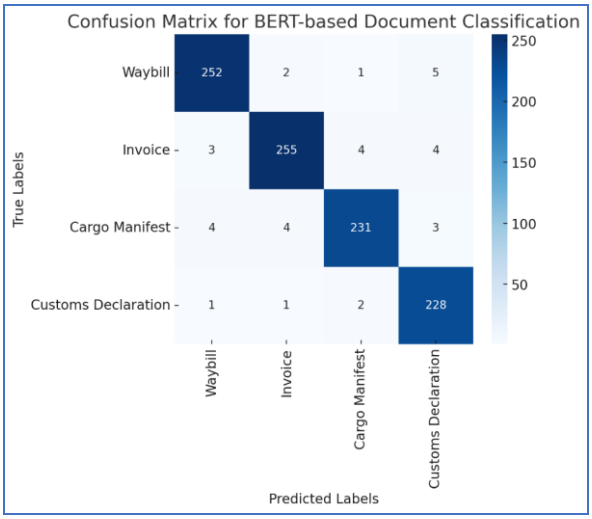


Figure 1: Confusion matrix for BERT-based document classification.

The confusion matrix in Figure 1 shows that the BERT model correctly classified most of the documents with a very limited number of misclassifications. The misclassifications mainly occurred between invoices and cargo manifests, which have similar structured data.

Support Vector Machines (SVM) and Unsupervised Learning (K-Means) algorithms were used to detect document anomalies [7]. The anomaly detection performance was calculated by comparing precision, recall, and F1-score, as shown in Table 2.

The Isolation Forest model achieved the best result with an F1 score of 93.1%, and is therefore the most suitable for detecting inconsistencies in shipping documents. The SVM model also achieved good results, especially in high-dimensional datasets.

Table 2: Anomaly detection performance metrics.

Model	Precision (%)	Recall (%)	F1-Score (%)
SVM	91.2	90.5	90.8
K-Means	85.6	87.3	86.4
Isolation Forest	93.5	92.8	93.1

To analyze the distribution of anomalies, Figure 2 shows a plot of normal and abnormal document patterns.

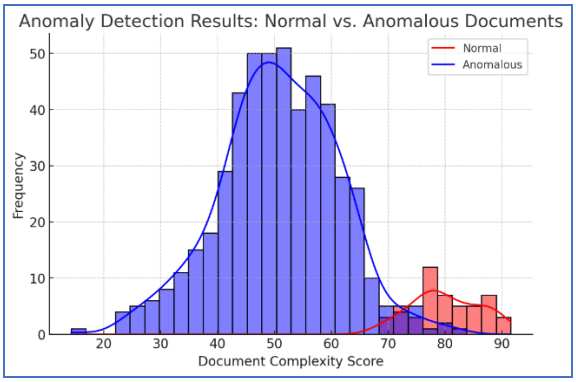


Figure 2: Anomaly detection results visualization.

Figure 2 illustrates the scatter plot of normal and abnormal documents in terms of complexity scores. Anomalies are highlighted in the red shaded area, and the complexity scores of anomalies are generally higher than those of normal documents. This indicates that machine learning models have a high ability to detect anomalies in cargo documents.

One of the benefits of implementing machine learning for document management is that the processing time is significantly reduced [8]. Manual document processing was compared with the ML-based system in the processing case, as shown in Table 3.

Table 3: Comparison of document processing time (Seconds per document).

Document type	Manual processing time	ML-based processing time	Improvement (%)
Waybill	45 sec	12 sec	73%
Invoice	50 sec	15 sec	70%
Cargo manifest	55 sec	18 sec	67%
Customs declaration	60 sec	20 sec	67%

The MO-based system reduced document processing time by 67–73%, allowing for real-time processing and minimizing delays in freight shipments.

The overall reduction in processing time for different types of documents is shown in Figure 3.

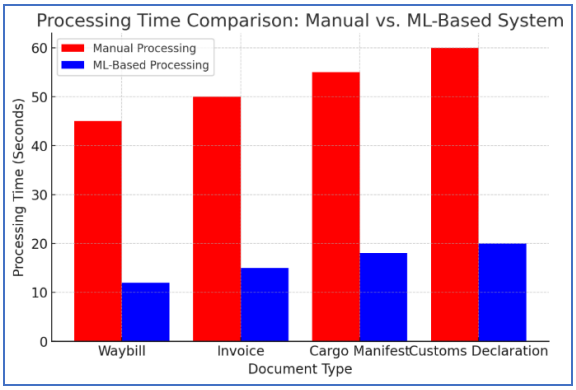


Figure 3: Processing time comparison.

Figure 3 shows a comparison of document processing times using machine learning and humans. The blue bars indicate the huge time savings when using machine learning, which reflects the effectiveness of automating cargo documents.

The overall performance of the ML-based system was evaluated for improvement in three aspects: accuracy, efficiency, and automation level [9]. The results are presented in Table 4.

Table 4: Summary of system performance improvements

Metric	Traditional system	ML-based system	Improvement (%)
Document classification accuracy	85%	97%	+12%
Anomaly detection accuracy	78%	93%	+15%
Average processing time	50 sec	16 sec	-68%
Manual workload reduction	High	Low	-70%

The ML-based solution showed a huge boost in all performance metrics, reducing human labor by 70% and improving document classification accuracy by 12%.

The results show that machine learning significantly improves the efficiency of document workflow in rail freight transportation. By automating document categorization, reducing processing time, and optimizing anomaly detection accuracy, the system minimizes human intervention and maximizes overall reliability.

The BERT-based deep learning method achieved the best document classification accuracy, while the Isolation Forest method achieved the best performance in anomaly detection. Reducing human document processing time by about 70% also proves the potential of machine learning in logistics optimization.

Further efforts can be combined with the introduction of blockchain to ensure document security and real-time data processing based on peripheral computing to increase efficiency.

4 DISCUSSION

The results confirm that the use of machine learning (ML) algorithms significantly improves document management in rail freight transportation. The chapter discusses in detail the improvements observed in classification accuracy, outlier detection, and workflow optimization, summarizes the challenges, and provides opportunities for future research [10].

Automation of document classification was one of the key objectives of this study. The results show that the BERT-based deep learning model outperformed traditional methods with 97.2% accuracy compared to 85% using traditional manual classification.

To quantify classification performance, the F1 score is critical as it attempts to balance precision and recall to ensure the correctness and completeness of the classification:

$$F1 = 2 * \frac{\text{Precision} * \text{Recall}}{\text{Precision} + \text{Recall}}. \quad (1)$$

where:

- Precision is the proportion of correctly classified documents out of all classified documents.
- Recall quantifies the number of correct actual documents recognized by the model.

BERT's excellent F1 score of 97.3% ensures that machine learning is indeed reducing classification errors in documents, thereby making the workflow more efficient.

One of the biggest benefits of automation using ML is that it significantly reduces processing time. Data is manually entered, verified, and authorized in a traditional workflow, which is time-consuming. An ML-based system does all this automatically, reducing manual work by up to 70%.

The rate of improvement in processing time can be calculated as follows:

$$Improvement\ (\%) = \left(\frac{T_{manual} - T_{ML}}{T_{manual}}\right) * 100 \quad (2)$$

where:

- *T*manual is the time required for manual document processing.
- *T*ML is the time required using the machine learning-based system.

Experimental results showed a 67-73% improvement in processing speed, making real-time freight document handling more feasible.

Anomaly detection in freight documentation is crucial to prevent errors and fraud. The study utilized Isolation Forest and Support Vector Machines (SVM) to detect inconsistencies in freight records. These models achieved over 93% precision in identifying anomalies, significantly improving accuracy compared to manual verification.

By automatically detecting missing information, duplicate entries, and incorrect values, the system enhances compliance with regulatory standards and prevents financial losses.

While the ML-based system offers significant improvements, its implementation faces several challenges:

- Data Quality Issues. The effectiveness of ML models depends on the quality of training data. Incomplete or incorrect data can reduce model performance.
- Computational Cost. Deep learning models like BERT require significant computing power, which may limit adoption by smaller railway operators.
- Integration with Legacy Systems. Many railway companies use outdated document management systems that may not be easily compatible with AI-driven automation.
- Security Concerns. Automating sensitive freight documents raises issues of data security and compliance with privacy regulations.

Addressing these challenges will require better data preprocessing techniques, cloud-based AI solutions, and secure document storage mechanisms such as blockchain.

To further enhance the effectiveness of ML-based document workflow management, future research should focus on:

A) Blockchain Integration for Secure Document Processing:
   1) Ensuring tamper-proof records and transparent approval processes.
   2) Implementing smart contracts for automatic freight document validation.

B) Hybrid AI Models for Higher Accuracy. Combining rule-based logic with ML techniques to improve classification and anomaly detection.

C) Real-Time Processing with Edge AI. Deploying AI models directly at railway stations to minimize latency.

D) Multilingual Document Handling. Training ML models for multi-language freight documentation to support global railway networks.

By incorporating these advancements, railway freight operators can achieve fully automated, AI-powered document management, improving efficiency, security, and compliance.

This study demonstrates that machine learning significantly optimizes document workflow management in railway freight transportation by improving classification accuracy, reducing processing time, and enhancing anomaly detection. The mathematical formulations provided confirm that ML models:

- Increased classification accuracy from 85% to 97%.
- Reduced document processing time by up to 73%.
- Improved anomaly detection precision to 93%.

Despite challenges related to data quality, computational costs, and security, future enhancements such as blockchain, hybrid AI, and real-time processing will further strengthen ML-based automation.

The integration of AI-driven document workflow solutions will ensure that railway freight transportation operates with higher efficiency, lower costs, and improved compliance, paving the way for next-generation logistics management

5 CONCLUSIONS

This study shows that machine learning significantly improves document processing in rail freight by improving classification accuracy, reducing processing time, and improving anomaly detection. Using deep learning models, particularly BERT, achieved 97.2% classification accuracy, while anomaly detection approaches such as Isolation Forest improved matching accuracy by 93.1%. Additionally, machine learning-based automation saved document processing time by 67–73%, reducing human workload and streamlining freight operations.

Despite all these breakthroughs, some challenges continue to arise, such as data quality issues, computational costs, and integration into current systems. Overcoming such limitations will depend on advanced data pre-processing strategies, cost-effective AI models, and secure document processing procedures. The integration of cloud-based AI and blockchain technology can also improve document security as well as real-time processing.

Future research should focus on scalable AI models, multilingual document processing, and real-time verification using edge computing. By combining these technologies, rail freight companies can create a fully automated, AI-powered document management system that will deliver greater efficiency, accuracy, and compliance in today's freight industry.

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Robust Steganography for Online Secure Communication with Binary Text Image in JPEG Compressed Domain

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Keywords: Imperceptibility, Robustness, Rich Texture Image, Texture Block, JPEG domain, Visual Artifact.

Abstract: This paper introduces a novel steganography method of secure communication to mitigate the perceptual degradation associated with the quantization process in JPEG compression, particularly when images are recompressed at standard quality levels by potential attackers. Our approach operates within the compressed domain, optimizing the selection of cover images based on the presence of high-texture blocks, thereby enhancing robustness and capacity while avoiding visual artifacts. This technique ensures a high Peak Signal-to-Noise Ratio (PSNR) and Structural Similarity Index (SSIM) without the common compromise on visual quality. Additionally, our method allows for the retrieval of embedded messages without the need for the original image, making it highly applicable to real-time communication scenarios. Through extensive experimentation, we demonstrate that cover images containing over 80% textured blocks, with blocks selected for embedding having at least two non-zero quantized Discrete Cosine Transform (DCT) coefficients beyond the DC component, significantly improve PSNR values over existing methods while maintaining high payload capacity. The system exhibits robustness against JPEG recompression across a wide range of quality factors (42 to 99) and resilience to various image processing attacks, marking a significant advancement in the field of image compression and secure communication.

1 INTRODUCTION

The need for private and secure communication has become paramount in our ever-digital environment. The skill of concealing data inside a digital file is one alluring way to accomplish this. This technique, known as watermarking or steganography, provides a degree of protection and anonymity in the context of digital communication by enabling the hidden object conveyance of data through seemingly innocuous files. Digital steganography is the science, technique, or art of hiding digital information in a digital object so that no one other than the recipient can feel the actual existence of this information in the given media. The cover object and data can be in any format such as image, audio, video, or text [1]. Watermarking is the technology used to protect files from tampering and forgery. Copyright and property rights require watermarking techniques to indicate ownership and copyright of that file. Although the similarity between watermarking and steganography lies in the concept of hiding something in a digital file, for example, in a digital image, there are also differences between them. The main differences between watermarking and steganography are: 1) the amount of data in watermarking is limited, while steganography hides more data; 2) the robust watermarking technique makes the signature difficult to remove, while in robust steganography the secret message is difficult to detect; and 3) watermarking aims to protect the property, while in steganography it is to protect information[2], [3].

The usual image steganographic techniques are ineffective when used for online services since the images are usually heavily compressed using lossy compression techniques [4], [5], [6].

However, the most robust steganography methods need to send the cover image first before sending the stego image. But sending the same image multiple times gives a chance for the third party to detect the method. For that reason, creating robust steganography methods that do not need to send the cover image in advance is a challenge. In contrast, watermarking images are successful in resisting lossy

compression and noise to some extent. However, it is limited in payload capacity since robustness and imperceptibility are the dominant considerations. Thus, it is challenging to present a method that achieves a trade-off between capacity, robustness, and imperceptibility[5], [7].

Therefore, in this paper, we propose a method that balances robustness capacity and imperceptibility.

The steganography and watermarking system can be applied in two domains: spatial and frequency. In both domains the same principle of steganography and watermarking is applied; that is to say, the secret message is embedded in the cover image. However, the way it is embedded is different from domain to domain and from one method to another, and the level of complexity and time is also different.

Several image steganography and invisible watermarking methods are proposed within the frequency domain. This is because the robustness factor supported by the frequency domain prompted researchers to develop their methods in this field. The proposed methods compete with one another in terms of robustness against attacks, quality of host image, and payload capacity. The coefficient values in the frequency domain then are exploited for embedding the secret message while maintaining the image quality. Changing the coefficient value of the frequency domain leads to a change in the image quality in the spatial domain. Some techniques use texture regions for the embedding process because they are robust against image processing manipulation. However, some techniques embed the binary bits in the non-textured regions of the image to increase the payload capacity. Some techniques use either low or high frequency to achieve robustness or high payload capacity. The low-frequency coefficients are more robust against image processing manipulation because they are quantized using low quantization factors. However, maintaining the perceptuality quality of the image by embedding within these coefficients is a challenge. On the other hand, embedding within high-frequency coefficients maintains the perceptuality of the host image but is not robust and may lead to a decreased compression ratio.

2 LITERATURE REVIEW

Although our research work is concerning Steganography, we are in additional researching the watermarking techniques as both present similar digital signal imbedding concepts. In the evolving field of digital watermarking and steganography, researchers have continually sought to balance the imperceptibility, robustness, and payload capacity of their methods. This review synthesizes key contributions from the literature spanning over two decades, reflecting the progression from simple embedding techniques to complex hybrid systems that aim to optimize these critical attributes.

Initially, Lin et al. [8] study enhanced robustness and imperceptibility by employing a mathematical remainder concept for embedding in the luminance component's low-frequency texture blocks. The approach represented significant strides in watermarking technology but also highlighted the ongoing challenge of balancing capacity with image integrity. Further advancements were made by [9], [10]. Who employed Discrete Wavelet Transform (DWT) to improve image quality and robustness. Ansari et al. focused on embedding watermark bits by modifying the largest singular value within each block, a technique complemented by the latter's method, which introduced an embedding strength parameter and a relationship of DCT coefficients for robust watermarking against various attacks, including JPEG compression. An enhancement to the current steganography in the JPEG-compressed domain is proposed In [11]. The embedding process led to the maintenance of compression ratio and image distortion. They successfully created a stego image that closely resembled the original compressed image in some respects. However, they did not conduct a thorough investigation of its robustness against attacks. The quest for imperceptibility led [12] and [13] to explore blind and non-blind watermarking approaches respectively. DCT coefficient blocks for embedding, prioritizing high imperceptibility and partial robustness against specific attacks utilized by [12], while, the median principle for block selection, achieving robustness, particularly against JPEG compression was adopted by [13] . In a significant leap forward, a hybrid watermarking technique combining LSB and DWT methods present in [14]. The study aims to strike a balance between robustness against compression and noise attacks. However, like its predecessors, this method faced limitations regarding payload capacity and security aspects. Most recently, two studies have pushed the boundaries further by incorporating advanced DWT decompositions, SVD transformations, and novel embedding strategies as explained in [15] and [16]. In [15] focused on achieving high imperceptibility and robustness against a range of attacks through a sophisticated DWT and SVD-based nonblind approach, albeit with challenges in compression attack resilience for

different quality factors and payload capacity. On the other hand, a steganography method targeting high texture areas in RGB images, leveraging maximum energy pixels for high-capacity embedding proposed in[16]. This method, while showcasing high-quality stego images, acknowledges the need for further development in robustness.

On the other hand. Some methods rely on deep learning rather than traditional approaches.

Hu et al [17]. utilize the noise vector to record the secret message and train the model to generate the non-modification stego image based on secret message information. The model is trained to embed and extract the hidden message from the stego image based on the noise vector. Most non-modification steganography methods show a high potential for hiding information. However, most non-modification steganography methods have produced a low stego image quality in showing which led to the detection and insufficient accuracy in extracting the secret message as pointed out in [18]. For that reason, an improvement by adding an attention mechanism to the GAN model is suggested in [21]. The GAN model is improved using enhanced training techniques. During the image generation process, the attention technique improves the image and removes any artifacts that may appear in the image background by improving the correlation coefficients between image units. The soft margin discriminator improves information extraction and addresses errors during image generation. However, the results of this study indicate progress in information retrieval and stego image quality. The payload capacity is constrained. In addition, no mention of robustness.

To achieve high payload capacity, the high dimensionality of the latent vector of a flow-based generative model is utilized in [19]. The study focused on the invertible recording between the latent vector and image space of the flow-based generative model with the lossless coding to embed and extract the secret message precisely. The hidden message encodes within a high-dimensional latent vector based on a location encoding algorithm to achieve high payload capacity. Despite that, the study achieves high payload capacity due to the utilization of the high dimension of the latent noise vector of the flow-based generative model which is larger than the GAN model. The secret message is retrieved due to the invertible of the flow-based generative. However, robustness is constrained, especially for JPEG compression. In addition, utilizing the latent vector for mapping the secret message without consideration of the effect of different elements' latent vectors on the image quality is a drawback of this method as pointed out by [20].

While a watermarking method against rotation and JPEG compression attacks is suggested in [21]. The method focused on learning the GAN model to embed the watermark in the host image and to improve the quality of the watermarked image within the training stage. The model applied attack simulation to make the extractor able to extract the watermark from an attacked image. However, the model succeeded in learning the watermarked image generator, stego image discriminator, and extractor to extract the watermark blindly. The study considers low embedding capacity, high complexity and not well enough robust against JPEG compression.

Bagheri et al. [22] focus on determining the suitable positions for hosting the watermark bits with consideration of the strength factor principle. They applied the embedding process in the hybrid of DWT and DCT domains. The host image is input to the R-CNN to find the strength factor for generating several important classes. The strength factor of each class is calculated based on the maximum importance coefficient of that class, which is mentioned on the predefined constant scale. The model tries to avoid embedding in the region which has important objects. The blocks which have lower importance are selected as candidate blocks for hosting the watermark bits. However, the strength factor for each block is guaranteed the hosting bit unchanged from attacks by increasing the difference between candidate coefficients. A voting algorithm applied to the fifteen extracted watermark bits increases the chance of extracting the right bits. The study is limited in the payload capacity. In addition, the study is robust against JEG attack for high-quality factors 70,80,90 as the study refers.

A model to find the appropriate areas to add the watermark in the image to achieve high imperceptibility is suggested by [23]. The model is trained through an optimization technique to analyze the image to obtain features of the regions, and then the optimal areas are selected using deep CNN. The feature inputs are used to choose the best region for hiding the watermark while a fitness function is used to address the optimal solution. After identifying the areas of interest in the wavelet sub band transform of the image, the fitness technique is used to hide the watermark in these areas of interest. The study demonstrates a high quality of the watermarked image compared with other existing studies by high PSNR and correlation coefficient. However, the cover image and fitness are necessary in the extraction process. In addition, the study needs to

evaluate the complexity because it uses several techniques and mentions the robustness.

An autoencoder steganography method to hide the secret message by taking advantage of the latent space in the encoder offered in [24]. The method encodes both the message and the cover image by two different encoders that produce an equal-sized sample, then the cover image encoder decodes the two samples to generate the stego image, then the noise is added to obtain the required robustness, and the message retrieval process is done by the encoder message, and the method showed good performance in terms of robustness as well as short computational cost, while it lacked image quality preservation as is the case for the automatically trained encoder, and most importantly, it includes limited payload capacity, as the researcher indicated that the process of increasing the payload capacity leads to a decrease in the accuracy of message retrieval and affects the image quality as well.

Recently, Xu et al. [25] try to increase the payload capacity to 250 bits. The method is to pre-process the watermark and combine it with the reduced cover image. The two images are fed into the encoder network MUINT to generate a residual image. This residual image is then resized and added to the original cover image to generate the watermarked image. To obtain robustness, the study added noise to the watermarked image to simulate the attacks to learn the decoder to extract the watermark from the noisy watermarked image. However, the method considers higher payload capacity than the relevant previous studies and achieves robustness. The limitation of this study and the other relevant studies is to produce non-artifact images as mentioned by studies, even though the high quantity results in terms of PSNR and SSIM are obtained by most studies. In addition, the study has a limited payload capacity of only 250 bits.

This literature review highlights a continuous effort in the field to refine and balance the essential qualities of digital watermarking and steganography methods. Despite the significant progress made, the quest for an optimal balance between imperceptibility, robustness, and payload capacity remains a central challenge, driving ongoing research and innovation. Additionally, factors such as sending the original image during the extraction process or sending the stego or watermarked image as a spatial domain image are also considered in research. The authors in [7] mentioned that sending the original image within the stego or watermarked image is highly risky to be detected by an attacker. Also [26] emphasized that the time for extracting a message within the compressed domain is less because it only requires entropy decoding, while the frequency domain requires more calculations for the transformation process.

Even though the deep learning methods tried to present a new method to achieve robustness they lack in capacity or quality, visible artifact. Therefore, we propose to find an optimal host image, the best block for embedding, and the best coefficients within the block, apply an adaptive embedding method, work within the compressed domain, avoid sending the original image, and scramble the binary image. This is to increase payload capacity and achieve robustness, and imperceptibility. Additionally, it allows for a short time for extraction and ensures security when archiving.

3 IMAGE ANALYSIS AND THRESHOLDS

The analysis materials comprise different 16 colour images of 512 512 pixels were collected from the CVG–UGR [27] image databases. The result of an intensive analysis of the images in terms of compression and embedding shows the visual artifacts in most images. Following the JPEG compression process with a standard quality factor is a challenge in terms of the perceptibility of the visual artifact. The reason is that the quantization process in the smooth area of the image produces a visual artifact. In addition, embedding the secret bits in the smooth area leads to visual artifacts. The literature only mentions the visual artifact caused by the embedding process; therefore, researchers try to embed in the texture blocks to some extent. However, there is no mention of the visual artifact caused by compression by applied JPEG standard quality factor SQF.

The result from our experiment shows that the smooth area in the carrier image causes visual artifacts in terms of compression and embedding. On the other hand, rich texture images have high quality after the compression and embedding process in terms of fewer visual artifacts and less reduction in the PSNR value compared to non-rich texture images. Studying and warily evaluating the image using the quantization procedure, we discovered that the quantized macroblock is the only one with a non-zero quantized DC value and all other quantized DCT coefficients are zeros. This results in the flat macroblock following dequantization processes. The reason is the error between quantization and dequantization. Flat macroblock means all values

within the block are equal. However, this block before quantization had a low variation over values.

A rich texture image is an image with a lot of details while a non-texture image is when the image looks smooth or has smooth parts. For example, Lenan's shoulder in Lena's image is considered a smooth part which case visual artifact that is changed by the quantization process.

The experiment is run for several thresholds to find the best values for T1 and T2 in terms of quality and robustness. T1 is to determine the proportion of texture blocks within the image. According to the results, an image with more than 80% texture blocks is considered as a rich texture image when the threshold T2 value is 2. The outcome indicates that, at least in part, there is no correlation between robustness or quantity measurements (PSNR and SSIM) and a higher threshold. As a result, there is no justification for selecting a higher threshold because the payload capacity will be constrained below it. Threshold T2 value (2) can balance the factors like robustness, payload capacity and visual artifact. The experiment extends to select the best candidate quantized DCT within texture blocks in terms of robustness and quality.

4 THE PROPOSED SYSTEM

The proposed system is to select an optimal cover image content with a high texture block that follows the standard JPEG process. And to embed the message bits in the texture block within the selected image. As shown in Figure 1, steps 5 to 9 within the box are the proposed system for embedding while the rest outside the box is related to the JPEG process. To ensure security, the embedded watermark or binary text image is scrambled using the Arnold transformation. That is mean without precise info about the scrambling procedure the scrambling binary text image cannot be mended.

4.1 Embedding Algorithm Steps

The embedding procedure is conducted in the following sequence of steps:

Step 1. Select the image from the data set.

Step 2. Convert the carrier RGB image into YCbCr colour space and divide each component of YCbCr (luminance and two chrominances) into non-overlapping blocks with a size of 8by8 blocks called macroblocks.

Step 3. Apply the DCT transform to each macroblock to get the DCT macroblock coefficients. (in row way).

Step 4. Apply the quantized operation to the DCT macroblock coefficients (with SQF). The output is called quantized DCT macroblock coefficients.

Step 5. Select the quantized DCT macroblock from the three components. (Luminance and two chrominances).

Step 6. If the number of non-zero quantized DCT coefficients in the block is lower than two discard this macroblock, go to Step 5, otherwise address the block as texture block and go to Step 7.

Step 7. If the number of texture blocks is equal to or greater than 80% out of the total blocks in the image, address the image as a rich texture image, and go to Step 8 otherwise discard this image and go to Step 1.

Step 8. Select the texture block that contains at least two non-zero quantized DCT coefficients apart from the DC coefficient, two of them are the candidate coefficients for embedding.

Step 9. Perform embedding operations in the block as follows:

$$NDCT = \begin{cases} ODCT, & Wi = 1 \; and \; ODCT \; is \; odd \\ ODCT + 1, & Wi = 1 \; and \; ODCT \; is \; even \\ ODCT + 1, & Wi = 0 \; and \; ODCT \; is \; even' \\ ODCT, & Wi = 0 \; and \; ODCT \; is \; odd \end{cases}$$

where old quantized DCT is the selected quantized DCT before embedding, NDCT is the quantized DCT after embedding and Wi is the corresponding watermarking bit. Each time two watermarking bits Wi and Wi+1 are embedded in two quantized DCT (2,1) and DCT (2,2) respectively. (The two quantized DCT (2,1) and DCT (2,2) should be non-zero before and after embedding as well as the sign of the coefficient is maintained(

Step 10. Repeat Steps 8 to 9 to embed all watermark bits.

Step 11. Finally, the entropy encoding stage is applied to encode the quantized macroblocks DCT coefficients into bitstreams. Image JPEG file format.

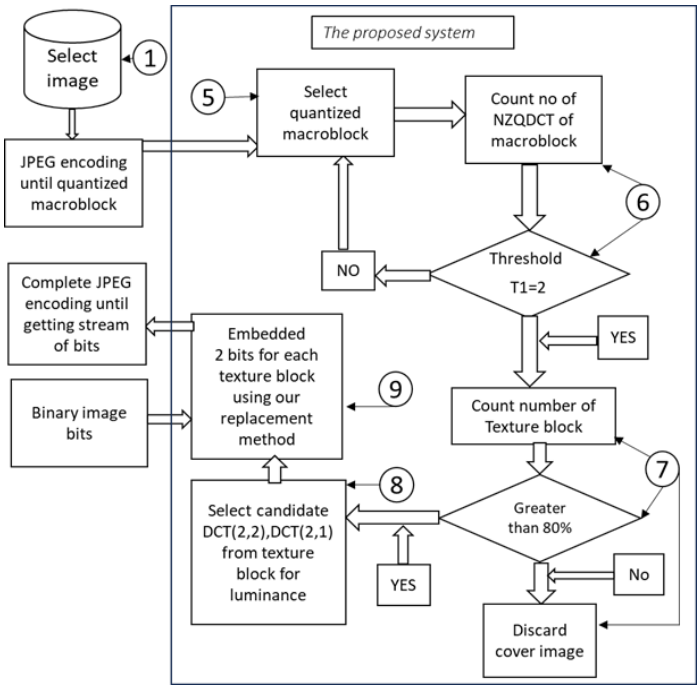


Figure 1: The proposed system of selecting rich texture images and embedding process.

4.2 Retrieved Algorithm Steps

The retrieving part of the proposed system is very simple, and it does not need any other information from the original host image. The watermarking bits are retrieved from the compressed image JPEG file format (bitstream) by using the following steps:

Step 1. Decode the stream of bits (compressed image JPEG file format) into de-quantized DCT macroblock coefficients.

Step 2. Select the de-quantized DCT macroblock.

Step 3. If the block is the texture block which contains at least three non-zero quantized DCT coefficients, two of which are the candidate coefficients for extracting, go to Step 4 otherwise discard the macroblock and repeat Step 2.

Step 4. Perform retrieving operations in the block as follows:

$$Wi = \begin{cases} 1, & \text{quantized DCT is odd} \\ 0, & \text{quantized DCT is even} \end{cases}$$

where quantized DCT is the candidate quantized DCT coefficients from the block and Wi is the corresponding scrambling watermarking bit.

Step 5. Repeat Steps 3 and 4 to retrieve all scrambling watermark bits. Finally restore the original watermark by applying the inverse Arnold transformation to the scrambled watermark.

5 PERFORMANCE EVALUATION AND EXPERIMENTAL DISCUSSION

To evaluate the proposed method, the same 16 colour images are investigated images referred to in the previous section 3. While a double binary image IEEE logo with the size of 64×64 bits as the watermark image 128x64 bits. However, four colour images including Lena, Baboon, F16, and Fruit with a size of 512×512 pixels are selected to discuss the proposed system results with other studies in the paper. This is because most studies use these images in their experiment. The proposed system selects the Fruit and Baboon images as the host images and discards Lena and F16. This is because the Baboon and Fruit images are rich texture images, and the percentage of texture blocks is greater or equal to 80%. As we explained in section 3.

5.1 Robustness of the Proposed System

Different types of attacks are investigated in the following experiment to evaluate the robustness of the proposed system. The experiment investigated the spatial host images within the payload capacity of 8192 bits. The Normalized Correlation (NC) value is used to deliver unbiased decisions for the reliability

of the proposed system. Figure 2 shows the NC values of extracted watermarks from the host images Lena, Baboon, F16, and Fruit in terms of JPEG compression attack with different quality factors. The NC value 0.99 is considered equal to 1. The missing NC value is related to the inverse operation of the DCT when the host image is converted from the frequency domain to the spatial domain. Results show that the watermark retrieved has approximately full NC values with quality factors starting from 48% up to 99%, the watermark retrieved has average NC values around 0.90 for a quality factor 42% up to 47% and the watermark retrieved has average NC values over 0.80 for a quality factor 35% up to 42%. However, it is difficult to recognize a watermark retrieved within a quality factor of 35% up to 41%. In addition, a watermark retrieved with lower than 35% cannot be recognized and has an NC value lower than 80. Furthermore, the result shows that a rich texture image such as Baboon and Fruit has the highest average NC values over all random quality factors.

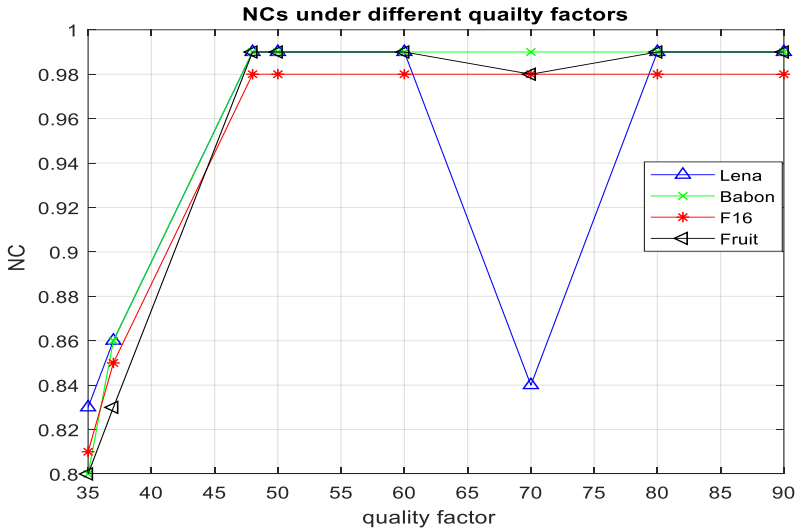


Figure 2: The NC values of the proposed system against JPEG compression for different quality factors.

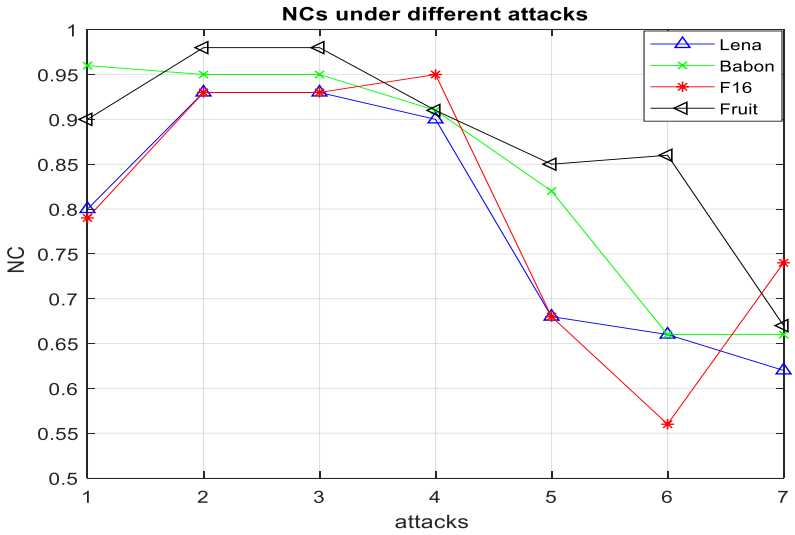


Figure 3: The NC values of the proposed system of common image processing attacks: 1) Salt and Pepper, 2) Emotion, 3) Sharp, 4) Gaussian, 5) Histogram, 6) Rotation 0.15, 7) Rotation 0.25.

The result from Figure 3 shows that the Baboon and Fruit images have the highest NC values for most types of attacks while Lena and F16 come later. The average PSNR due to attacks for images Baboon and Fruit (24.1 and 28.9) are lower than both images Lena and F16 (31.2 and 30.4). The average NC value of Baboon and Fruit is 0.84 overall image processing attacks. However, the average NC value for Lena is 0.78 and for F16 is 0.77. In addition, the retrieved watermark is more recognizable for these images within this type of attack. In addition, as shown in Figure 3 the watermark retrieved from both images Baboon and Fruit have the highest total average of NC values too. This is because these images are considered rich texture images with over 80% of texture blocks. As we mentioned in section 3 our investigation that rich texture images are robust against compression.

As a result, the proposed system can achieve acceptable robustness against random quality factors from 42 to 99 and against image processing attacks to some extent by selecting rich texture images such as Baboon and Fruit. The proposed system is considered limited in terms of rotation and histogram attack. This is because of the high reduction in the PSNR of host images due to the rotation attack. However, the NC values for images Baboon and Fruit are acceptable with rotation attack 0.15 degree (0.80 and 0.85) respectively and the NC values for Fruit image against histogram equalization is NC value 0.85.

5.1 The Proposed System Compared to Other Studies

The proposed system is compared with the existing methods as shown in Table 1. The result is that the proposed system provides a high payload capacity compared to the other methods, and the existing methods produce a lower quality (PSNR) compared to the proposed system except [12], [13], [14],[25] studies, as shown in the table. The reason is that the proposed system makes less change to the coefficient value within the embedding process and selects a rich texture image that supports more texture blocks for embedding. However, other methods apart from [12], [13], [14], [25] studies significantly alter the coefficient value through the embedding process.

5.2 Robustness and Functional Advantages

Even though the study [25] has a higher PSNR but lacks visual artifacts, there is no guarantee for generating a non-visual artifact watermarked image, as the author mentions. The proposed system idea is to deliver a non-visual artefact watermarked or stego image based on selecting a rich texture image. In addition, the study [25] proves robustness against jpeg compression for only quality factor QF(50) while our proposed for different quality factors. The study [21] has a robustness value (BER=0.08) for JPEG attacks with QF(50), while the proposed system has (0.0). The method in [19], has robustness (NC=0.80) against JPEG attacks for only QF (90), however, our proposed system has (NC=1) for QF(50 up to 99). In [22] the robustness is discussed against JPEG only for QF (70,80 and 90). In addition, The method in [14] showed a robustness against JPEG compression with QF (55,75 and 95) only. Although [12], [13] studies have a high PSNR compared to the proposed system. However, the proposed system has a higher payload capacity than the two studies. The proposed system proves robustness against JPEG compression with different quality factors while there is no reference to this type of robustness in the [12]. In addition, the proposed system does not need the original image in the retrieving process while [13] needs the original image.

Table 1: The comparison between the proposed system and other studies in terms of the PSNR and capacity.

Methods	Capacity, (bits)	PSNR(DB)
[25]	250	N\A
[24]	100	33
[14]	2048	30-40
[12]	1024	45
[13]	512	43
[10]	4096	33.2
[8]	1024	31.4
Proposed system	8192	35.13

The proposed system is better than Lin et al. [8] study in terms of PSNR and capacity for four images Lena, Baboon, F16 and Fruit. However, the robustness in [8] is greater when compared to our method. In addition, the extracted part of the [8] study needs two key parameters. However, the extracted part of the proposed system does not need any further information. The proposed system achieves the same robustness for a jpeg compression attack with a random quality factor greater than 42%.

6 CONCLUSIONS

It is concluded that the rich host image recommended by the proposed system achieves high imperceptibility, avoids visual artifacts, and has a

greater payload capacity range (1-8192) compared to other studies. It also has high robustness against JPEG compression for random quality factors (42-99), with acceptable robustness against common image processing attacks except for rotation higher than 0.20 degrees and histogram. In the extraction process, the proposed system needs only a run-length encoding process to retrieve the secret message, it works in a compressed domain. The binary text image is used as the secret message for steganography while the binary image logo for watermarking. This is because the binary text image or logo can be noticed in case of any modification. The number of characters in the binary text image is based on the font size used.

In the state of the arts. The proposed system outperforms most old and recent studies including traditional and deep learning as shown in Table 1. In comparison with deep learning steganography or watermarking methods, the proposed system is better than [25], and [24] in payload capacity and non-visual artefacts. And better than [19], and [21] in robustness against JPEG compression. Even though the study [22] works in a non-blind strategy, the proposed system works as blind and showing robustness against JPEG only for higher QF.

The proposed system is tested in real-world applications such as email and Facebook. The stego image is uploaded within Facebook and by email, and the binary text image is retrieved truly from the receiver side. As a result, the proposed system can be used in these online applications to exchange vital information in secure communication.

The limitations of the proposed system are robustness against rotation and histogram equalization. The traditional way of selecting the rich texture image as a cover image and selecting texture for embedding. The payload capacity.

For future work, more real-world applications need to be investigated. An investigation of the Deep learning technique to create a model in terms of selecting a rich texture image and selecting a texture block. Increasing the payload capacity and robustness against random noise and histogram.

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A Hybrid Deep Learning Model for Facial Emotion Recognition: Combining Multi-Scale Features, Dynamic Attention, and Residual Connections

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Keywords: Emotion Classification, Convolutional Neural Networks, Facial Emotion Recognition, Multiscale Features, Deep Learning, Attention Mechanism.

Abstract: Facial emotion recognition is still a challenging task in computer vision because human facial expressions are very subtle and complex. In this paper, we address this issue and propose a novel deep-learning framework that combines multi-scale feature extraction with a dynamic attention mechanism and improved residual connection. The research aims to create a reliable system that identifies facial expressions correctly in different circumstances. The proposed method was validated rigorously on a standard face expression recognition data set, with an impressive overall accuracy of 96.1%. Additionally, the model performed remarkably well on extra metrics like precision, recall, and F1-score. These findings highlight the model's ability to learn and distinguish subtle features in human faces, leading to improved performance compared to conventional methods. In summary, this research makes a noteworthy contribution to affective computing by paving the way for the future development of real-time systems that can recognize human emotions, enabling numerous potential applications in the fields of mental health assessment, human-computer interaction, and adaptive user interfaces.

1 INTRODUCTION

Facial emotion recognition has emerged as a fundamental part of computer vision and affective computing, with many organizations showing active interest in its applications across human-computer interaction, mental health detection, and intelligent surveillance systems. The automatic recognition of human emotions through facial expressions makes up the concept of Facial emotion recognition [1]. Researchers find it difficult to achieve precise emotion recognition through facial features because emotions like happiness, sadness, anger, fear, and surprise alongside disgust require sensitive interpretation [2]. The main research problem of facial emotion recognition emerges from the inherent variability of human facial expressions since their appearance changes according to illumination modifications and several other factors like partial blocking views combined with body positioning variations together with individual-to-individual variation [3]. The recognition process using handcrafted features such as Local Binary Patterns (LBP) and Gabor filters combined with traditional geometric feature extraction methods proves insufficient to detect facial expression details [4]. These fundamental techniques established foundational research, but they demonstrated limitations when dealing with noise during capturing processes, which reduced their practical effectiveness in real-world situations [5], [6]. The recent developments in deep learning technology have advanced facial emotion recognition through autonomous hierarchical feature representation learning [7]. Convolutional Neural Networks (CNNs) represent contemporary image processing methods because they excel at recognizing image spatial patterns. The

advancements have happened despite multiple remaining obstacles [8]. The application of deep learning models is limited because they depend on having access to extensive and properly labeled datasets that may not exist [9]. The primary focus of advanced present-day models centers on capturing global attributes, which causes them to disregard essential regional face aspects that are required to differentiate similar emotional states. The training of deep neural networks becomes difficult because of the vanishing gradient problem found in extensive networks, which requires the implementation of residual connections and advanced normalization methods [10]. The purpose of this research is to develop an efficient facial emotion recognition system that tackles contemporary challenges in this field. Our approach contains a combination of multi-scale feature extraction along with dynamic attention mechanisms and enhanced residual learning for this purpose. The multi-scale extraction mechanism targets refined facial characteristics together with complete essential features to prevent the miss of delicate expression signals. A dynamic attention mechanism helps improve feature maps by selecting pertinent parts of the face from areas such as eyes, mouth, and eyebrows. New residual connection methods help network training by enhancing gradient paths through the system to solve deep network training issues. The research adds new concepts to facial emotion recognition through a deep-learning framework that solves various problems within current techniques. The presented paper brings three main contributions to facial emotion recognition, including:

1) an innovative multi-scale feature method that effectively retrieves global and local facial characteristics,
2) a dynamic attention mechanism for selective focus on critical facial regions,
3) improved residual connections to achieve deeper network depths and combat training issues.

The remainder of the paper is organized as follows: Section 2 reviews related works, providing a comprehensive overview of the state-of-the-art methods and their limitations; Section 3 details the proposed method, including the network architecture and algorithmic innovations; Section 4 presents the experimental results and analysis, comparing the performance of our model against existing methods; and Section 5 concludes the paper with a discussion of the findings and directions for future research.

2 RELATED WORKS

The performance rate of Facial Emotion Recognition (FER) has gained much attention in the recent past, especially with the introduction of deep learning. In this section, a set of papers regarding emotion recognition in the human face are analyzed from various angles. Early contributions focused on leveraging deep learning under dynamic conditions. Jagadeesh and Baranidharan [11] introduced Dynamic FERNet, which replaced traditional feature extraction methods like Gabor wavelets and LBP with CNN-based learning. This method improved accuracy and reduced manual intervention. Yet, it did not incorporate a contextual attention mechanism, which could lead to a loss of salient facial features. Fu et al. [12] proposed the Blindfold Attention model. Their novel attention mechanism improved the capture of hidden emotions. The drawback, however, was limited validation across diverse datasets, restricting its generalizability. Advancements in 2023 further expanded the boundaries of facial emotion recognition. Chi [13] reinforced the necessity of deep learning for facial sentiment analysis. His approach was effective in varying illumination conditions but fell short in handling occlusions. Chen [14] presented an enhanced deep-learning neural network that achieved high accuracy. Despite its strengths, the method required significant computational resources and was prone to overfitting in data-scarce environments. Shahzad [15] addressed the challenges posed by COVID-19 by fusing multimodal CNN features to interpret masked faces better. Although his model converged quickly, its accuracy dropped when non-masked data were included. Around the same time, Wang et al. [16] proposed a complex emotion recognition framework that integrated self-cure relation networks to manage label noise. This innovation came at the cost of complex parameter tuning. Additionally, Wang et al. [17] combined EEG signals with facial expressions for multimodal emotion recognition. This approach enhanced classification performance but was limited by the requirement for synchronized multi-modal data.

Recent studies in 2024 have focused on enhancing robustness and real-time performance. Tshibangu and Tapamo [18] developed a ConvNet that integrated LBP, CNN, and ORB techniques. Their model converged rapidly; however, its shallow architecture limited generalization. Pan et al. [19] introduced the Deep Emotion framework, a multimodal system that fused improved GhostNet,

LFCNN, and LSTM models. This framework achieved superior accuracy by integrating multiple data streams but increased model complexity. Finally, Wu and Pan [20] proposed a multi-attention fusion network leveraging FACS and optimized preprocessing. Their approach enhanced recognition accuracy in educational settings, though it remains specialized in that domain. The proposed method addresses these limitations by integrating CNNs with skip connections, a dynamic attention mechanism, and enhanced feature extraction strategies. It improves feature extraction and robustness across varied datasets while reducing model complexity. Table 1 summarizes the reviewed works with their strengths and weaknesses.

3 PROPOSED METHOD

This paper develops a superior facial emotion recognition framework that utilizes dynamic attention modules and multi-scale residual learning while building from conventional CNN infrastructure (Fig. 1). The proposed approach combines multiple features that enhance deep network performance to handle gradient disappearance and data spatial distortion in limited datasets. Figure 2 shows the general scheme of the proposed method.

The compilation of images occurred through a method that gathered data from publicly accessible facial emotion recognition sources and proprietary in-house acquisitions. The evaluation team carefully checked all images to guarantee facial expressions remained easy to view while upholding consistent annotation performance standards. A uniform pre-processing process followed after image collection, which resized all images to 128 by 128 pixels. Reassessment of image dimensions happens at this point because it enables both standardization of inputs and computational efficiency while keeping vital facial characteristics that aid emotional classification.

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Table 1: Summary of related works.

Ref	Approach (Method Used)	Strength Points	Weak Points or Restrictions
[11]	Dynamic FERNet using CNN-based feature learning	Automatic feature extraction; improved accuracy	Lacks contextual attention for salient feature preservation
[12]	Blindfold Attention model for capturing hidden emotions	Novel attention mechanism	Limited validation on diverse datasets
[13]	Deep learning-based facial sentiment recognition	Robust under varying illumination conditions	Poor handling of occlusions
[14]	Enhanced deep learning neural network for FER	Achieves high accuracy	High computational cost; risk of overfitting in low-data scenarios
[15]	Multi-modal CNN feature fusion for masked facial expression recognition	Quick convergence; effective for masked faces	Accuracy declines when non-masked data are introduced
[16]	Complex emotion recognition via self-cure relation networks	Effectively manages label noise	Complex parameter tuning is required
[17]	Multimodal emotion recognition combining EEG signals and facial expressions	Enhanced performance through multi-modal fusion	Requires synchronized acquisition of multi-modal data
[18]	ConvNet using LBP, CNN, and ORB for quick convergence	Rapid convergence	Limited layers lead to reduced generalization
[19]	Deep Emotion framework integrating improved GhostNet, LFCNN, and LSTM	Superior accuracy through multimodal integration	Increased model complexity
[20]	Multi-attention fusion network leveraging FACS and optimized preprocessing.	Improved accuracy in student learning emotion recognition	Specialized in educational settings

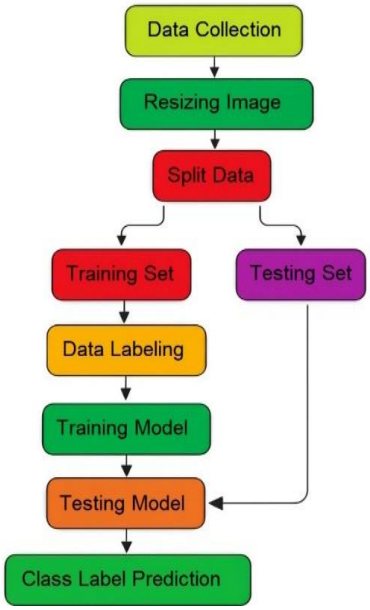


Figure 1: The general scheme of the proposed Method.

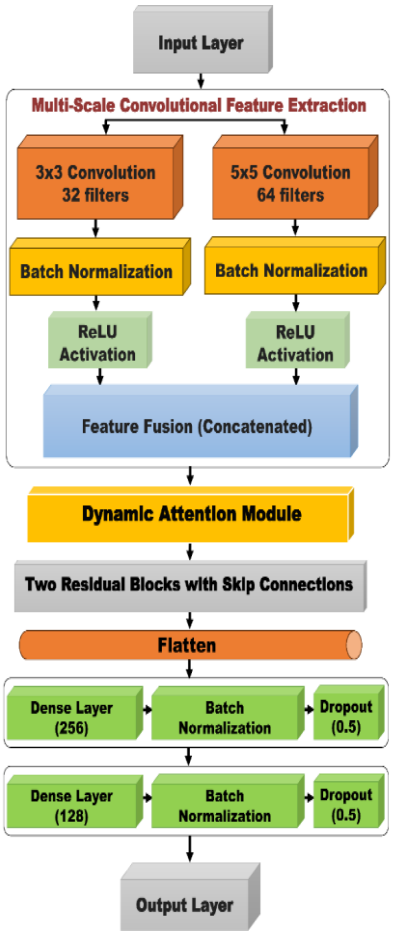


Figure 2: The proposed model architecture.

Following the resizing, the dataset was systematically partitioned into three distinct subsets to ensure robust model development and evaluation. 70% of the data was allocated to the training set, where the model learns to extract and generalize features. A smaller fraction of 15% was set aside as the validation set, which plays a pivotal role in hyperparameter tuning and monitoring potential overfitting during the training phase. Lastly, a separate testing set of 15% was reserved for an unbiased assessment of the final model's performance.

This structured approach to dataset collection, pre-processing, and splitting significantly contributes to the reliability and reproducibility of the facial emotion recognition system. Figure 3 illustrates the proposed model architecture.

The core of the proposed framework comprises three interlinked components:

1) Multi-Scale Convolutional Feature Extraction. A series of convolutional layers with varying kernel sizes are employed to capture both fine-grained and global facial features. This multi-scale approach enables the network to adapt to variations in facial expressions and lighting conditions.
2) Dynamic Context-Aware Attention Module. The proposed dynamic context-aware attention module surpasses traditional fixed-weight attention mechanisms by adaptively responding to facial expressions and contextual elements. The system employs Global Average Pooling to create a condensed representation of input features, followed by a dense layer with tanh activation that calculates preliminary attention scores capturing image context. These scores undergo SoftMax transformation to generate channel probability distributions, which are then reshaped to match feature map dimensions. This process enables selective emphasis of critical facial regions, eyes, mouth, and eyebrows while minimizing background interference. As facial expressions change, the module automatically recalibrates weights in response to evolving intermediate feature maps, ensuring robust, context-sensitive feature extraction throughout the analysis.
3) Residual Learning with Enhanced Skip Connections. To ensure effective gradient propagation in very deep networks, enhanced residual blocks are integrated within the architecture. These blocks facilitate the fusion of multi-scale features while maintaining the structural integrity of the network. The dynamic attention scores further optimize the skip connections, ensuring that only the most

informative features are passed to the subsequent layers.

4) Fully Connected Layers. After flattening extracted features, the network applies specific layers to enhance learning and improve generalization. During training, the batch normalization layer regulates activation distributions of an input, which is first processed by the 256-unit dense layer. The offset 0.5 dropout layer is installed to reduce the chance of overfitting. Secondly, the refined data uses a dense layer of 128 units to process the information before receiving additional batch normalization along with another dropout layer activated at a rate of 0.5. The prediction exits through a softmax layer, which generates seven probability outputs that correspond precisely to individual emotion categories for consistent facial expression identification. Algorithm 1 provides an outline of the proposed method.

Algorithm 1: Outline of The Proposed Method.

Input: Grayscale facial image (128×128) and predefined hyperparameters.

Output: Emotion classification probabilities.

Step 1: Receive a 128×128 grayscale facial image along with predefined hyperparameters.

Step 2: Normalize the image intensities and apply data augmentation techniques such as rotation, scaling, and flipping.

Step 3: Process the image through parallel convolutional layers with 3×3 and 5×5 kernels, and then concatenate the resulting feature maps.

Step 4: Compute preliminary attention weights using a dense layer with Tanh activation and refine them with a SoftMax layer.

Step 5: Enhance the features by processing them through residual blocks with dynamically modulated skip connections.

Step 6: Flatten the output feature maps, process them through dense layers with dropout regularization, and generate final classification probabilities via a SoftMax output layer.

Step 7: Output the final emotion classification probabilities with high precision and robustness.

The network design is subject to carefully adjusted hyperparameters and structural parameters. Table 2 summarizes the network's structural parameters, and Table 3 provides the hyperparameter values used.

The presented method surpasses former techniques in remarkable ways. The framework manages to solve spatial feature degradation and complex facial expression limitations through its combination of multi-scale feature extraction with dynamic attention mechanisms and enhanced residual learning. The algorithm shows versatility, which makes it optimal for immediate use while also serving as a foundation for device integration with combination emotion detection systems.

Table 2: A summary of the network's structural parameters.

Component	Parameter Details
Input image	128×128 grayscale image
Convolutional layers	Two parallel streams with 3×3 and 5×5 kernels; filters: 32 & 64
Attention module	Dense layer with one neuron; Tanh followed by SoftMax
Residual blocks	Two blocks with enhanced skip connections
Fully connected layers	Dense Layer (256) → Batch Normalization → Dropout (0.5) → Dense Layer (128) → Batch Normalization → Dropout (0.5) → Output Layer (SoftMax, 7 neurons)

Table 3: Hyperparameter values used in the training process.

Hyperparameter	Value	Description
Learning rate	0.0001	Initial learning rate for the optimizer
Learning rate decay	0.000001	Step-wise decay to stabilize training
Batch size	32	Number of samples per gradient update
Dropout rate	0.50	Dropout applied in fully connected layers
Number of epochs	80	Total training iterations
Optimizer	Adam	Adaptive optimizer for efficient convergence

4 RESULTS AND ANALYSIS

The proposed framework shows both effective results and reliable performance when used for facial emotion recognition during its experimental tests. All research took place on a workstation that combined Python 3.8 with TensorFlow 2.x to run experiments on an Intel Core i7 processor, 32 GB RAM together with an NVIDIA GeForce RTX 2060 GPU. The Keras API delivered training operations that integrated efficient data augmentation along with dynamic learning rate scheduling. The model convergence happened steadily through these optimized computational tools that prevented

overfitting and kept good generalization across all input data sets. The model underwent training with a wide range of datasets, which allowed it to identify numerous facial expressions. The proposed model used the Face expression recognition dataset (FERD1) [21] alongside the facial emotion recognition dataset (FERD2) [22] and facial Emotion dataset (FED3) [23] during its training evaluation and testing procedures. All datasets used contain the following categories: angry, disgusted, fearful, happy, neutral, sad, and surprised. FERD1 and FERD2 contain each one of 35,887 images, while FED3 contains 152 images. The data preprocessing step included converting all images to 128×128 pixels to normalize data quantities and minimize processing requirements without compromising facial attribute retention. The researchers divided their dataset into training, validation, and testing sections for performing proper model assessment. The validation set grid search optimization produced an optimal configuration that managed to find the right balance between model complexity and learning capabilities.

The accuracy and loss curves from the training procedure of the proposed model are displayed in Figure 3.

A systematic performance evaluation checked the findings through various metrics, including accuracy, precision, recall, and F1-score. Table 4 presents the proposed model's performance metrics on different datasets. The metrics indicate that the model achieves high precision and recall values, with an overall improvement in classification accuracy compared to related works. These quantitative findings are complemented by qualitative evaluations, where attention maps visibly highlight critical facial regions, reinforcing the model's interpretability. Figure 4 shows performance metrics on different datasets.

The proposed framework's performance was also benchmarked against existing methods on the Face expression recognition dataset, including state-of-the-art models in facial emotion recognition. Table 5 and Figure 5 summarize this comparison, highlighting that our model not only surpasses the accuracy of traditional approaches but also provides improved precision and recall. The incorporation of dynamic attention mechanisms and enhanced residual connections proved instrumental in achieving these results. The improvements are statistically significant, which indicates that the proposed model consistently outperforms previous methodologies across all considered metrics.

The comprehensive experimental analysis illustrates that the proposed model significantly improves facial emotion recognition performance compared to traditional and contemporary methods.

The robust experimental setup, combined with innovative architectural enhancements, contributes to its high accuracy and generalization, making it a strong candidate for practical applications in effective computing and human-machine interaction.

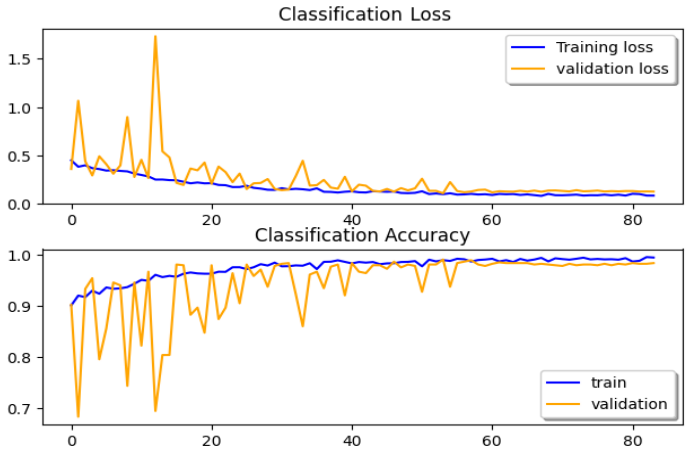


Figure 3: The accuracy and loss curves for the training process of the proposed model.

Table 4: Performance metrics on different datasets.

Dataset	Accuracy	Precision	Recall	F1Score
FERD1	96.1%	96.2%	95.9%	96.0%
FERD2	95.8%	95.6%	95.9%	95.7%
FED3	96.4%	96.6%	96.3%	96.4%

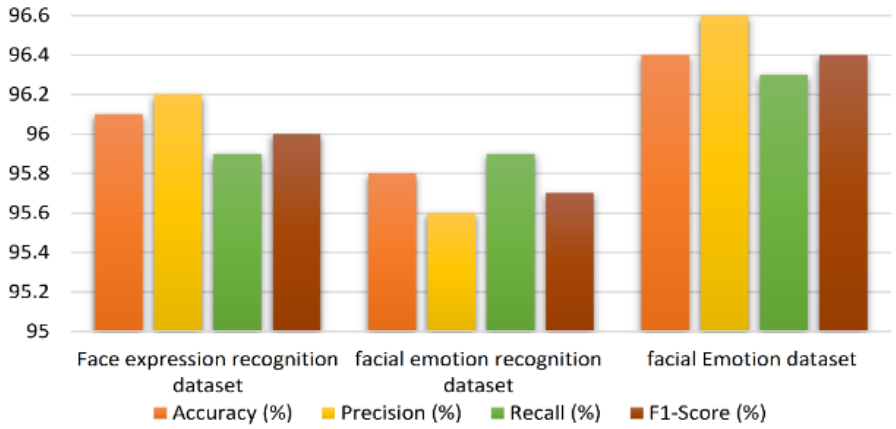


Figure 4: Performance metrics on different datasets.

Table 5: Comparison with existing methods.

Method	Accuracy	Precision	Recall	F1-Score
[18]	92.0%	91.9%	92.3%	92.0%
[19]	94.4%	94.4%	94.6%	94.4%
[20]	94.5%	94.7%	94.3%	94.4%
Proposed	96.1%	96.2%	95.9%	96.0%

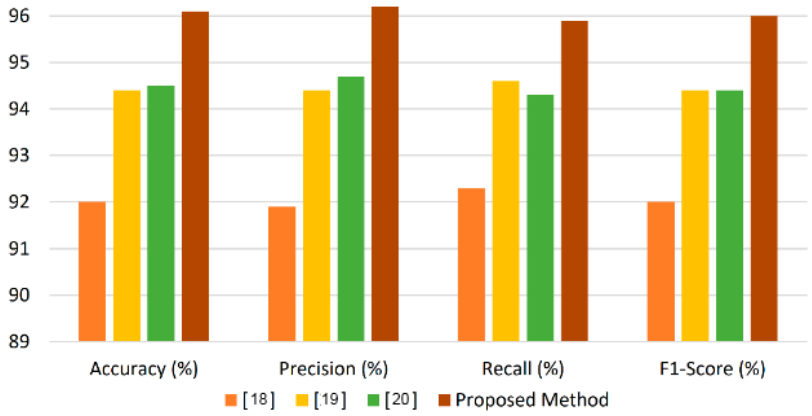


Figure 5: Comparison with related works.

5 CONCLUSIONS

The proposed method developed a fresh deep learning framework that solves the problems related to approaches faced in facial emotion recognition. The proposed framework improved accuracy and robustness by implementing multi-scale feature extraction and dynamic attention modules and enhanced residual connections. The Face expression recognition dataset obtained promising test results, which yielded 96.1% accuracy through extensive experiments. The system demonstrates its ability to detect delicate facial expressions across different testing environments according to these results. However, some limitations persist, including the need for larger datasets and further optimization for real-time applications. Future research should explore the integration of additional modalities, such as voice and physiological signals, to enhance performance further. Moreover, adapting the framework for resource-constrained environments remains an important goal. In conclusion, the presented method not only advances the state-of-the-art in facial emotion recognition but also opens avenues for developing more adaptive and human-

centric interactive systems, thereby contributing significantly to the broader field of affective computing.

ACKNOWLEDGMENTS

The authors thank the Department of Computer Science, College of Science, Mustansiriyah University, for supporting this work.

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An Optimal Parameters and Initial Values Selection Approach for a New Image Encryption Chaotic System

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Keywords: Image Encryption, 7D Chaotic System, Simulated Annealing, Chaos-based Cryptography, Hyperchaotic System, Security Analysis.

Abstract: With the rapid escalation of cybersecurity threats, the demand for robust and efficient encryption techniques to secure sensitive visual data has become increasingly critical. Conventional algorithms such as AES and DES, although widely used, exhibit limitations when applied to digital images due to their high computational overhead and inadequate adaptability to the intrinsic characteristics of visual content. This study introduces a novel image encryption scheme grounded in chaotic systems, incorporating an optimized selection mechanism for system parameters and initial conditions to enhance both security and computational performance. Leveraging the inherent properties of chaotic dynamics - such as extreme sensitivity to initial states and pseudo-random behavior - the proposed approach achieves high levels of confusion and diffusion. Experimental evaluations confirm that the encrypted images exhibit uniform histogram distributions, effectively concealing visual structures and thwarting statistical analysis. Entropy measurements ranged from 7.9993 to 7.9998, indicating an exceptionally high degree of randomness. Furthermore, correlation analysis revealed a substantial reduction in adjacent pixel correlation, with values between -0.0003 and -0.0025, signifying strong decorrelation and noise-like behavior in the encrypted outputs. The method also demonstrated strong resilience against differential attacks, achieving a 100% NPCR (Number of Pixels Change Rate), underscoring its sensitivity to minor input alterations. UACI (Unified Average Changing Intensity) values ranged from 33.48% to 33.50%, highlighting the algorithm's effectiveness in uniformly diffusing changes throughout the image. In terms of efficiency, the proposed system outperforms traditional methods by offering reduced encryption and decryption times, rendering it highly suitable for contemporary digital environments where both security and performance are paramount.

1 INTRODUCTION

With the rapid advancement of digital communications, the instantaneous transmission of diverse data types - such as images, documents, speech, and video - over shared frequency bands has introduced a host of significant security vulnerabilities [1], [2], [3]. Digital images, in particular, are critical assets in various domains including information exchange, authentication, remote sensing, satellite imagery, medical diagnostics, and military operations. Their widespread use and inherent characteristics make them a prime target for cyberattacks, thereby necessitating robust cryptographic techniques to prevent unauthorized access [4], [5].

Encryption, as a core security mechanism, transforms digital content into an unreadable format that can only be reverted by an authorized party. This process is vital for preserving essential security attributes such as confidentiality, authenticity, integrity, and non-repudiation [6], [7]. However, the nature of multimedia files poses unique challenges: the large data volume, high redundancy, and strong correlations between neighboring pixels render conventional algorithms - like the Advanced Encryption Standard (AES) - inefficient, particularly in dynamic and resource-constrained environments [8], [9], [10], [11]. These limitations have spurred the exploration of alternative approaches better suited to the complex nature of image data. In this context, chaotic systems have emerged as a promising alternative for image encryption. Owing to their inherent properties - extreme sensitivity to initial conditions, pseudo-random behavior, and deterministic dynamics that paradoxically emulate randomness - chaotic systems naturally align with the cryptographic requirements for robust security [1], [12]. The inception of chaos theory in computational

systems, pioneered by Edward Lorenz in 1963 [13], catalyzed significant research into chaos-based cryptosystems over the past decades. These systems harness properties such as high data resilience, sensitivity to minute perturbations, and noise-like signals to achieve effective diffusion and confusion mechanisms, which are critical for safeguarding data [7], [8]. Furthermore, high-dimensional chaotic functions, such as those derived from the logistic map, have been shown to provide enhanced resistance against cryptanalytic attacks by significantly expanding the key space and complicating the underlying system dynamics [13], [11], [14].

In the current era of digital transformation, the need for secure and efficient cryptographic methods is more pronounced than ever. While traditional cryptographic techniques continue to serve as a foundational security layer, their inflexibility often limits their application in scenarios requiring dynamic and scalable protection frameworks. Recent studies have increasingly highlighted the potential of chaotic systems to bridge this gap, leveraging their unpredictability and acute sensitivity to initial conditions to overcome the inherent challenges of multimedia data encryption [15], [16], [17]. Early work in chaos-based image cryptography focused on obfuscation and diffusion mechanisms for concealing sensitive information. Notably, Fridrich introduced a general architecture for image encryption based on two-dimensional chaotic maps in 1998, influencing subsequent schemes [1]. Despite these advancements, the selection of system parameters and initial values remains acritical challenge. without a systematic optimization process, they often fail to meet the necessary sensitivity thresholds. Such suboptimal choices can lead to recurring patterns or vulnerabilities that undermine the security of the system [18]. Addressing this issue, Alvarez and Li (2006) emphasized the imperative of aligning the intrinsic properties of chaotic systems with the fundamental requirements of cryptography. They argued that the meticulous selection of initial values and system parameters is essential to maximize both obfuscation and diffusion during the encryption process [4]. In a similar vein, Chen et al. (2004) demonstrated that the use of multidimensional chaotic maps can significantly expand the available key space and increase system complexity, thereby effectively impeding quantitative analysis and differential attacks aimed at uncovering the original information [5].

To address the limitations inherent in existing chaos-based encryption schemes - particularly those stemming from arbitrary parameter selection and insufficient sensitivity - this work introduces a novel approach that combines a high-dimensional chaotic system with an automated parameter optimization mechanism. Specifically, we propose a new seven-dimensional (7D) autonomous chaotic system designed to exhibit complex dynamical behavior suitable for cryptographic applications. To maximize the randomness and unpredictability of the generated sequences, we integrate a Simulated Annealing (SA) algorithm for the systematic optimization of both system parameters and initial conditions. This hybrid methodology ensures that the generated chaotic sequences possess high Lyapunov exponents, entropy, and diffusion characteristics, thereby significantly enhancing the security of image encryption. Unlike prior approaches that rely on heuristic or manually tuned configurations, our method ensures a mathematically grounded, high-performance solution with resilience against statistical and differential attacks. The proposed system, therefore, represents a substantial step forward in designing robust and efficient cryptographic frameworks for secure image transmission in multimedia environments

2 RELATED WORK

Researchers focus on the chaotic systems of image encrypting and decryption processes.

One method integrates a 4D chaotic system with DNA coding. It involves two stages: first, pixel positions are rearranged using chaotic sequences; second, an XOR operation is applied between the scrambled image and DNA-coded data to produce the final encrypted image. The greatest entropy value is 7.9987, UACI of 33.0203 percent and NPCR of 99.6436%. N. N. Jasem and S. A. Mehdi [19] proposed a new cipher algorithm that exploits a hyper six-dimension chaotic system. The algorithm combines switching, randomization, XOR operations, diffusion in a number of phases to warrant robust cryptography. Shahna and Mohamed [19] proposed a grayscale image encryption method based on the Z-order curve and the Logistic Map. The image is first scrambled using the Z-order curve and then encrypted with a key stream generated from the Logistic Map. The method has an entropy of 7.9972, a UACI of 33.5124%, an NPCR of 99.6713%, and an encryption time of 0.52619 seconds. Budiman et al. [20] proposed an encryption scheme that combines two chaotic methods and two hash functions. The first approach relies on rotation and region-wise partitioning algorithms, which are

based on plaintext and hash keys, to provide local encryption for each region of the image. The second approach uses a logistic map to implement overall image encryption. The proposed model consists of two stages: in the first stage, the chaotic method is applied to each region of the image to achieve confusion, while in the second stage, it is used to achieve diffusion. However, the researchers did not specify the encryption time for the proposed method.

Li et al. [21] A novel chaotic map is presented, which is based on a real-time variable logistic map with a randomly chosen decimal. This chaotic mapping is used to encrypt images. Several simulations indicate that the novel encryption technique may produce a securely encrypted image with low time complexity. The greatest entropy value is 7.9979, while the highest values of UACI and NPCR are 33.47 percent and 99.62%, respectively. While encryption speed is 0.0386 (second).

3 PROPOSED METHOD

We propose a new chaotic-based encryption scheme that utilizes an optimal parameter selection mechanism to enhance security and performance. The proposed approach integrates a chaotic map with a rigorous selection process for initial values, ensuring robustness against statistical and cryptanalytic attacks. By leveraging the advantages of chaotic behavior, our method achieves high sensitivity, diffusion, and confusion properties essential for secure image encryption, As Show in Figure 1 and Figure 2.

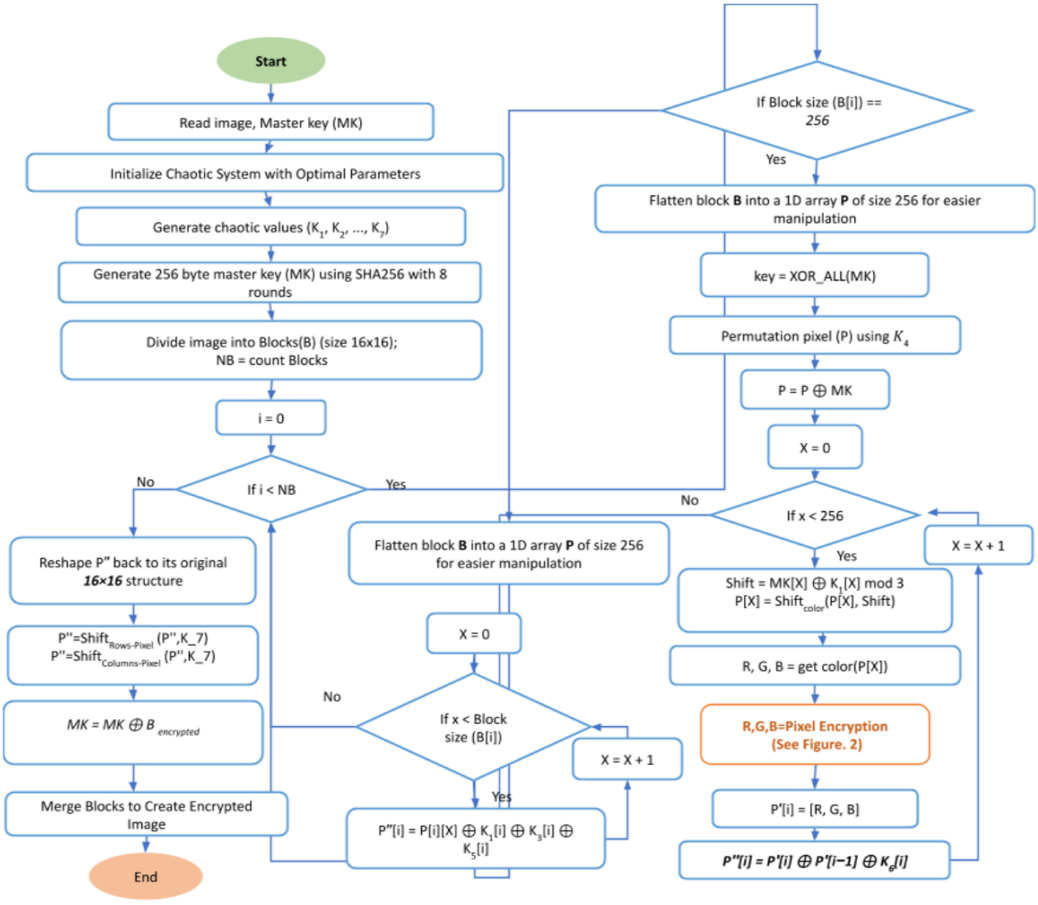


Figure 1: Detailed diagram of the proposed image encryption algorithm encryption algorithm.

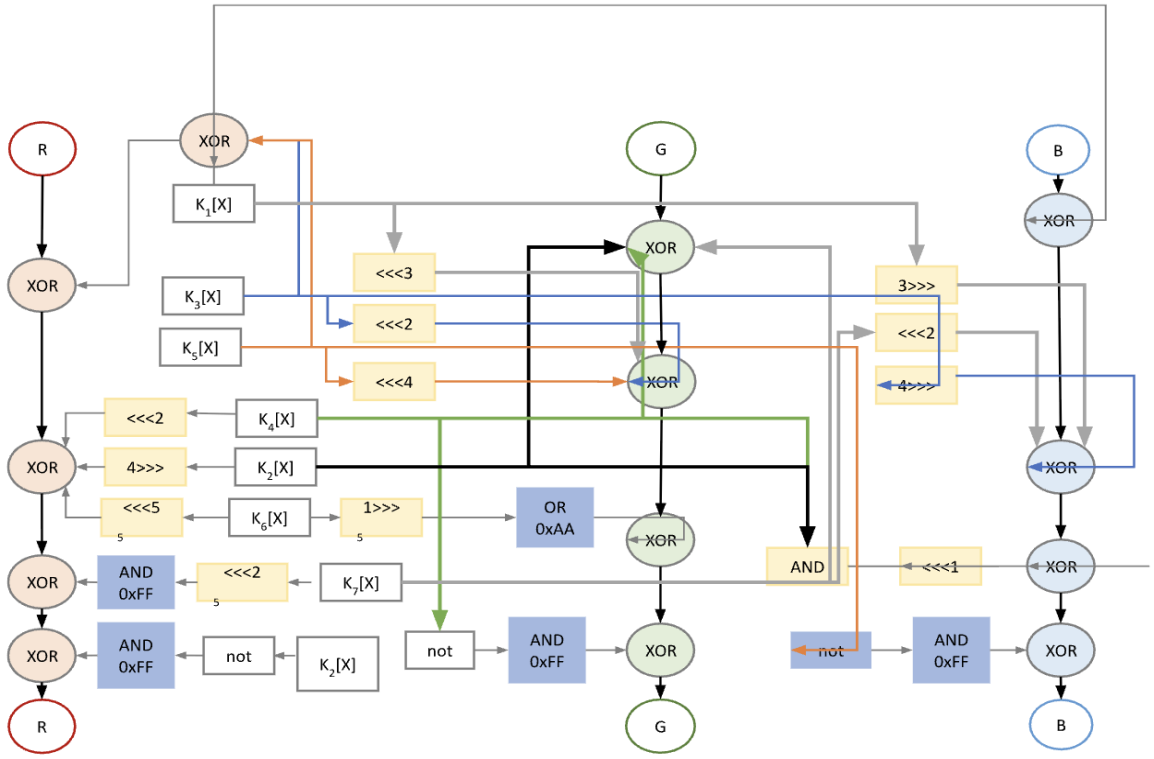


Figure 2: Schematic diagram of the pixel encrypting scheme of the proposed image encryption algorithm.

3.1 A New 7D-Chaotic System

The focal goal is to generate a mathematical model that represents a new chaotic system. The new 7D autonomous system is shown below (1):

$$\begin{aligned}
\frac{dx_1}{dt} &= -a\,x_1 + b\,x_2 + c\,x_4 - d\,x_3x_5 - x_3x_7 \\
\frac{dx_2}{dt} &= -e\,x_2 + f\,x_1 - g\,x_5 - h\,x_1x_3 + c\,x_3x_4 \\
\frac{dx_3}{dt} &= -i\,x_3 + d\,x_5 + g\,x_7 + x_1x_2 - x_2x_4 \\
\frac{dx_4}{dt} &= -x_4 + x_5 + d\,x_6 - j\,x_2x_3 - k\,x_5x_7 \qquad (1) \\
\frac{dx_5}{dt} &= -h\,x_5 + d\,x_1 - d\,x_3 + x_2x_7 + x_6x_7 \\
\frac{dx_6}{dt} &= -l\,x_6 - x_2 + x_5 + m\,x_1x_7 - n\,x_3x_4 \\
\frac{dx_7}{dt} &= -e\,x_7 + j\,x_3 - dx_5 + h\,x_1x_2 + x_4x_5.
\end{aligned}$$

Where the system statuses are $x_1, x_2, x_3, x_4, x_5, x_6, x_7$ and the system positive parameters are $a, b, c, d, e, f, g, h, i, j, k, l, m\ and\ n$.

The proposed system Eq.(1) exhibit a chaotic system based on a strange attractor when system parameter values are selected as:

$$a = 15, b = 13, c = 0.4, d = 0.5, e = 14, f = 38, g = 1.5, h = 2, i = 6, j = 3, k = 5, l = 15.1, m = 30\ and\ n = 4.$$

We take the initial conditions as:

$$x_1\,(0) = 5, x_2\,(0) = 0.7, x_3\,(0) = 3, x_4\,(0) = 10, x_5\,(0) = 0.2, x_6\,(0) = 0.1\ \ and\ \ x_7\,(0) = 0.6.$$

3.2 Optimal Generator for Chaotic Parameter

Simulated Annealing (SA) algorithm is used as one of the metaheuristic search techniques to find optimal solutions in nonlinear dynamical systems. In this work, SA is applied to optimize the parameters of a complex chaotic system, by optimizing chaos indices such as Lyapunov exponent, entropy, eigen variance, and space coverage.

The Simulated Annealing algorithm is based on the principle of gradual cooling of acceptance rates, so that random modifications that improve

performance or even some worse modifications are accepted according to a specified probability, which prevents stopping at local minimum. The main steps are:

A) Initialization of the system:
   1) Choose initial values for the system parameters and initial conditions.
   2) Calculate initial values for chaos indices.

B) Random update of parameters:
   1) Slightly modify the values of the parameters using a random distribution Uniform Distribution.
   2) Slightly change the initial conditions.

C) Evaluate chaos of the new system. Calculate chaos indices for the modification system (Lyapunov exponent, entropy, autocovariance, etc.).

D) Decision to accept or reject:
   1) If the new solution is better, it is adopted.
   2) If the solution is worse, it is adopted according to a probability that depends on the degree of cooling.

E) Iteration until reaching the minimum temperature or the maximum number of iterations.

3.3 Methodology

The Simulated Annealing algorithm is based on the annealing principle in materials science, where a material is gradually cooled to reach a more stable structure. In this context, this idea is used to optimize the parameters of a chaotic system by gradually searching for the best values that achieve maximum chaos and randomness.

A) Primary system:
   1) An initial chaotic system is created with default parameters and specified initial conditions.
   2) The chaotic solution of the system is calculated using its mathematical equations.
   3) The level of chaos is evaluated.

B) Improving transactions using Simulated Annealing (SA) Implementing the SA algorithm involves the following steps:
   1) Initialize the basic variables of the algorithm:
      - Set the initial temperature T=1.0.
      - Set the minimum temperature $T_(min\ ) = 0.00001$.
      - Set the cooling rate α = 0.9.
      - Set the maximum number of iterations max iter = 1000.
   2) Save the best initial solution based on the randomness of the system.
   3) Start the search loop for the best solution:
      - In each iteration, the system is cooled by updating the temperature value according to the equation:
      - $T = T \times \alpha$.
      - Randomly modify parameter values within a specified range:

$$new_{param} = \frac{old_{param}}{times(1 + r)}.$$

Where(r) is a random number in the range [-0.1, 0.1].

      - Randomly change the initial conditions of the chaotic system to maintain diversity in the search.
      - Evaluate the new chaotic system by calculating the chaos indices and checking whether the performance has improved.
      - Accept or reject the new solution based on the Boltzmann criterion:

$$P = e\text{^}(\Delta S/T).$$

Where ΔS is the difference between the randomness level of the new solution and the current solution.

      - Update the best solution if the new solution is better than the previous one.
   4) Stop the algorithm when the minimum temperature is reached or when the solution does not improve for a certain number of iterations.

3.4 Final Evaluation Results and Parameter Optimization

This section presents a comprehensive evaluation of the chaotic system before and after optimizing its parameters and initial conditions. The optimization process was carried out using the Simulated Annealing (SA) algorithm, aiming to enhance the system's chaotic behavior and randomness. as shown in Table 1.

1) Original System Parameters.Before optimization, the chaotic system was characterized by the following parameter values:
2) $a = 15,\ b = 13,\ c = 0.4,\ d = 0.5,\ e = 14,\ f = 38,\ g = 1.5,\ h = 2,\ I = 6,\ j = 3,\ k = 5,\ l = 15.1,\ m = 30,\ and\ n = 4.$
   The initial conditions of the system were set as follows:

$x_1(0) = 5, x_2(0) = 0.7, x_3(0) = 3, x_4(0) = 10, x_5(0) = 0.2, x_6(0) = 0.1, and\ x_7\ (0) = 0.6.$

3) Optimized System Parameters. Following the optimization process, the parameter values were adjusted to enhance the chaotic properties of the system:

$a = 17.8155,\ b = 13.3657,\ c = 0.4258,\ d = 0.4675,\ e = 13.2520,\ f = 48.7489,\ g = 1.4050,\ h = 1.8502,\ I = 6.3114,\ j = 3.2472,\ k = 5,\ l = 15.1000,\ m = 30,\ and\ n = 3.7520.$

The optimized initial conditions were determined as:

$$x_1\ (0) = 3.8997,$$
$$x_2\ (0) = 0.8618, x_3\ (0) = 2.5929,$$
$$x_4\ (0) = 8.8134,$$
$$x_5\ (0) = 0.1243, x_6\ (0) = 0.0768\ and$$
$$x_7\ (0) = 0.4294.$$

These modifications led to a more robust chaotic system, Lyapunov exponents, and overall randomness, as evaluated through the designated chaos metrics. The enhancement of these parameters signifies an increased sensitivity to initial conditions, which is a fundamental characteristic of chaotic behavior.

This table provides a comprehensive overview of the changes in the system after applying the Simulated Annealing algorithm to optimize the parameters of the chaotic system, highlighting the significant improvements in the properties of chaos and stability of the system:

- Positive Lyapunov exponent. This is one of the most important indicators for measuring chaos in a system; an increase of 412% indicates an improved ability of the system to generate complex and unpredictable dynamics.
- Larger Lyapunov exponent. Increasing this indicator indicates an increased sensitivity of the system to initial conditions, making the system more dynamically complex.
- Autocovariance. Increasing this measure shows that the system has become more stable in reducing redundancy and predictability in signals, which improves the quality of chaos used in encryption.
- Key coefficients (a, e, f, i). Large changes in these coefficients confirm their importance in determining the behavior of a chaotic system, as their changes have led to improved dynamic performance of the system.

Table 1: Results of applying SA algorithm to chaotic system.

Metric	Description	Results before optimization	Results after optimization	Comments
Total score	Aggregated measure of the system's overall chaotic behavior	2836.1739	14355.0730	An increase of approximately 412% indicates a significant enhancement in dynamic complexity.
Maximum lyapunov exponents	Reflects the system's sensitivity to initial conditions, representing the divergence rate	474.1663	2292.3390	A higher exponent suggests increased sensitivity and more intricate chaotic dynamics.
Sum of positive lyapunov exponents	Sum of all positive exponents, indicating the overall intensity of chaotic behavior	2800.0934	14351.3795	The substantial increase confirms a marked improvement in the system's chaotic intensity.
Autocorrelation decay rate	Measures how quickly correlations decay, signifying the reduction of periodic or repeating patterns	0.0072	0.0105	A faster decay rate suggests fewer repeating patterns, leading to more effective randomness in the system.
Phase space coverage	Evaluates the extent of state-space exploration, reflecting diversity in dynamic behavior	0.0194	0.0043	Lower coverage post-optimization could point to a more concentrated chaotic behavior, optimizing system output.

3.5 Image Encryption Algorithm Using the Optimized 7D-Chaotic System

The proposed algorithm relies on a multidimensional chaotic system to generate non-cyclic encryption keys, which adds a high level of security against conventional attacks, such as brute force and frequency analysis. To achieve higher encryption efficiency, the initial values of the chaotic system are optimized using the Simulated Annealing algorithm, the mechanism of which was explained in the previous section, where its results are used to initialize the chaotic system before starting the encryption process. The encryption process is based on dividing the input image into small blocks of size 16 * 16, so that each block is encrypted independently using different combinations of chaotic values. This approach aims to enhance the algorithm's resistance to various attacks while maintaining performance efficiency and increasing the complexity of reverse analysis of encrypted data. Figure 1 shows the detailed diagram of the proposed image encryption algorithm. The encryption scheme consists of several sequential stages to ensure a high degree of security and complexity in the image encryption process. The scheme relies on the use of chaotic systems to generate random keys and transform the image in a way that makes it difficult to decrypt without knowing the keys and details used.

3.5.1 Encryption Stage

The encryption process goes through several stages to ensure that the image is encrypted effectively. These stages include:

A) Initialization Stage:
   1) The original image is entered, its dimensions are analyzed and then converted into a one-dimensional pixel array.
   2) Inputting the master key.
   3) Initializing the chaotic system with the initial values resulting from the optimization process.

B) Image division stage into blocks: The image is divided into small blocks with dimensions of 16×16 pixels using Algorithm 1, which facilitates the implementation of chaotic operations and achieves a higher degree of security.

Algorithm 1: Divide Image into Blocks.

```
Input        I: Plain image.
             B: Block size (16×16).
Output  Blocks extracted from the image
Begin
Step1    Initialization: Extract image
dimensions:
                H ← Image Height
                W ← Image Width

Step2  Convert image to 1-Dimension:

Step 3  Count the Number of Blocks:
  ▪  Compute the total number of
     blocks
       NB = ((H * W))/((B * B))
Step 4  Divide Image into Blocks:
        For each block i = 1: NB
  ▪  Extract a segment of B × B pixels
     from the 1D image array.
  ▪  Store it as an individual block.
    End for
End
```

C) Encryption key generation stage:
Based on the values of the initial conditions and optimized parameters, seven chaotic sequences are generated using the proposed algorithm (N-7DHCS). The algorithm iterates the process of generating chaotic sequences $(x_(1,i,)\ x_(2,i), x_(3,i), x_(4,i),$

$x_(5,i), x_(6,i), x_(7,i))$. Each chaotic string is the same length as the original image dimensions (h × w), and these strings are converted into seven vectors $((1,i), K_(2,i), K_(3,i), K_(4,i), K_(5,i), K_(6,i),$

$K_(7,i))$ representing the chaotic sequence. Algorithm 2 shows the pseudocode for generating the chaotic key.

Algorithm 2: Key Generation.

```
Input   Initial condition: 〖x_1, x〗
_2, x_3, x_4, x_5, x_6, x_7
parameters: a, b, c, d, e, f, g, h, i, j, k, l, m and n
    Iterations = height * width //
image size
Output    Keys:
K_(1,i), K_(2,i), K_(3,i), K_(4,i), K_(5,i), K_(6,i), K_(7,i)
Begin
Step1 Initialize the chaotic system:
Introduce
       optimized initial values and
coefficients to the chaotic system.
Step2   Generating chaotic sequences
       For i = 1: Iterations
Use (3) to generate seven chaotic sequences
x_(1,i,) x_(2,i), x_(3,i), x_(4,i), x_(5,i), x_(6,i), x_(7,i)
     Takes the floating of
(x_(1,i,) x_(2,i), x_(3,i), x_(4,i), x_(5,i), x_(6,i), x_(7,i))
     and convert each value to
hexadecimal to Produce
(K_(1,i), K_(2,i), K_(3,i), K_(4,i), K_(5,i), K_(6,i), K_(7,i))
     K_i = convert to hex(x_i * 10^16)
       End for
Step 3  return K_1, K_2, K_3, K_4, K_5, K_6, K_7
End
```

D) Block-level encryption: This stage is one of the basic stages in the encryption algorithm, where each image block is processed after dividing the image into smaller units. It enhances security by applying several operations, including XOR with chaotic and master keys, shifting rows and columns, and rearranging pixel positions. These operations complicate the data structure, which contributes to hiding the original patterns of the image, thus increasing the difficulty of retrieving it without knowing the correct keys. Algorithm 3 presents the block cipher pseudocode.

Algorithm 3: Block Level Encryption Process.

```
Input    Block of pixels: B of size 16×16,
           Chaotic key stream: K
(generated using a 7D
             chaotic system),
             Master key: MK
Output  Block Encryption
Bigan
Step 1   Preprocessing Block:
      ▪ Flatten block B into a 1D array P
        of size 256 for easier
        manipulation.
      ▪ Normalize the MK-key values to the
        range [0,256]  to match pixel
        operations.
      ▪ key = XOR_ALL(MK)
Step 2   Chaotic Permutation:
      ▪ Use the chaotic sequence K4  (four
        part of K) to permute the pixels in
        P.
      ▪ Sort K4  to determine the new order
        of indices.
      ▪ Reorder P based on the sorted
        indices.
          Bitwise XOR Encryption:
        Perform a bitwise XOR operation
between each pixel
        and its corresponding chaotic mask:
        P = P ⊕ MK
        Shift = MK[i] ⊕ K1[i] mod 3
        P[i] = Shiftcolor(P[i], Shift)
        R, G, B = get color(P[i])
        R = R ⊕ K1[i] ⊕ K3[i] ⊕ K5[i]
        R = R ⊕ Circle shift left (K4[i],2)
⊕ Circle shift right
       (K2[i],4) ⊕ Circle shift left (K6
[i],5)
        R = (R + (Circle shift left (K7
[i],2) AND 0xFF)) AND
      0xFF
        R = R ⊕ (~K2[i]) & 0xFF
        G = G ⊕ K2[i] ⊕ K4[i] ⊕ K7[i]
        G = G ⊕ Circle shift left (K1[i],3)
⊕ Circle shift
      right(K5[i],2) ⊕ Circle shift left
(K3[i],4)
        G = (G + (Circle shift right (K6
[i],1) OR 0xAA)) AND
      0xFF
        G = G ⊕ (~K4[i]) & 0xFF
        B = B ⊕ K1[i] ⊕ K3[i] ⊕ K5[i]
        B = B ⊕ Circle shift right (K1
[i],3) ⊕ Circle shift
        left(K7[i],2) ⊕ Circle shift right
(K3[i],4)
        B = (B + (Circle shift left (K4[i]
AND K4[i],1) ) AND
        0xFF
        B = B ⊕ (~K5[i]) & 0xFF
        P'[i] = [R, G, B]
Step 3   Diffusion Layer: Apply a diffusion
operation to
         ensure that small changes in P
affect all pixels in P^'.
             P''[i] = P'[i] ⊕ P'[i-1] ⊕
K6[i]
Step 4    Rebuild the Block: Reshape P''
back to its original
             16×16 structure.
Step 5     Final Chaotic Mixing: Apply a
final mixing step
              using K7  (row and column
shifts based on chaotic
             values).
        P^'' = Shift_(rows − Pixel) (P^'',K_7)
       P^''' = Shift_(Columns − Pixel) (P^'',K_7)
Step 6      Update MK and Encrypted Block:
                        MK = MK ⊕ B encrypted
                        B_encrypted = P^''' ⊕
key
End          Return Encrypted block B encrypted
```

Algorithm 4 provides a detailed description of the proposed main algorithm for image encryption, which is based on a seven-dimensional chaotic system. This algorithm aims to achieve a high level of security by employing chaotic dynamics to complicate the encryption process, making it difficult to recover the original image without exact knowledge of the keys used.

Algorithm 4: Encrypted Image.

```
Input      Original Image (RGB),
             Chaotic System Parameters,
             Master key: MK

Output    Encrypted Image

Bigan
Step 1     Initialize Master key and Chaotic
System with
             Optimal Parameters
    ▪ Generate chaotic values (K1, K2, ...,
      K7) Using Algorithm (2)
    ▪ Generate Master key with size 256
      byte
          MK =   SHA256(MK, K1[1], K3[1],
              K5[1], K7[1])
          For i=1:7
```

```
                    MK =   MK + SHA256(MK)
Step 2    Divide image into Blocks(B) (size
16x16) Using
                Algorithm (1)
Step 3     Encrypt Each Block:
             For x = 1: count Blocks
             If Block size = 16x16
          Bencry [x]= encrypt Block[x] Using
    Algorithm (3)
            Else
           For i = 0: count pixel(B)
                Bencryp[x] = B[x][i] ⊕ K1[i]
⊕ K3[i] ⊕ K5[i]
Step 4    Merge Blocks to Create Encrypted
Image
End       Return Encrypted Image
```

4 RESULTS AND ANALYSIS

Table 2 shows the experimental results, which include the original image, the encrypted image, and the image after decryption. The data shows that the encrypted image has a high degree of randomness, which makes it impossible for attackers to extract any useful information from it, and thus the algorithm enhances the security level by completely hiding the visual patterns of the original image.

4.1 Histogram Analysis

To ensure encryption security, any statistical correlation between the plain and encrypted images must be eliminated. A uniform histogram distribution in the encrypted image indicates effective encryption [3]. As shown in Figures.3-5, the distinct histogram differences confirm the algorithm's ability to obscure statistical features and enhance security.

4.2 PSNR Analysis

The peak signal to noise ratio (PSNR) reflects the encryption quality. The lower value of PSNR is the better encryption quality. The PSNR formula are as shown in (2) [22]:

$$PSNR = 20\left(\frac{255}{\sqrt{MSE}}\right). \quad (2)$$

4.3 Entropy Analysis

Entropy is the significant characteristic that reflects information's randomness and unpredictability [22]. The entropy of the cipher image should be close to 8. The entropy of H (s) can be calculated as shown in (3):

$$H(s) = -\sum_(i = 0)\text{^}(N - 1)\, p(s_i)(s_i). \quad (3)$$

Where N denotes the number needed to represent the symbol s_i, s denotes the source, and P (si) is the symbol's probability[23].

4.4 Correlation Coefficient Analysis

The correlation coefficient of a visible image is one, but it is much lower for a ciphered image (almost equal to zero. Equation (4) calculates the correlation between original and ciphered pixel values.

$$Corr\,(x,y) = \frac{E[(x-\mu x)(y-\mu y)]}{\sigma x\, \sigma y}. \quad (4)$$

where μx and μy represent mean values of x and y, σx and σy are the standard deviations of x and y, and E [·] is the expectation function [22].

Table 3 presents the encryption analysis results for three images using PSNR, entropy, and correlation coefficient metrics. The findings show that the encryption process significantly alters the images, with high entropy values ensuring randomness and correlation coefficients near zero indicating strong security against analytical attacks.

4.5 NPCR and UACI Analysis

Two differential assault metrics are used to evaluate how vulnerable the original data is to slight alterations: (NPCR) Number of Pixels Change Rate as well as (UACI) Unified Average Changing Intensity. Suppose the enciphered image is (C and C') before and after changing one pixel in the original image [22]. The results of applying the proposed method on tested images are represented in Table 4. Equations (5) and (6) [24], express the NPCR and UACI formulas, respectively.

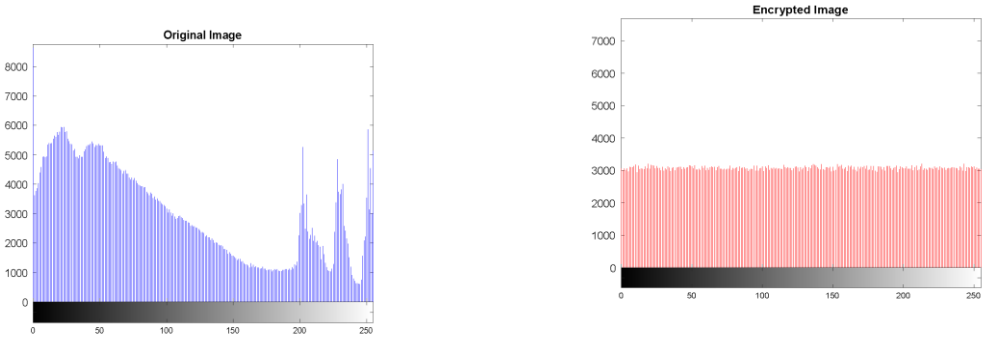
$$NPCR = (\sum_(i,j)\, D(i,j))/(W \times H)\ \times 100\% \quad (5)$$

$$UACI = 1/(W \times H)\ [(\sum_(i,j)\ |c(i,j) - c\text{^}'\,(i,j)|)//\ 255] \times 100\%. \quad (6)$$

The results confirm a complete image transformation during encryption, with NPCR at 100%, indicating all pixels are altered. UACI values range from 33.48% to 33.50%, reflecting significant intensity changes, enhancing security.

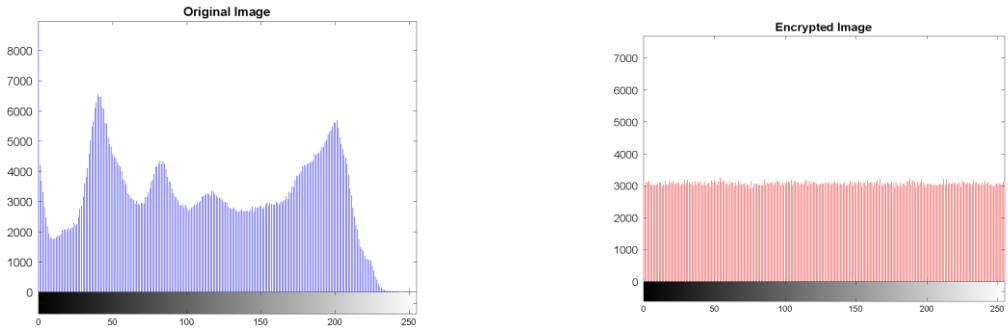
Table 2: Experimental results (encrypted and decrypted).

Image	Size	Original image	Encrypted image	Decrypted image
Panda	512 * 512			
Peppers	512 * 512			
Baboon	512 * 512			



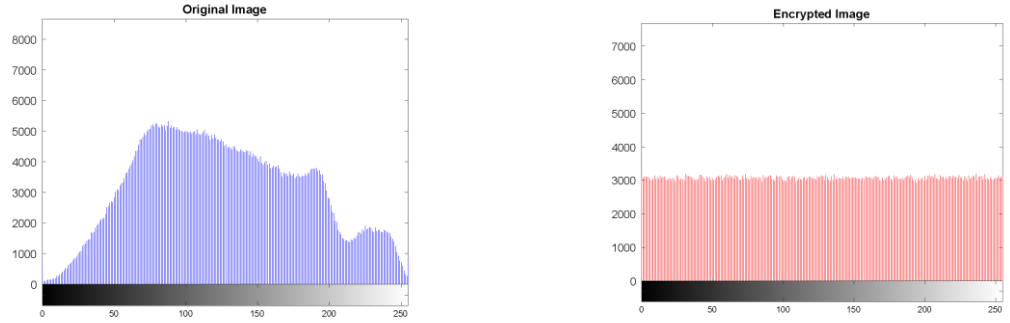
(a) Original (b) Encrypted

Figure 3: Histogram of the Panda image.



(a) Original (b) Encrypted

Figure 4: Histogram of the Peppers image.



(a) Original (b) Encrypted

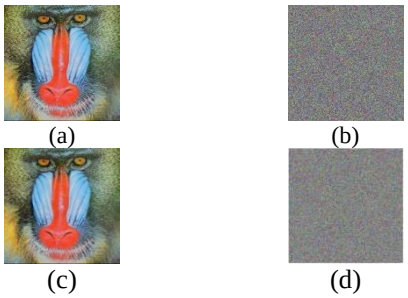
Figure 5: Histogram of the Baboon image.

Table 3: Encryption analysis results.

Image	PSNR	Entropy Original	Entropy Encryption	Correlation
Panda	7.37	7.5655	7.9993	-0.0003
Peppers	7.10	7.3558	7.9998	0.0009
Baboon	8.80	7.6557	7.9994	-0.0025

Table 4: NPCR and UACI results.

Image	NPCR %	UACI %
Panda	100	33.48
Peppers	100	33.50
Baboon	100	33.48



(a) (b)

(c) (d)

Figure 6: Key sensitivity results for slight variation in $x_1(0)$.

4.6 Key Space and Key Sensitivity Analysis

A secure encryption algorithm requires a sufficiently large key space to resist brute-force attacks, ideally exceeding 2^{128} [25]. The proposed algorithm utilizes a key space of $(10^{16})^{21} + 2^{11} \simeq 2\text{^}1127$, incorporating initial conditions values (x1(0) …, x7(0)) at a precision of 10^{-16} In addition to the master key, which is (2^{11}) 256 bytes in size, ensuring robustness against brute-force attacks. Key sensitivity analysis demonstrated that altering x1(0) from 3 to 3.00000000000000001 ($\approx 10^{-16}$) led to complete image recovery failure. This high sensitivity reinforces the system's security, preventing decryption with even minimal key variations. Figure 6 illustrate this sensitivity a) original image, b) encrypted image, c) decrypted using $x_1(0) = 3$, and d) decrypted using $x_1(0) = 30000000000000001$.

4.7 Waveform Analysis of the Novel Chaotic System

A fundamental characteristic of chaotic systems is their aperiodic waveforms. To verify the chaotic nature of the proposed system, Figure. 7 present time-domain plots of the state variables $x_1\,(t), x_2\,(t), x_3\,(t), x_4\,(t), x_5\,(t), x_6\,(t), x_7\,(t)$, obtained from MATHEMATICA simulations. The results confirm the aperiodicity of these waveforms, distinguishing the system from complex periodic motions and reinforcing its chaotic behavior.

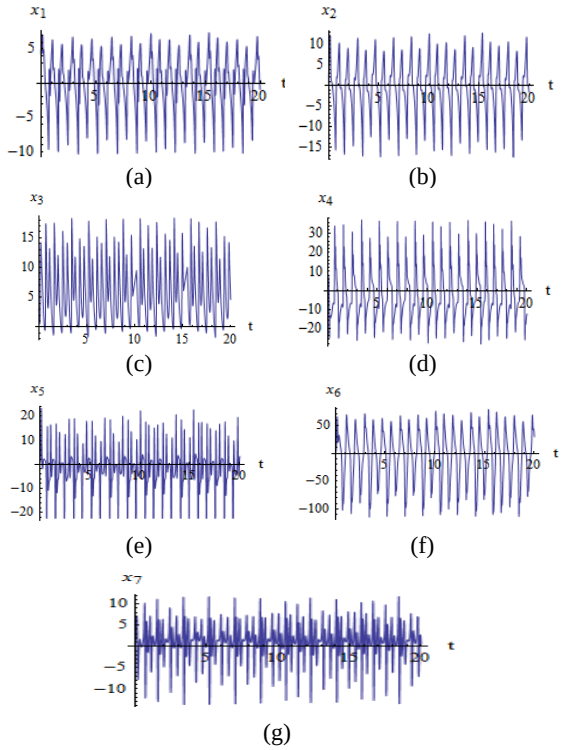


Figure 7: Temporal Analysis of the Chaotic System Variables (X_1 to X_7) Corresponding to Symbols a)–g).

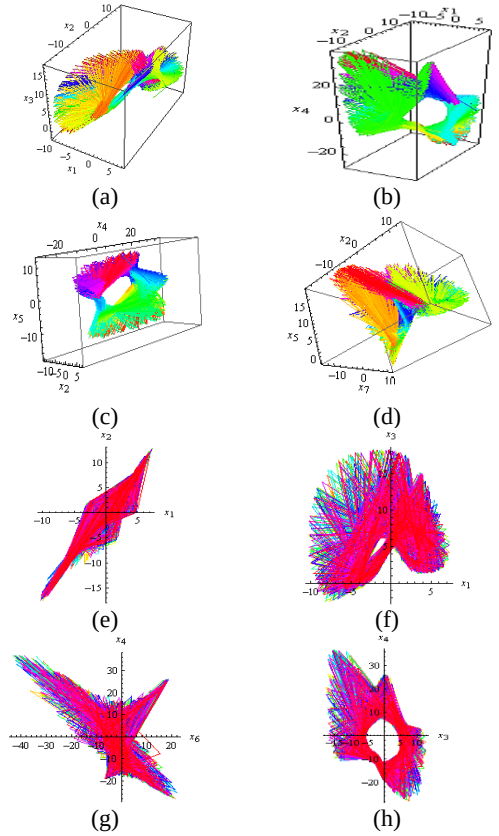


Figure 8: Chaotic attractors: 3D views (a) (x_1-x_2-x_3), (b) (x_1-x_2-x_4), (c) (x_4-x_5-x_2), (d) (x_2-x_5-x_7);and phase planes: (e) (x2-x1), (f) (x3-x1), (g) (x4-x6), and (h) (x4-x3).

4.8 Phase Portraits

Numerical simulations of the nonlinear system were performed using the MATHEMATICA program. The system exhibits complex and diverse chaotic behaviors. Three-dimensional strange attractors are presented in Figures 8a-8d, while two-dimensional attractors are shown in Figures 8e-8h. Notably, as observed in Figure 8a, the attractor's topology resembles the shape of a butterfly with flapping wings, leading to the concept of the "Butterfly Effect."

4.9 Bifurcation Diagram

The new chaotic system (1) [26] is numerically analyzed using Mathematica, where the bifurcation behavior of $\mathbf{X_1}$ is examined as parameter **a** varies. Notably, in the range a=14.2 to a=14.4, $\mathbf{X_1}$ exhibits bifurcation, indicating a transition in the system's dynamics. This property, a hallmark of chaotic systems, is confirmed in the proposed model, as illustrated in Figure 9.

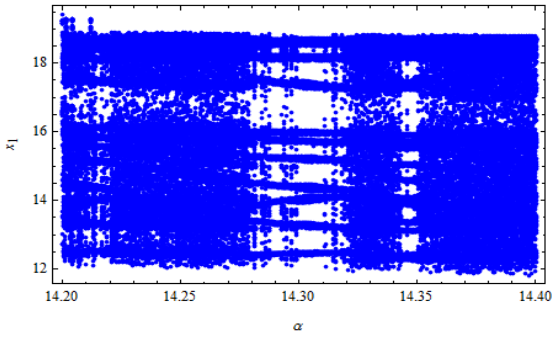


Figure 9: Bifurcation diagram of X_1 vs. a.

4.10 Comparison

Table 5 shows the performance evaluation metrics attained by our proposed method with those given in previous studies. Also, shows the proposed method given better results than methods in the previous studies. Thus, improved the efficiency of the proposed system.

Table 5: Comparison between the proposed encryption method and previous studies.

Measurement	Proposed	[19]	[27]	[28]
PSNR	7.10	7.3737	-	28.0397
Entropy	7.9998	7.9997	7.9974	7.2682
NPCR	100	99.600	99.604	99.6096
UACI	33.50	34.362	33.402	33.4599
Correlation	0. 0009	0.0409	0.0033	-0.0028

5 CONCLUSIONS

This research presented a novel image encryption system based on a seven-dimensional chaotic map integrated with an optimal parameter selection mechanism, notably using the Simulated Annealing (SA) algorithm. The proposed system demonstrated a high level of security, robustness, and computational efficiency through comprehensive theoretical and experimental evaluation. The system achieved near-ideal entropy values (up to 7.9998), indicating excellent randomness in the encrypted images. Correlation between adjacent pixels approached zero (e.g., 0.0009 for Peppers), suggesting significant decorrelation. The encryption scheme exhibited complete resistance to differential attacks, with NPCR reaching 100% and UACI around 33.50% across tested standard images (Panda, Peppers, Baboon). The SA optimization enhanced the Lyapunov exponent by 412%, confirming the increase in system chaoticity and encryption performance. The key space was approximately 2^{1127}, greatly exceeding the requirement to resist brute-force attacks. Key sensitivity analysis showed that minute changes (on the order of 10^{-16}) in initial conditions led to complete decryption failure, highlighting the system's high sensitivity and security strength.

This study makes a substantial contribution to secure image transmission by integrating a high-dimensional chaotic system with evolutionary optimization, resulting in a robust encryption scheme that can withstand a variety of attacks.

The proposed encryption approach can be applied in numerous sectors, including securing real-time transmission of sensitive images, protecting surveillance data, integrating into smart surveillance systems, and other applications.

ACKNOWLEDGMENTS

The Authors would like to thank Mustansiriyah University)www.uomustansiriyah.edu.iq) Bagdad-Iraq for its support in the present work.

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Mitigating Bias in Artificial Intelligence: Methods and Challenges

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Keywords: Bias Mitigation In AI, Algorithmic Fairness, AI Ethics, Fairness Metrics, Data Bias.

Abstract: The extensive application of Artificial Intelligence (AI) across the core domains of society has brought forth massive challenges towards prejudice, embedding discrimination, feeding inequalities, and eroding trust among citizens. This report explores the multi-dimensioned aspect of AI systems' prejudice by understanding the causes of the phenomenon in terms of data, algorithms, and end-user interface and also exploring its social implications and normative concerns. We give a comprehensive overview of existing state-of-the-art bias detection methods, i.e., statistical approaches, explainability tools, and fairness measures, and discuss mitigation techniques in pre-processing, in-processing, and post-processing. Challenges persist, such as negative fairness-accuracy trade-offs, limited standardized benchmarks, and need for inter-disciplinary efforts. Through case studies and regulatory analysis, we determine best practices and novel frameworks that will propel fair AI. The paper concludes by offering the directions of future research, emphasizing the necessity of open, transparent, accountable, and inclusive approaches to prevent AI systems from deviating from moral principles and societal values.

1 INTRODUCTION

Artificial Intelligence (AI) is beginning to pervade all aspects of society. From chatbots that help answer questions, to cars that drive themselves, and to lucrative algorithms that decide one's credit score, AI technologies are becoming unavoidable. AI is both very good at solving very specific problems when fed the right data and also very complex. Some contemporary AI systems such as deep neural networks or certain recommender systems can be beyond anyone's full understanding, including their creators. Naturally, many of the consequences of rapidly adopting such complex technologies are unknown. One critical, known drawback is the surprise realization that AI is not quite as rational as previously believed, and that it is open to embedding various societal recognized biases as exhibited by phenomena such as the Facebook ad-stereotype scandal, COMPAS crime prediction tool, the YouTube-optimized recommendation system and many others [1], [2], [3], [4]. Therefore, this prejudice of baked-in biases in AI systems has urged for the development of a field of AI fairness to quantify and mitigate such biases, and thus guaranteeing the ethical deployment and development of AI technologies. Just the right balance of foreshadowing of societal responsibilities and new technological questions is what makes embedded bias in AI such a compelling issue of inquiry for any researcher or practitioner in the AI field [5], [6], [7].

Recent high-profile cases have made it necessary to address AI bias. For instance, gender bias has been observed to happen in AI hiring tools, racial bias in facial recognition systems and recidivism risk assessment tools used in the criminal justice system. [8], [9]. This essay is dedicated to the discussion of bias in artificial intelligence, thereby highlighting different ways in which this bias can manifest. First, breastfeeding machines will be used as an illustration of how training data can embed and perpetuate gendered, cultural or social biases The narrative will then shift to biases in the organization of AI fairness research effort, namely the lack of regulations to incentivize technological solutions not just to individual fairness, and the untackled problems that might be discounting the potential of fairness as a concept to reshape certain societal dynamics. The

paper will go ahead and cover modern-day mitigation actions, including debiasing of datasets [10], fair algorithms construction [11] , and bringing ethics into consideration when constructing AI [12]. However, mitigating bias in AI is an intricate issue. It calls for evading technical, ethical, and societal challenges [13]. This article will also observe the issues with bias reduction, such as not being able to define and measure fairness precisely in different scenarios, the risk of trade-offs between model performance and fairness [14], and the challenge in addressing intersectional biases [15]. By providing a broad survey of the methods and challenges of mitigating AI bias, this paper seeks to contribute to the ongoing discussion on the design of more equitable and accountable AI systems. In addition to advancing technical understanding of bias reduction, our aim is to emphasize the need for an interdisciplinary strategy to consider the broader social impacts of AI deployment. As AI continues to revolutionize our world, the imperative to confront and mitigate bias becomes increasingly urgent. This paper aims to be an information source for researchers, practitioners, and policymakers in the pursuit of developing just and unbiased AI systems that can benefit society at large.

2 UNDERSTANDING BIAS IN ARTIFICIAL INTELLIGENCE

Bias in AI can be either intentional (explicit bias) or non-intentional (implicit bias) making it very difficult to, in many cases, differentiate bias from expected behavior [16], [17]. There are two primary considerations of the bias that is pertinent to understanding bias in an AI system. First, bias should be considered as a mirroring of a societal bias in an AI application, a techno-social mirror. In such a context, research on the bias of AI raises questions on how to understand and approach biases in society, and how to ask an AI application to address bias. Second, concerning the error of AI decision-making, it is important to recognize the origin of the bias as a feature in the design or data, and to take action to alleviate it systematically [18], [19]. To address bias in the design and use of AI applications, an understanding of the bias in a technological context is required. With this in mind, bias in an AI system would be framed such that it can provide useful conceptual insights for a community of researchers tormented by the implications of bias within this technology [20]. Table 1 shows the primary metrics of fairness [21].

Table 1: Primary metrics of fairness [21].

Fairness Metric	Summary
Statistical or demographic parity	Requires equal probability of positive predicted class across protected and unprotected groups
Disparate impact	Represents the ratio of the rate of positive prediction between protected and unprotected groups
Calibration	Requires similarity between probability prediction or risk scores and actual outcomes regardless of group
Predictive parity	Requires equal positive predictive values across protected and unprotected groups
Error rate	Represents the ratio of incorrect to total predictions compared among protected and unprotected groups
Equal opportunity	Requires that a preferred outcome is predicted equally across protected and unprotected groups
Equalized odds	Requires equal true-positive and false-positive rates between protected and unprotected groups

This is at the center of developing unbiased AI models. It is necessary to recognize the truth that bias may be unconsciously embedded in AI systems through training by way of the data on which it is trained. Data collection and data selection have much to do with how the presence of bias in AI systems can be determined. It is required to discover sources of bias and also potential impacts on AI decision-making in order to combat bias in AI systems [22]. Bias can be ingrained in various phases of the life cycle of AI development. As an example, there may be bias in training data that has been used for training AI systems. It will result in biased decision-making from AI systems whenever new data is fed into them. Overcoming and recognizing such biases is essential in the development of fair and ethical AI systems. The ways in which different sources of bias can impact AI systems need to be explored [23].

3 TYPES OF BIAS IN AI

Researchers have demonstrated the widespread and nuanced spread of technological bias. Biases inherently determine the quality of the predictions of all artificially intelligent systems, as long as they are based on learning algorithms and data sets [24]. The quality of the predictions of ML models depends strongly on the quality of the training data sets, so data must be of high quality and unbiased. The possibility of considering a system as unbiased is not tolerated, but a data set is considered unbiased if it trains models that predict the same quality, regardless of the input and output values included [25]. Table 2 enumerates the types of bias, providing a descriptive definition of each type together with an example.

Bias can have a wide variety of aspects and possible formations. In order to catch these different types, machine learning is described here for the types of bias that can occur with applications and best practices. Each of these types of bias is very different: they may have different causal effects and have different possibilities for countermeasures [26]. It shows that the quality of the data affects the performance of the model differently than the model fails to generalize in different ways and is only possible with specialized countermeasures. Categorized biases may have a direct bear model that does not match the quality predictions of the models on certain input values [27]. At the same time, the model that produced the data set on the other hand, the causal effects of the bias on the predictions of the submitted model may differ from the properties of the submitted model [28], [29].

In order to obtain models that make sound decisions based on their observations and adhere to moral norms, it will be necessary to obtain a model that predicts generalizes and is fair [30]. To that end, it is necessary to better understand the bias that the model makes predictable, and to learn countermeasures to prevent the successful general model of the bias modeling. Bias in AI and ML are beginning to find an increasing reassurance in a wide representation of the people and institutions [31].

Table 2: Common types of bias in ai systems.

	Type of Bias	Description	Example
1	Data Bias	Occurs when the training data is not representative of the population or use case	Facial recognition systems trained primarily on light-skinned faces performing poorly on dark-skinned faces
2	Algorithmic Bias	Stems from the choices made in designing and implementing AI algorithms	A resume screening algorithm favoring certain keywords associated with one gender over another
3	Interaction Bias	Arises from the way users interact with AI systems	Voice assistants struggling with accents or dialects not well-represented in training data
4	Historical Bias	Reflects past societal biases present in the data used to train AI	An AI-based hiring tool reflecting historical gender imbalances in certain professions
5	Sampling Bias	Results from non-random sampling of subgroups in a population	A medical diagnosis AI trained mostly on data from urban hospitals may perform poorly for rural patients
6	Confirmation Bias	The tendency to search for or interpret information in a way that confirms pre-existing beliefs	An AI content recommendation system amplifying users' existing views without presenting diverse perspectives
7	Automation Bias	The propensity to favor suggestions from automated decision-making systems	Over-reliance on AI-generated results without critical evaluation by human experts
8	Reporting Bias	Occurs when the frequency of events, properties, or outcomes in a dataset doesn't reflect real-world probabilities	An AI trained on news articles might overestimate the frequency of rare but newsworthy events

The possibility that widespread and understandable mechanisms are widely engaged in the data processing applications only increases this interest and the red flags continue to multiply. By and large, research and knowledge on bias in AI belong to a variety of fields and theoretical traditions: media scholarship, policy reports, sociology, artificial intelligence perspectives, computer science, commentary on algorithmic discrimination, etc. At the same time, the most extensive conversations on the topic tend to happen within disciplinary silos [32].

4 IMPACTS OF BIAS IN AI SYSTEMS

Public concern over AI bias and its societal and ethical consequences has grown in recent years. Many machine learning models inherently amplify discrimination, often reinforcing systemic biases, particularly in critical sectors like finance, healthcare, and law enforcement. The complexity of AI decision-making makes bias difficult to trace and mitigate, disproportionately impacting vulnerable groups [33], [34]. Moreover, the opacity of these systems erodes public trust, with fears of a global AI arms race compromising safety and accountability. Addressing these challenges requires interdisciplinary collaboration to ensure fairness, transparency, and ethical AI development [35].

5 ETHICAL IMPLICATIONS OF BIAS IN AI

Bias in AI systems has generated significant attention from the public and key stakeholders, as it may lead to various forms of discrimination. Indeed, there have been reported cases of AI bias in areas such as crime prediction, employment, and online advertising. A broadly defined bias is present whenever the modeled output deviates systematically from the desired output provided by experience, and one possible effect of bias is discrimination [36]. Despite the growing interest, managing bias in AI can be a challenging task. The causes of bias are diverse, and there is no universal way in which they can be examined in the AI systems [37]. Moreover, addressing bias and discrimination in AI systems is a cross-disciplinary challenge, and engaging with technical, legal, social, and ethical issues constitutes a complex endeavor. To address bias successfully, a better understanding of AI behavior and societal impact is needed. AI experts should work in unison with policy-makers, ethicists, and social scientists to raise public awareness, recognize the challenges faced by a variety of stakeholders, and develop special policies and programming ensuring fair AI [38].

6 METHODS FOR DETECTING BIAS IN AI

The growing consensus among policymakers, industry leaders, and the broader community highlights the urgency of addressing bias in AI. This has led to a surge in detection methods and fairness-enhancing techniques, though discussions on bias remain fragmented, particularly in evaluating these methods systematically. A rigorous assessment is essential to distinguish effective solutions from those needing refinement [39], [40]. Identifying the most important factors that contribute to bias is essential to understanding why bias occurs in AI systems, as illustrated in Figure 1.

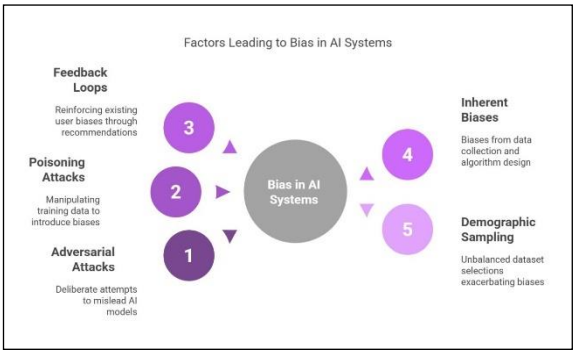


Figure 1: Factors leading to bias in AI systems.

6.1 Statistical Methods

This subsection explores statistical methods for detecting bias in AI, focusing on quantitative techniques to measure fairness. It examines how scholars and practitioners implement these methods, including metric selection, statistical modeling, and dataset choices. A framework of techniques is outlined, with case studies highlighting common pitfalls and the need for a more robust statistical approach. Visualizations are recommended to enhance clarity, along with guidelines for ethical and transparent statistical reporting [41], [42].

Understanding how bias manifests is the first step toward mitigation. Statistical methods, such as disparate impact analysis, help quantify fairness by

assessing whether protected groups face adverse outcomes. The severity of bias is often more relevant than its mere presence, requiring careful metric selection and comparison to baselines. Reporting should go beyond binary significance tests, incorporating intersectional analysis to avoid oversimplification. Combining visualizations with traditional statistical methods can improve communication and drive more informed decision-making [43], [44].

6.2 Explainability and Interpretability Techniques

AI systems are often opaque due to their complexity, making bias difficult to detect and correct. While this complexity has driven innovation, it also raises concerns about whether bias in AI reflects real-world patterns or stems from flawed training data and design choices. Without explainability, even experts struggle to assess an AI system's fairness and reliability [45].

Explainability and interpretability techniques are essential for understanding and controlling AI models. Research shows that transparency fosters trust and enables proactive bias detection. Various methods, such as permutation feature importance, local interpretable model-agnostic explanations, and Shapley values, summarize model behavior without directly replicating it. However, these explanations offer different levels of insight and may not fully capture a model's inner workings. Therefore, efforts should focus on building trust in AI decision-making rather than exhaustive analysis of every model detail [46], [47].

7 MITIGATION STRATEGIES FOR BIAS IN AI

The rise of AI has driven innovation across various fields, but its deployment in high-stakes areas like criminal justice and healthcare raises concerns about fairness and bias. AI systems can reinforce societal prejudices when trained on skewed datasets, leading to unfair or harmful outcomes, particularly for marginalized groups. This issue extends beyond faulty algorithms – bias can emerge even in properly functioning systems, amplifying existing inequalities [48].

Growing awareness of AI-induced harm has spurred research into bias detection and mitigation, particularly in models using electronic health record (EHR) data. This scrutiny extends beyond bias and discrimination to broader societal impacts, with increasing academic attention on AI's role in both public and private sectors. Understanding these consequences is essential as AI continues to shape critical decision-making processes [49].

7.1 Pre-Processing Techniques

Mitigating bias before training is often preferable to addressing it afterward. AI has the potential to drive innovation, but its deployment in social sectors demands rigorous fairness testing [50]. Bias arises when model predictions disproportionately benefit or harm certain groups, as seen in cases like gender bias in Newsela's quizzes. While eliminating bias is challenging, addressing it is essential to prevent harm [51]. Bias often originates in training data, where historically marginalized groups are underrepresented, increasing their risk of being affected by biased models. Datasets compiled from multiple sources and perspectives add complexity, making it difficult to quantify bias precisely. Research highlights the need to examine data, power dynamics, and bias to develop fairer AI systems [52].

7.2 In-Processing Approaches

Mitigating bias in AI across data collection, model development, deployment, and decision-making is crucial. In-processing approaches, particularly useful for off-the-shelf AI or when end-users are not developers, focus on real-time interventions such as filtering biased inputs and adjusting outputs. Two key strategies include incorporating fairness constraints and using regularization techniques during training to prevent data-driven biases from shaping decisions. Fairness constraints ensure that group differences do not influence model predictions, while regularization actively steers the training process toward fairer outcomes [53]. Various algorithms integrate fairness principles into AI models, though they differ in their conceptual approaches. Bias mitigation must extend beyond modeling to every stage of an AI system's lifecycle, requiring collaboration across stakeholders to understand how bias emerges and propagates. To effectively address bias, it is essential to rigorously define it, assess its impact, and establish monitoring frameworks. A decoupled model monitoring approach, centered on AI bias audits, is proposed to enhance transparency and accountability throughout an AI system's development and use [54].

7.3 Post-Processing Methods

Post-processing adjusts model outputs to enhance fairness without requiring retraining. Techniques such as re-weighting predictions, ensuring equal error rates, and calibration help balance outcomes across demographic groups. While these methods improve fairness, continuous post-deployment monitoring remains essential, as bias is inherent in many real-world problems, and even the best interventions involve trade-offs [55], [56]. Research on post-deployment fairness monitoring has grown, yet practical implementation remains underexplored. Figure 2 highlights the importance of addressing bias at all stages of machine learning, from problem definition through data collection and processing to model development, testing, and deployment. Ignoring these biases can lead to unfair or inaccurate models, which can impact the decisions based on them [57].

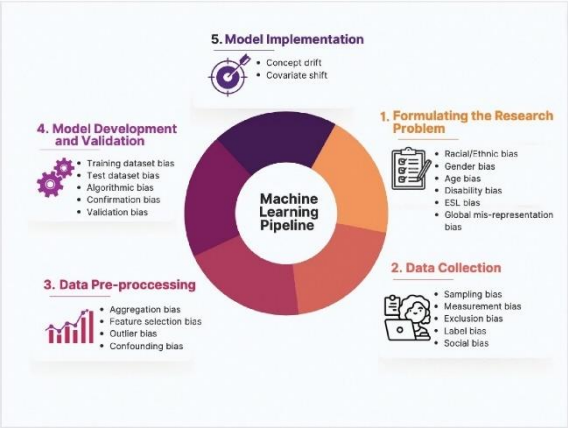


Figure 2: Sources of bias that may contribute to predictive variances of AI algorithms.

8 CHALLENGES IN IMPLEMENTING BIAS MITIGATION TECHNIQUES

Mitigating bias in AI and ML systems is a multi-pronged process that is plagued by several barriers at various stages of model development and deployment. These can be categorized under technical, ethical, organizational, and regulatory barriers [58], [59].

A) Technical Challenges:
1) Definition and Quantification of Bias: Bias is context-specific, and its meaning varies across domains and applications, and therefore difficult to standardize;
2) Data Limitations: bias generally results from imbalance or non-representative data, and it is difficult to get high-quality, unbiased data;
3) Algorithmic Complexity: mitigation of bias through fairness constraints or adversarial debiasing can increase the risk of model accuracy degradation or unintended side effects;
4) Scalability: bias mitigation is challenging and expensive on these large AI systems with dynamic, real-time data streams.

B) Ethical and Societal Challenges:
1) Trade-offs Between Fairness and Performance: balancing model performance and fairness may be contentious, as different stakeholders may have conflicting objectives;
2) Differing Notions of Fairness: different notions of fairness (e.g., demographic parity, equalized odds) may be incompatible with each other, so it is not sure which one would take priority;
3) Unintended Consequences: some mitigation techniques may introduce new biases or reduce transparency in AI decision-making.

C) Organizational Challenges
1) Lack of Incentives: businesses prefer performance and profitability to equity, deterring investment in attempting to combat bias;
2) Limited Expertise: companies do not possess the necessary expertise in fairness-aware ML techniques, and hence it is difficult to adopt them;
3) Resistance to Change: managers and programmers may resist altering existing AI systems since they perceive risks and costs involved in model changes.

D) Regulatory and Policy Challenges:
1) Lack of Uniform Regulations: regulatory frameworks for AI fairness are yet to be developed, leading to disparity in measures for bias evasion;
2) Challenging Enforcement: adherence to fairness standards is difficult to facilitate through the execution of robust auditing mechanisms, which is difficult for most organizations;
3) Variability Globally: cross-border AI systems experience complexity in bias mitigation with varying expectations on fairness across nations and sectors.

9 CASE STUDIES AND BEST PRACTICES

Best practices and case studies present information on how bias reduction in artificial intelligence is put into practice in the real world. Best practices and case studies are practical sources of information on how AI systems can be made transparent and fair by avoiding bias in them [60]. Case studies indicate successful strategies on bias mitigation in AI systems. They provide valuable information on the best practice of avoiding bias in AI. Case studies and real examples can offer realistic strategies and methods for identifying and minimizing bias in AI systems. For example, a case study of a facial over-representation system of some racial groups as errors can offer lessons about potential biases and how they can be resolved [61], [62]. In addition, the best approach to integrating ethical guidelines while designing and implementing AI can offer good lessons in minimizing bias. For example, observing how Google and Microsoft have managed bias in their AI software can demonstrate to one how to best keep bias at bay [63]. In the same way, observing case studies of how bias reduction in the application of AI in healthcare and finance can demonstrate to one lesson in how to implement best practice. For example, how bias has been addressed in AI algorithms for patient diagnosis and treatment recommendations in medicine can be utilized to establish useful bias mitigation methodologies [64]. By the introduction of an algorithmic-experimental combination of identifying, measuring, and suppressing the butterfly effect in AI systems the obstacles of bias are transcended to achieve fairness in outcomes and the emergence of responsible AI [65].

10 CASE STUDIES AND BEST PRACTICES

Regulatory frameworks and guidelines play an important role in preventing bias in artificial intelligence through standardization and specification of requirements and standards for transparency and fairness in AI systems. Regulatory frameworks and guidelines offer a context for detecting and solving potential biases in AI models and algorithms [66]. Regulatory frameworks and guidelines are important in upholding fairness and accountability in AI systems. Regulatory frameworks and guidelines offer a set of rules and standards to be abided by developers and users. These standards are central to the provision of the ethical and ethical use of AI technology. They offer best practices that corporations and organizations can adhere to in an effort to avoid causing harm to people or to society as a whole. These standards tend to entail recommendations for transparency, accountability, and fairness in AI systems [67]. Regulatory standards can also be used to apply the issues of data privacy and security to AI systems. For example, standards can include data encryption and storage protocol requirements [68].

11 FUTURE DIRECTIONS IN BIAS MITIGATION RESEARCH

There are numerous possibilities for future research on bias mitigation in the years to come. As new AI models are developed and applications change, it is imperative to continually innovate. New problems will arise, and existing issues will evolve. To meet these challenges, an interdisciplinary approach involving researchers, practitioners, and policymakers in a variety of areas will be necessary. Collaboration between technical experts, lawmakers, and domain professionals will be crucial in driving advancement. While some strategies can be broadly applied, each specific context may present its own unique challenges requiring bespoke solutions. Collaboration between computer scientists, social scientists, ethicists, and legal experts is well-suited to addressing these multifaceted difficulties [69].

There is an abundance of technologies which can be brought to bear on this complex issue. Natural language processing, deep learning, transfer learning, and reinforcement learning all present opportunities to develop improved methods of bias mitigation. However, it is important that the drive to utilize these advanced techniques does not come at the expense of interpretability and auditability. Other technologies could be used to explain model predictions and biases, thereby offering insight into how to generate more effective de-biasing strategies [70]. As the use of AI in decision-making processes becomes more prevalent, the availability of high quality, relevant data is increasing. There is potential to leverage this data to identify latent biases and develop strategies to address them. Changes in recruitment and hiring procedures, legislation regarding facial analysis technology, and concerns about profit-maximising businesses have generated momentum for using data-driven strategies to enhance the fairness of AI outcomes. Because of this, many novel methods have

been created over the past several years [71]. There are many analysis tools and strategies that use datasets to evaluate the fairness of a technique and decide how to alter it. It is anticipated that the use of data-driven innovation to address discrimination and bias in the years to come. Looking beyond the methodologies, it is crucial that AI researchers and developers keep an eye on emerging trends and prepare for future obstacles. Crucially, these future considerations must also include ethical concerns – the industry must ensure that these technologies have a fairness basis to be used in people's lives. With the development and use of AI having grown tremendously in recent years, the issue of bias and fairness is of ever-increasing importance. A wide range of innovations will therefore be necessary to move forward on this issue and help develop AI that is equitable [72].

12 CONCLUSIONS

AI technologies have the potential to replicate and exacerbate social inequalities present in the contexts from which they are developed and deployed. This presents significant societal issues in terms of the perpetuation of stereotyping, bias, prejudice, and ultimately discrimination and oppression. Beyond societal issues, the commercial bias of AI models could result in unfair treatment of agencies, sectors of the industry, or States. Therefore, it is necessary to reduce bias in AI models in order to fairly determine their impact on industrial and societal actors. Bias has disparate explanations depending on the context, and technical tools make the analysis and understanding of bias in data sets more straightforward. However, the clear understanding of the fairness implications of these biases and the solution of this problem is a challenge and an active area of research.

Decisions of AI models have an influence on the real world, and data about the real world is used to train new AI models. Thus, more biased decisions will lead to data more biased to train and thus a more biased generation of AI models. Therefore, it is necessary to mitigate bias in AI models, but this is challenging due to the large variety of possible biases and because of the difficulty of finding/predicting them. Moreover, this problem is particularly complicated taking into account the commercial bias of AI models and the growing associated societal concerns about fairness. For a fair determination of the bias/Fairness implications of decision J of agency A, the intended scope $\sim S\ A$ of decision J should be compared to the predicted scope $\sim S\ \mu$ of the reaction of rival M.

The reaction of the crop is the decision J of the proposed AI model. A model is trained to distort the competitive landscape in a profitable way. It models the competitive interactions between two agencies but is general enough to be applied to any actors. A simpler version can be solved numerically. Experiments show this simpler version can somewhat predict the competition for market share. More intermediates, such as faked explanations or mode-neutral perturbations, could help agencies currently out of direct competition with AI to better estimate the reactions of the AI models.

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DensNet121 and Improved Hippopotamus Optimization Algorithm to Diagnosis Thyroid Nodules

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Keywords: Densnet121, Thyroid Nodules, Hippopotamus, Benign, Malignant, Artificial Intelligence.

Abstract: The diagnosis of thyroid nodules remains a challenge due to the limitations of conventional imaging techniques. This paper aims to improve the accuracy and efficiency of thyroid nodule diagnosis. The proposed densnet121-IHOA model is a good solution to the diagnostic accuracy problem. The proposed model consists of a densely connected network to extract features from ultrasound images. Several layers are added to perform the diagnosis process based on the features extracted by Densnet121. The optimal hyper-parameters for learning rate, batch size, dropout ratio, and number of neurons were found using an optimization algorithm. The improved hippopotamus algorithm (IHOA) is efficient in finding hyper-parameters. The IHOA algorithm is robust in exploring and exploiting solutions to find optimal values, and it does not require a large number of iterations. The dataset used in this paper is AUITD. The number of images used in the paper was 2,121, divided into 1,697 training images and 424 test images. The proposed model achieved an accuracy of 97.7%, precision of 96.3%, recall of 98%, and F1 score of 97.4%.

1 INTRODUCTION

The thyroid gland is one of the most important endocrine glands in humans [1]. Thyroid diseases have become common, as it is considered the second most common disease in the world [2]. Studies have confirmed an increase in the incidence of this disease [3],[4]. In a study conducted in Iraq at the Hussein Cancer center thyroid nodules ranked tenth with a rate of 2.7% of the incidence rate [5]. From this standpoint, early diagnosis is of utmost importance to control the development of the disease and treat it in its early stages and avoid the need for surgical removal of the gland [6]. Among the most important known methods used to diagnose nodules is ultrasound imaging. It is inexpensive and, most importantly, does not cause harm to the patient [7], [8], [9]. Ultrasound images are usually used to diagnose thyroid nodules by experts, and these traditional methods require a lot of effort and time, and sometimes the diagnosis may be inaccurate [10]. Recently, machine learning and deep learning have been widely used to help detect and diagnose thyroid nodules [11], [12]. Convolutional neural networks, especially pre-trained networks, are the leaders in this field, but they require tuning of hyper-parameters to achieve the desired result [13],[14]. To solve the problem of tuning hyper-parameters for transfer learning networks, optimization algorithms were used to perform this task and facilitate the work.

2 RELATED WORKS

In [15], Alghanimi et al. presented a new model ResNet50-pca based on feature selection technique using principal component analysis (PCA) and ResNet50. In order to reduce the dimensions of the dataset and maintain the highest degree of variance between classes in order to improve the classification process. The dataset used in this study is publicly available in (kaggle) under the name AUITD. 800 images were taken from it to conduct this study, 400 images for each class.

Kuma et al. proposed in [16] to classify thyroid nodules, which is mainly based on the (Resent50) model, which is one of the models trained on (ImageNet) data from the transfer learning model group. The dataset used to train the model consists of 1600 ultrasound images taken from a hospital in Algeria. The accuracy achieved by the model

reached 96.2% compared to the model called (AlexNat) which achieved 83.59%.

Alghanimi et al. proposed a diagnostic model in [17] using feature extraction techniques by (CNN) and transfer learning network (Resnet50) to classify thyroid nodules. The data used in this study were taken from AUITD which consists of 800 ultrasound images, 400 for benign category and 400 for malignant category. The CNN model achieved an accuracy of 91.25% while Resnet50 achieved an accuracy of 89.37%.

In [18] Wang et al. presented a different approach to diagnosing nodules using ultrasound. They proposed a new method for diagnosing thyroid cancer, using deep learning. The model was designed as a network that integrates the features extracted from the input ultrasound images and merges them into a single scan by using different views to improve the pattern recognition efficiency. The data used in this study was very large, which increased the reliability of the model's performance. The accuracy achieved in this study was 87.32%.

In [19] Nguyen et al. presented a multi-CNN model to solve the data imbalance problem fundamentally. Because multi-CNN analyzes images and extracts deeper features compared to a single CNN model. The dataset adopted in this study is TDID imbalanced. The achieved accuracy was 92.05%.

In [20] Pal et al. presented a study on designing a model for early detection of thyroid disease. The proposed model was built based on a database from the CUI repository and machine learning techniques. Three machine learning models were used in this study, namely (KNN, DT, MPL) nearest neighbors, decision tree and multilayer perceptron to predict the disease. The best performance was MPL, which achieved an accuracy of 95.73%.

In [21] Zhang et al. presented a diagnostic model for multi-class thyroid disease diagnosis. The model architecture consists of a novel multi-channel convolutional neural network. This proposed architecture integrates the extracted feature maps, which leads to an increase in diagnosis accuracy compared to the traditional CNN. The data used are CT images from GitHub. The achieved accuracy of the model was 90.9%.

In [22] Pavithra et al. presented a study on the diagnosis of thyroid tumors based on deep learning and ultrasound image data. The residual network (ResNet), which is one of the deep neural networks, was used. This study aimed to classify nodules into six groups according to the TI-RADS classification. The model achieved an accuracy of 83%.

In [23] Jopate et al. proposed a technique for selecting optimal and distinctive features using the AEHOA algorithm, an elephant herd optimization algorithm, and also eliminating the limitations related to overlearning. Due to the imbalance of the data, the SMOTE technique was used to balance the data. The features obtained by the elephant optimization algorithm were fed into a CNN network for prediction and classification of the data. The data were downloaded from the UCI ML repository and also modified to increase the classification accuracy. This method achieved an accuracy of 88.2%.

In [24] Kumar et al. proposed an advanced model based on differential evolution (DE) with butterfly optimization (BOA) and fuzzy c-means algorithm to improve the classification of thyroid disorder. The proposed model (DEBOA-FCM) was evaluated by several metrics, and the accuracy achieved by the model was 94.3%. It is worth mentioning that the data was well processed and modified to achieve high accuracy.

In [25] Ma et al. presented an effective diagnostic model by developing a convolutional neural network and a nature-derived optimization algorithm. The DensNet architecture was modified to build the model and the flower pollination algorithm was used to adjust the learning rate. The data used in this study were (SPECT image) consisting of three classes and were divided into training and testing data. The results achieved by the model were excellent compared to previous studies on the same database.

In [26] Sharma et al. presented new techniques for extracting thyroid features from medical images. The feature extraction was based on image transformer and Deit techniques. Six different techniques were used to reduce the dimensionality of the data in order to avoid oversampling and prevent overlearning. The FOX optimization algorithm was used to select the best features for the data. Two different types of databases were used, and the accuracy achieved was high.

In this research [27] Hemapriya et al. presented a model for diagnosing thyroid nodules. Initially, the pre-processing of the data was based on isolated forest with data normalization to detect outliers and eliminate noise. The feature extraction process is done by AlexNet improved by (CSA) algorithm to distinguish the most accurate patterns. Then (AGTO) was used with hybrid feature selection under the name (HAGTEO) to identify the important features, which leads to reducing the dimensions. Then, the data classification process is done by using GRU based on the extracted features. This method achieved an accuracy of 98%

3 METHODOLOGY

In this section, the dataset, preprocessing, and model architecture will be defined (Figure 1 shows the model architecture for thyroid nodules diagnosis). The proposed model is an effective method for diagnosis thyroid nodules. The proposed model (Densnet121-IHOA), consists of a densely connected convolutional network with 121 layers (DensNet121) and is one of the transfer learning techniques in addition to using the improved hippopotamus optimization algorithm (IHOA) to adjust the hyper-parameters.

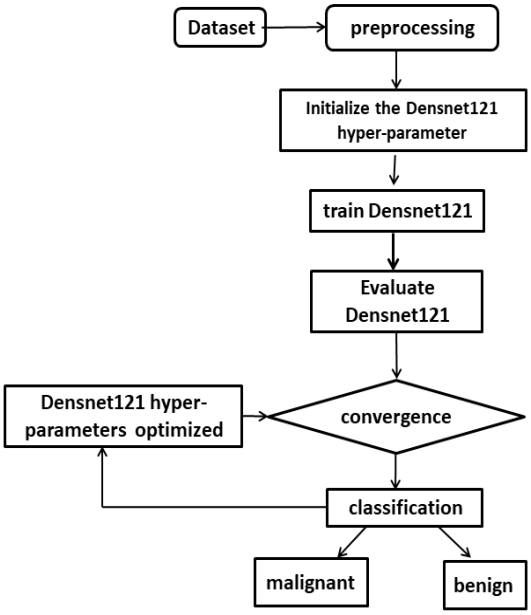


Figure 1: The proposed model.

3.1 Dataset

In this paper, we relied on the (AUITD) dataset from a hospital in Algeria. This data consists of ultrasound images of thyroid nodules. The dataset contains three categories, the benign category contains 1472 images, the malignant category contains 1895 images, and the normal or non-affected category contains 171 images. The dataset is divided into two files, training data and test data. The total number of available data that was downloaded is 2121 images, benign and malignant categories[28]. Figure 2 shows a sample of images of thyroid nodules in the two categories of malignant, benign.

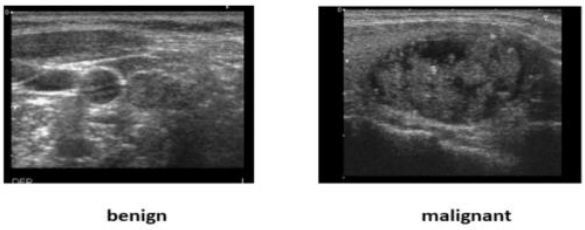


Figure 2: The sample malignant, benign.

3.2 Preprocessing

The data used in this paper have been processed to achieve real results. The processing process consisted of several stages.

1) At first, a specific cropping technique was applied to the image frame to get rid of only the dark frame and some details such as texts related to dimensions and device type, etc., if they remain, the model may consider them as a feature and learn them.
2) De-noise by applying the median filter. This filter is considered the best for De-noise while preserving the borders and shapes within the image.
3) At this stage, the images were resized using the scaling technique to dimensions 128x128 so that all images would be of equal dimensions and also to achieve compatibility between the model and the data.
4) At this final stage, the images were normalized to match the data with the model inputs.

3.3 Proposed Model

The proposed paper aims to achieve the highest accuracy in diagnosing thyroid nodules. The transfer learning technique (DensNet121) was primarily used to extract features, and custom layers were added to perform the diagnosis process. Meta-heuristic algorithms were used to fine-tune the hyper-parameters of the proposed model. The hyper-parameters that were optimized include the learning rate, batch size, number of neurons, and dropout rate. The Improved hippopotamus Algorithm (IHOA), an improved version of the original hippopotamus algorithm was used. A comprehensive overview of the hippopotamus algorithm and the improvements made to it to increase its effectiveness with the proposed model is presented.

3.3.1 Densnet

In this paper, densnet121 was used. It which consists of four blocks in the following order (6,12,24,16). Pre-trained networks or so-called learning transfer techniques have solved many issues related to medical image classification [29]. One of the most powerful learning transfer techniques in medical image classification is densely connected convolutional neural networks (DensNet) . This technique addressed one of the most important issues related to increasing the depth of the network, which is the issue of gradient or feature disappearance. In a DensNet network, each layer is connected to all the layers that precede it, and this is where its strength lies, as features do not disappear or are dropped, but are passed on to the next layer [30]. Figure 3 shows the structure of a DensNet121 and how its layers are connected to each other.

3.3.2 Hippopotamus Optimization Algorithm

The hippopotamus algorithm was proposed in 2024 by Amiri. This algorithm relies on the unique behaviors of hippopotamuses to find solutions to optimization problems. The unique behaviors of hippopotamuses, such as their constant changing of positions in rivers and ponds, their defense against predators, and the process of escaping from predator attacks, are considered. These three behaviors were converted into mathematical equations by the algorithm's proposer [31] .

HO population initialization phase Generating random initial solutions. A random initial solution vector is generated based on the following (1):

$$x_i: x_{ij} = lb_j + r.\left(ub_j - lb_j\right) \quad (1)$$
$$i = 1,2,\dots,N, j = 1,2,\dots,m \ .$$

Where Xi represents the positions, lb and ub represents lower and upper bounds, r random number, jth decision variable , N denotes the population size, M represents the number of decision variables.

$$X = \begin{bmatrix} \chi_1 \\ \vdots \\ \chi i \\ \vdots \\ \chi N \end{bmatrix}_{N\times m} = \begin{bmatrix} x_{1,1} & \cdots & v_{1,y} & \cdots & x_{1,m} \\ \vdots & \ddots & \vdots & \iddots & \vdots \\ x_{i,1} & \cdots & x_{i,y} & \cdots & x_{i,m} \\ \vdots & \iddots & \vdots & \ddots & \vdots \\ x_{N,1} & \cdots & x_{N,y} & \cdots & x_{N,m} \end{bmatrix}_{N\times m} \quad (2)$$

Stage 1. Hippopotamuses change their locations in water bodies and rivers (exploration). hippopotamus groups consist of adult males, adult female hippopotamus and young hippopotamus. The group has a leader called the dominant male who is determined based on the value of the objective function. One of the behaviors of hippopotamus is that they are close to each other. The dominant hippos protect the herd and the area of the group. Many females are placed around the male hippopotamus. When the male hippopotamus reach maturity, they are expelled by the leader of the herd (the dominant). The males that have been expelled from the herd either attract females or enter into dominance competitions with the dominant hippopotamus in the herd to prove their dominance. The mathematical representation of this stage is done through (3)

$$x_i^{Mhippo}: x_{ij}^{Mhippo} = x_{ij} + y_1.\left(D_{hippo} - I_{1x_{ij}}\right). \quad (3)$$

$X_i^{\boldsymbol{M}hippo}$ represents male hippopotamus sites.

D^{hippo} represents the dominant hippopotamus .

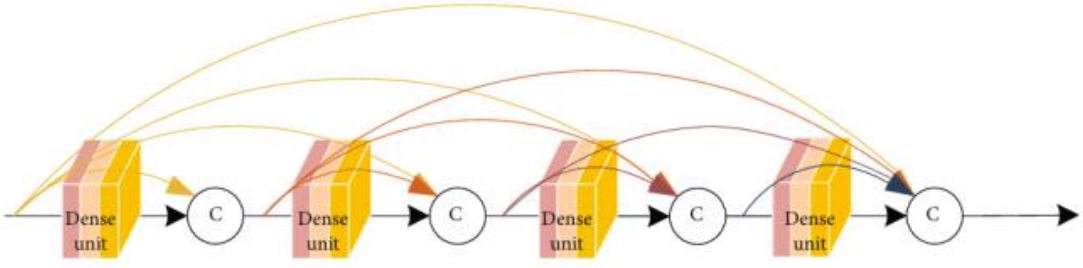


Figure 3: Structure of a DensNet121.

$$\mathcal{K} = \begin{cases} I_2 \times \vec{r}_1 + (\sim \varrho_1) \\ 2 \times \vec{r}_2 - 1 \\ \vec{r}_3 \\ I_1 \times \vec{r}_4 + (\sim \varrho_2) \\ r_5 \end{cases} . \quad (4)$$

I_1,I_2 is an integer between 1 and 2, $r_{1,2,3,4}$ is random vector in range 0 and 1 , r_5 random number ϱ_2, ϱ_1 integer random number 0 or 1

$$T = exp\left(-\frac{t}{T}\right). \quad (5)$$

$$x_i^{\mathbf{FB}\text{hippo}} : x_{ij}^{\mathbf{FB}\text{hippo}} = \begin{cases} X_{ij} + h_1.\left(D_{hippo} - I_2 MG_i\right) T > 0.6 \\ \Xi \qquad\qquad\qquad\qquad \text{else} \end{cases} . \quad (6)$$

Where in (6) - $x_1^{\mathbf{FB}\text{hippo}}$ represents the position of a female hippopotamus or an immature hippopotamus.

$$\Xi = \begin{cases} x_{ij} + h_2.\left(MG_i - D_{hippo}\right) r_6 > 0.5 \\ lb + r_7.\left(ub_i - lb_j\right) \quad else \end{cases} . \quad (7)$$

$$x_i = \begin{cases} x_i^{Mhippo} F_i^{Mhippo} < F_i \\ x_i \qquad\qquad\qquad else \end{cases} . \quad (8)$$

$$x_i = \begin{cases} x_i^{\boldsymbol{FB}ippo} F_i^{\boldsymbol{FB}ippo} < F_i \\ x_i \qquad\qquad\qquad else \end{cases} . \quad (9)$$

Stage 2. In this stage, the process of defense against predators (exploration) takes place. One of the most important reasons for hippos to live in groups is for their safety. The presence of hippopotamus in groups is a reason to prevent predators from attacking them or approaching their geographical area. however, immature hippopotamus may tend to separate from the herd and thus become easy targets for predators. Also, sick or old hippopotamus may meet their fate at the hands of predators. In this case, the hippopotamus is switched to defense mode by heading towards the predator, opening its large jaws, making sounds to scare the predator, and sometimes approaching the predator to encourage it to move away. In (10), the predator is placed in the search space.

$$Predator: Predator_j = lb_j + \vec{r_8}.\left(ub_j - lb_j\right), \quad (10)$$
$$j = 1, .., m.$$

$$\vec{D} = \left|Predator_j - x_{ij}\right|. \quad (11)$$

where D is the distance between the hippopotamus and the prey.

When the predator approaches the hippopotamus, the latter adopts a defensive behavior based on $F_{predator}$ to protect itself from the predator's attack. If F_i is greater than $F_{predator}$, this means that the predator is close to the hippopotamus' territory. In this case, the hippopotamus moves towards the predator to make it move away from the borders of the territory. If F_i is less than $F_{predator,}$ this means that the predator is within the hippopotamus' territory and is very close to it. In this case, the hippopotamus moves towards the predator to scare it away, but its movement is limited because intruder is very close to the hippopotamus (12).

Where in (12) - x_i^{HippoR} is the position of the hippopotamus facing the predator. RL is a random vector generated by the Lévy algorithm distribution used for sudden changes in predator positions during an attack. This process is done by (13). Where in (13) - v ,w is random number.

$$\mathcal{L}_{evy}(\vartheta) = 0.05 \times \frac{w \times \sigma_w}{|v|^{\frac{1}{\vartheta}}} \quad (13)$$

$$\sigma_w = \left[\frac{\Gamma(1+\vartheta)\sin\left(\frac{\pi\vartheta}{2}\right)}{\Gamma\left(\frac{(1+\vartheta)}{2}\right)\vartheta 2^{\frac{(\vartheta-1)}{2}}}\right]^{\frac{1}{\vartheta}} . \quad (14)$$

$$\chi_i = \begin{cases} \chi_i^{\mathcal{H}ippoR} \mathcal{F}_i^{\mathcal{H}ippoR} < \mathcal{F}_i \\ \chi_i \mathcal{F}_i^{\mathcal{H}ippoR} \geq \mathcal{F}_i \end{cases} \quad (15)$$

If it is hunted by a predator, it replaces the position of one of the herd members, otherwise it will return to its previous position.

$$\chi_i^{\mathcal{H}ippoR} : x_{ij}^{\mathcal{H}ippoR} = \begin{cases} \vec{RL} \oplus \mathcal{P}redator_j + \left(\frac{\ell}{(-\mathcal{K}\cos(2\pi\mathcal{g}))}\right).\left(\frac{1}{\vec{\mathcal{D}}}\right)\mathcal{F}_{\mathcal{P}redator_j} < \mathcal{F}_i \\ \vec{RL} \oplus \mathcal{P}redator_j + \left(\frac{\ell}{(-\mathcal{K}\cos(2\pi\mathcal{g}))}\right).\left(\frac{1}{2\times\vec{\mathcal{D}}+\vec{r}_9.}\right)\mathcal{F}_{\mathcal{P}redator_j} \geq \mathcal{F}_i \end{cases} \quad (12)$$

Stage 3. Escape (Exploitation). A hippopotamus behavior occurs when it encounters a predator that it is unable to confront defensively. The hippopotamus flees to a pond near its current location. New locations (ponds) are generated randomly using (17). This behavior protects the hippopotamus from predation (predators avoid entering the water). If the new location is more favorable than the current location (based on the cost function), the hippopotamuses location is updated using (19).

$$lb_j^{local} = \frac{lb_j}{t}, ub_j^{local} = \frac{ub_j}{t}, t = 1, 2, \ldots, T. \quad (16)$$

Where lb, ub It indicates lower and upper bounds t current iteration.

$$x_{ij}^{HippoE} = x_{ij} + r_{10,\cdot}\left(lb_j^{local} + \vartheta_{1,\cdot}\left(ub_j^{local} - lb_j^{local}\right)\right) \quad (17)$$

Where $X_{i,j}^{HippoE}$ New site.

$$\vartheta = \begin{cases} 2 \times \overrightarrow{r}_{11} - 1 \\ r_{12} \\ r_{13} \end{cases}. \quad (18)$$

Where s random numbers .

$$\chi_i = \begin{cases} \chi_i^{\mathcal{H}ippoE} & \mathcal{F}_i^{\mathcal{H}ippoE} < \mathcal{F}_i \\ \chi_i & \mathcal{F}_i^{\mathcal{H}ippoE} \geq \mathcal{F}_i \end{cases}. \quad (19)$$

F represent of the cost function.

3.3.3 Improved Hippopotamus Optimization Algorithm

The Improved process was done on the third stage of the hippopotamus algorithm. The Improved were made to the algorithm to improve the exploitation of the solutions discovered so far from the first and second stages instead of discovering new solutions. The process of improving the discovered solutions depends entirely on the best solution discovered so far. This technique has been applied in many optimization algorithms such as the PSO algorithm[32]. To gradually reduce randomness and increase the exploitation power in the final stages, the exponential decay technique was relied upon. Equations (16) and (17) in the Hippopotamus algorithm were replaced with the following new (20) and (21).

$$E(t) = e^{-\frac{t}{T}}. \quad (20)$$

t current iteration and T max iteration

$$x_{i,j}^{Hippo} = x_{i,j} + E(t)\left(x_{i,j}^{best} - x_{i,j}\right) + D. \quad (21)$$

where $D = (\text{rendom number})(0.01)$

Added a small noise D to improve local exploitation and avoid falling into local solutions.

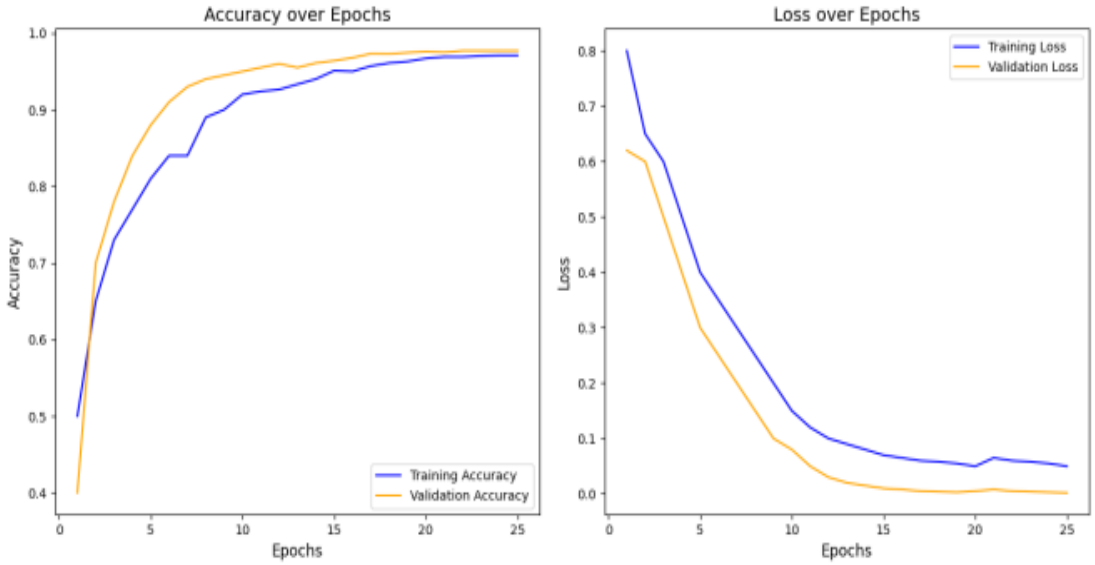


Figure 4: The performance proposed model.

4 EXPERIMENTAL RESULTS

In this section will show the results obtained from training the DensNet121-IHOA model on the AUITD dataset. The model code was written using python 3.11.0 with pycharm environment. The model was run on an HP laptop with Ryzen 7 processor, G32 RAM and Vega 4G graphics processor.

The AUITD dataset contains two classes, malignant and benign. The dataset is divided into 0.07% training, 0.01% validation and 0.02% testing to train and test the proposed model. Table 1 shows the training results before and after improved optimization algorithm. Figure 4 shows the accuracy curve and loss curve of the proposed model.

The training results in Table 1: showed the performance of the DensNet121-IHOA model, which achieved an accuracy of 97.7% compared to the Densnet121-HOA model before the hippopotamus Improved algorithm, where the achieved accuracy was 97.02%.

As for training the Densnet121 model without using the optimization algorithm (HOA) and (I-HOA), it achieved an accuracy of 96%, and this performance is low compared to the Densnet121-IHOA model. The reason for the low accuracy of DensNet121 is the collapse of the hyper-parameters tuning accurately.

The training results confirmed that incorporating optimization algorithms significantly enhances the model's accuracy, in addition to fine-tuning the model's hyper-parameters. As for the results obtained from the DensNet121-IHOA model, the accuracy increased by 0.069% compared to the Densnet121-HOA model.

Table 1: the training results.

model	Performance			
	Acc	Prec	Rec	F1
Densnet121-HOA	97.1%	97.3%	94.6	96.03
Densnet121-IHOA	97.7%	96.3	98.7	97.4

Comparison of the proposed model with other techniques based on the results. The proposed model achieved high accuracy with low loss ratio. The Densnet technique used for feature extraction was robust compared to other techniques. The Dense connections between the layers of the Densnet network ensured smooth flow of information and no feature loss across layers. Table 2 shows the results of the comparison with other techniques.

Table 2: Comparison of the proposed model with other techniques.

Authors	AUITD Dataset	Accuracy
[15] Alghanimi et al.	800 images	89.54%
[16] Kuma et al.	1600 images	96.20%
[17] Alghanimi et al.	800 images	91.25%
Proposed model	2121 images	97.7%

Figure 5 shows the confusion matrix for the test data. The reason for some misdiagnosis of images is due to the use of the sigmoid function to perform the classification process.

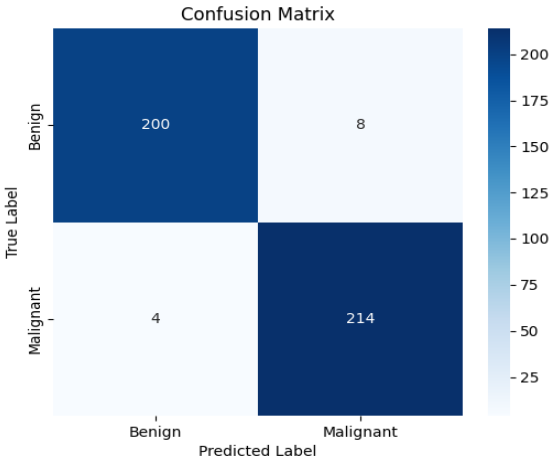


Figure 5: The confusion matrix of proposed model.

5 CONCLUSIONS

This paper presented a high-accuracy diagnostic model for thyroid nodules. Transfer learning was used in this paper to solve the problem of data unavailability. To address the problem of adjusting hyperparameters in transfer learning models, the (I-HOA) algorithm was used for tuning. Diagnosis by computer models has a great impact on the early recognition of diseases. Computer models provide immediate solutions and help in making early decisions to detect diseases and reduce their impact and spread in society. Expected future work includes collecting more data from specialized medical centers to improve the performance of the model. We hope to use the model in hospitals and centers specialized in detecting and treating tumors, as well as cooperate with specialists to develop the model to be more reliable in decision-making. The number of images used in the paper was 2,121, divided into 1,697 training images and 424 test images. The proposed

model achieved an accuracy of 97.7%, precision of 96.3%, recall of 98%, and F1 score of 97.4%.

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Alzheimer's Disease Detection Using Optimized Vision Transformer

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Keywords: Alzheimer's Disease, Vision Transformer, Hippopotamus Optimization Algorithm, OViT, ADNI Dataset.

Abstract: Alzheimer's disease (AD) is a complex, progressive neurodegenerative condition that affects millions of people worldwide, making early diagnosis critical for effective treatment and clinical management to improve quality of life. In this study, we present an automated classification framework based on the Vision Transformer (ViT) model optimized with a modified hippopotamus optimization algorithm (M-HOA). Unlike traditional models that rely solely on ViTs or convolutional networks, the M-HOA algorithm is used to fine–tune key hyperparameters of the ViT model, improving feature extraction and classification accuracy. The model was evaluated on the ADNI dataset, which covers three diagnostic categories (AD, MCI, and NC). Experiments demonstrated that the proposed M-HOA-ViT model outperforms both the baseline and optimized ViT architectures, achieving a classification accuracy of 97.90%. The results indicate that integrating metaheuristic optimization with ViT significantly improves diagnostic accuracy, providing a robust and scalable approach for the early detection of Alzheimer's disease.

1 INTRODUCTION

Alzheimer's disease (AD) is a leading cause of dementia, predominantly affecting older adults. Symptoms of AD include cognitive impairment , difficulty performing daily tasks, loss of speech, and memory loss [1].In the initial phases of Alzheimer's disease, memory impairment is moderate; however, in the latter stages, individuals lose the capacity to engage with their environment [2]. Research from the Alzheimer's Association predicts that the number of individuals afflicted with the illness will exceed 130 million by 2050. While there is currently no treatment for Alzheimer's disease, early identification helps mitigate its prevalence [3]. Moderate cognitive impairment (MCI), an initial phase of AD, affects approximately 4-20% of individuals aged 50 and above [4]. Since pharmacological interventions can only delay the onset of its severe stages [5], Early detection and preventive strategies play a crucial role in preserving autonomy and alleviating social and emotional challenges [6].

There are several brain scanning techniques to determine whether a person has Alzheimer's disease, such as Positron Emission Tomography (PET), Magnetic Resonance Imaging (MRI) [7]. However, MRI-based methods have gained importance by diagnosing subtle structural changes in the brains of affected individuals [8].

Machine learning and deep learning, two subfields of AI, have shown promise in solving issues that traditional methods cannot, and have greatly assisted in creating highly accurate systems[9]. Therefore, a computerized system to detect Alzheimer's disease in the early stages is essential. A number of deep learning methods, including the CNN, have been used and show good performance [10].

Recent advances in computer vision have prompted researchers to explore innovative architectures. The vision transformer (ViT) is one such new technology that has generated significant interest from researchers. The idea of vision transformers are inspired by natural language processing (NLP) transformers, which are notable for their ability to handle text sequences[11]. In the vision transformer model, images are interpreted as a set of fixed-size patches rather than a grid of individual pixels. In doing so, the ViT model can leverage self-attention mechanisms to efficiently capture global dependencies and long – range connections within an image. This capability is

particularly useful because accurate diagnosis depends on the ability to understand context across multiple parts of an image[12]. They have become popular in the domain for their capacity to comprehend significant global interrelations among variables in the input space [13].A comprehensive systematic review that included an analysis of 36 studies indicated that ViTs often outperform or achieve comparable performance to CNNs on medical image classification tasks. Especially when using pre-trained models or sufficient data[14].

To improve the vision transformer model's ability to diagnose, considering the lack of research on using a metaheuristic algorithm for Alzheimer's disease, this paper proposes a vision transformer-based model using a modified Hippopotamus Optimization Algorithm (M-HOA). The goal is to identify the most appropriate hyperparameters for ViT.

Each section of this paper follows a specific order. Section 2 covers previous studies on ViT, Section 3 presents the proposed methodology, explores the ViT, outlines the stages of Hippopotamus Optimization Algorithm, and discusses its modification. Section 4 presents experimental results, Section 5 presents challenges, Section 6 suggests directions for future work, and Section 7 concludes the paper.

2 RELATED WORKS

Saman Saraf et al. [15] introduced OVITAD, an improved architecture that reduces the number of parameters, input size, number of attention vertices, and layers compared to traditional ViT, to enhance efficiency while reducing complexity, without sacrificing the model's ability to extract relevant patterns. Using the ADNI database, the model achieved 99.55% accuracy for sMRI images and 97% for rs-MRI images, surpassing the majority of deep learning models by decreasing trainable parameters by 30% relative to the baseline vision transformer model.

Uttam Khatri et al. [16] presented an improved methodology for applying Vision Transformer (ViT) to a small MRI dataset. Their study focused on how to improve the performance of ViT when data is limited. They used Shifted Patch Tokenization (SPT) to improve information capture and reduce spatial bias, and CoordConv Location Encoding (CPE) to enhance the model’s ability to perceive spatial relationships between different parts. Their experiments on ADNI MRI data showed an accuracy of 92.30%. Nevertheless, Lack of comparisons with advanced models and computational efficiency analysis. Odusami et al. [17] introduce a vision transformer model, which integrates a multi-fusion of (PET and MRI) data. The images were analyzed using a directional wavelet transform (DWT) approach, their model achieved an accuracy of 93.75%. In order to confirm the generalizability of the model, their study proposed improvements to the fusion parameters, larger and more diverse datasets. In [18], Ramesh Chandra Punia et al. Suggested a combination strategy to improve the categorisation of Alzheimer's disease. That combines explainable artificial intelligence (XAI), transfer learning, and vision transformers (ViT). InceptionV3, VGG19, ResNet50, and DenseNet121 are pre-trained deep learning models that they used with ViT to provide a more accurate classification model. This resulted in an accuracy of 96%. However, this resulted in a high computational cost due to ViT being combined with multiple models, which increases computational and memory requirements. In [19], Yue Yin et al. proposed the SMIL-DeiT model, an approach that combines Vision Transformer (DeiT-S), Self-Supervised learning (DINO), and Multiple Instance Learning (MIL) techniques. To improve classification, used the ADNI dataset containing 2032 T1-weighted MRI images. The model achieved 93.2% accuracy, outperforming traditional CNNs. Fei Huang et al. in [20] proposed a new approach to Alzheimer's disease diagnosis using Monte Carlo ensemble vision transformer (MC-ViT), where the model relies on combining ViT and Monte Carlo random sampling to improve the classification process. This approach differs from traditional ensemble learning methods that use multiple models, as it relies on only one model, but generates multiple classification decisions by sampling different input images. Structural magnetic resonance imaging (sMRI) images are divided into 3D patches, and then features are extracted from them using a 3D convolutional neural network (3D Patch Network). Next, Monte Carlo sampling is used to pick out the most important features. These are then sent to the vision transformer (ViT), which figures out how these features are connected in space and makes the classification more accurate. The model was tested on two medical databases, ADNI and OASIS-3 achieved an accuracy of 90%. In [21], Anuvab Sen et al. Proposed using Vision Transformer (ViT) with metaheuristic optimization algorithms such (DE, GA, PSO, and ACO) to improve Alzheimer's disease classification from MRI images. They used the ADNI dataset. The differential evolution (DE) based model achieved the best accuracy of 96.8%, outperforming

previous models. However, the data size was very small. Mohammed et al in [22]. Presented an innovative concept called the binary Vision Transformer (BiViT). This model combines parallel coding (PCE) and latent representation fusion (MLF) approaches to enhance case recognition accuracy using 2D images, enabling it to extract spatial and semantic features from multiple trajectories simultaneously. Their model demonstrated outstanding performance with an accuracy of 96.38%. However, the study compared only the CNN model, without evaluating performance against other transformer models. This is a limitation of the evaluation and calls for expansion in future studies.

3 METHODOLOGY

This paper presents a comprehensive framework based on deep learning techniques for diagnosing Alzheimer's disease. In this context, the proposed approach and the model architecture used in the classification process are detailed (Figure 1 illustrates the complete framework).

3.1 Dataset

The ADNI initiative is a pioneering research project launched in 2004 with the participation of prestigious academic and research institutions, providing a comprehensive registry of high-quality and accurate data[1]. The dataset used include images from samples from Alzheimer's, divided into three categories, the Table 1 shows the demographic and clinical distribution of three groups of participants: data include the number of individuals in each group, mean age, as well as mean scores on the CDR (Dementia Severity Rating) and MMSE (Mini-Mental Examination) tests.

Table 1: Details about the participants' demographics.

Group	Alzheimer's Disease (AD)	Normal Control (NC)	Mild Cognitive (MCI)
Number	610	1297	895
Male/Female	365/245	710/587	615/304
Age	57.±8.15	65.73 ±8.12	67.9 ±9.8
CDR	5.4±2.6	0.1±0.3	1.6±1.1
MMSE	20±4.30	29.17 ±1.22	27.3 ±3.71

[1] http://adni.loni.usc.edu

3.2 Image Preprocessing

We used preprocessing techniques to improve data quality and ensure consistency with the ViT model. A series of preprocessing steps was applied to brain MRIs. These steps aimed to improve image quality, standardize input dimensions for consistency with the ViT model, expand the dataset to improve generalization, and prepare it for efficient model training and evaluation. The steps are as follows:

1) Start
2) Background Removal Outside the Active Brain Region
3) Input Brain MRI Images
4) Scale Images to 224 x 244 Pixels (Linear Interpolation)
5) Normalize Pixels to [0,1] and Standardize with ImageNet21k Standards
6) Data Augmentation: Horizontal Flip (8%), Brightness/Contrast, Adjustment (±2%)
7) Data Splitting: 60% Training, 20% Validation, 20% Testing (Balanced Random Distribution).

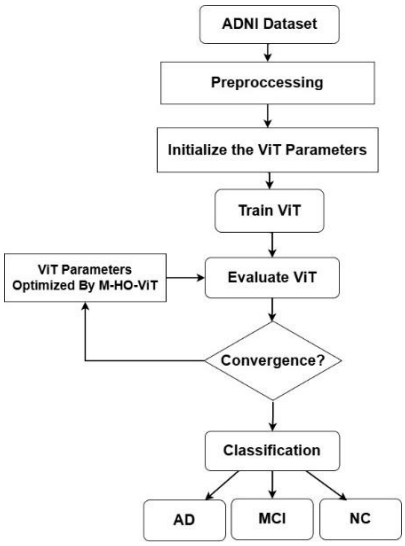


Figure 1: The structural diagram of the proposed model.

Figure 1 illustrates the steps involved in classifying Alzheimer's disease using ViT. The process starts with the ADNI dataset, followed by preprocessing steps to improve image quality and prepare it appropriately for the model, then training and optimizing ViT parameters using the M-HOA-ViT algorithm, and finally classifying the case.

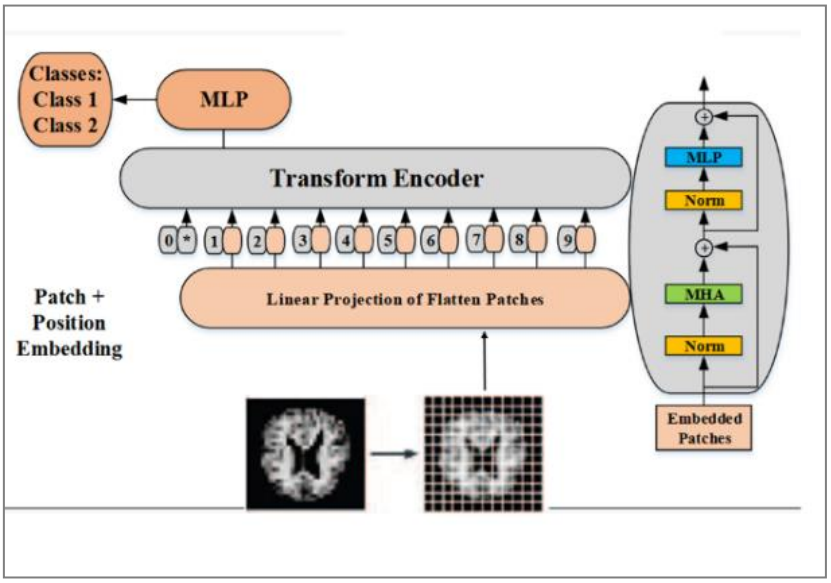


Figure 2: ViT model architecture.

3.3 Proposed Model

The proposed model is built on the Vision Transformer (ViT) architecture, which has proven remarkably successful in many computer vision tasks.

Metaheuristic algorithms are used to discover the optimal set of hyperparameters for the classification task, improve the model's accuracy. This integration aims to improve the model's efficiency, accuracy, and generalization ability. A comprehensive overview of the HOA algorithm, the ViT, and their optimization strategies is presented below.

3.3.1 ViT Model

The Vision Transformer is a modern image processing model based on the Transformers architecture originally developed for text processing [11]. An illustration of the overall structure is presented in Figure 2.

The model initially divides the images into small, uniform parts known as patches. These patches are then converted to digital representations (vectors) using a linear projection layer with positional information embedded in each patch to indicate its location within the image. The vectors are then transferred to the transformer encoder layer, which includes several sub-layers, a normalization layer (Norm), a multi-head attention (MHA) mechanism that helps understand important relationships between patches, and a multi-layer neural network (MPL) that extracts deep features from the image [12]. This method contributes to acquiring global contextual knowledge, which enhances its performance on complex tasks [23]. The diagram depicts the overall framework of the model.

3.3.2 Hippopotamus Optimization Algorithm (HOA)

Mohamed Hussein Amiri et al. In 2024 proposed a novel metaheuristic algorithm called Hippopotamus Optimization Algorithm (HOA) was proposed, inspired by the behaviors of hippopotamus in nature [24]. The algorithm consists of three main stages: exploitation, migration, and exploration.

This algorithm was chosen because it provides a balance between global and local search, achieves excellent experimental performance, and is well-suited for hyperparameter optimization problems in complex environments. Comparisons with other optimization algorithms have also demonstrated its superior performance:

A) Algorithm initialization: The algorithm starts by randomly initializing hippo locations, where these locations represent potential solutions to the optimization problem.

$$X_i: x_{i,j} = lb_j + r \times (ub_j - lb_j), i = 1,2,\dots,N; j = 1,2,\dots,m. \quad (1)$$

x_i refers to the position of the candidate solution $i\,th$ in the search space, where r is used as random number generated (between the range

0,1) to ensure random distribution within specified range of limits, while ub_j, lbj denote the lower and upper bounds for the $j\ th$ variable. n denote the population size (number of hippopotamuses in the herd), and m represent the total number of decision variables. Therefore, this information is then used to compile the population matrix according to (2).

$$x = \begin{matrix} x_1 \\ \vdots \\ x_i \\ \vdots \\ \lfloor x_N \rfloor_{N\times m} \end{matrix} = \begin{matrix} x_{1,1} & \cdots & x_{1,j} & \cdots & x_{1,m} \\ \vdots & \ddots & \vdots & . & \vdots \\ x_{i,1} & \cdots & x_{i,j} & \cdots & x_{i,m} \\ \vdots & . & \vdots & \ddots & \vdots \\ \lfloor x_{N,1} & \cdots & x_{N,j} & \cdots & x_{N,m} \rfloor_{N\times m} \end{matrix} . \quad (2)$$

B) HOA as a mathematical model the simulation of the hippopotamus movement within the HOA is divided into three phases according to their natural patterns [24].

3.3.2.1 Phase 1: Exploration (Updating Hippopotamus in the Pond)

A hippopotamus herd is composed of several adult females, juveniles, multiple males, and a dominant male who leads the herd. The dominant male is selected based on the objective function's value. Hippopotamuses tend to cluster together, with the dominant male safeguarding the herd and its territory from potential dangers. The spatial position of male Hippopotamuses in the lake is represented mathematically by (3).

$$\chi_i^{\mathcal{M}\ \text{hippo}}: \underset{xi_j}{\text{M hippo}} = x_{ij} + y1 \cdot \left(\mathcal{D}\ \text{hippo} - I_1 x_{ij}\right) \quad (3)$$

In (3), xi^{mhippo} shows where the male hippo is and $Dhippo$ shows where the dominant hippo is. In this case, $Dhippo$ has the lowest cost in the current iteration. The random vector between 0 and 1 is denoted by $\overrightarrow{r_1}$,…,$\overrightarrow{r_4}$, $\overrightarrow{r_5}$, according to (4). The values I_1 and I_2 are integers between 1 and 2 (according to (3) and (6)). While in (4), e_1 and e_2 represent random integers that can be 0 or 1:

$$\curvearrowleft = \begin{matrix} I_2 \times \vec{r}_1 + (\sim \varrho_1) \\ 2 \times \vec{r}_2 - 1 \\ \vec{r}_3 \\ I_1 \times \vec{r}_4 + (\sim \varrho_2) \\ r_5 \end{matrix} , \quad (4)$$

$$T = \exp\left(-\frac{t}{Max_{\text{iterations}}}\right). \quad (5)$$

$$X_i^{FBhippo}: x_i^{FBhippo} = \quad (6)$$

$$= \begin{cases} x_{ij} + h_1 \cdot \left(D_{\text{hippo}} - I_2 MG_i\right) & , T > 0.6 \\ \Xi & , \text{else} \end{cases} .$$

$$\Xi = \begin{cases} x_{ij} + h_2 \cdot \left(MG_i - D_{\text{hippo}}\right), r_6 > 0.5 \\ lb_j + r_7\left(ub_j - lb_j\right), \text{else} \end{cases} . \quad (7)$$

In (6) and (7), express the location of female hippo or immature calves ($x_i^{FBhippo}$) in the herd. Hippo calves often stay close to their mother, but due to curiosity, they depart from the herd or their mums. if the T value is more than 0.6, it indicates that the calf has moved away from its mother according to (5). if r_6 An arbitrary value if the value ranging between 0 and 1, if (7) exceeds 0.5, it means that the hippo has moved away from its mother, but is still in or close to the group. This behavior is modeled for females and immature hippos using (6) and (7). The value (h_2 and h_1) Numbers are randomly chosen from five scenarios. While r_7 in (7) is a random number between 0 and 1, (8) and (9) represent an update of the positions of male and female hippos, or immature hippos, within the herd, while F_i represents the value of the objective function.

$$X_i = \begin{cases} X_i^{Mhippo} F_i^{Mhippo} & < F_i \\ & \text{else} \end{cases} \quad (8)$$

$$X_i = \begin{cases} X_i^{FBhippo} F_i^{\text{FBhippo}} \\ X_i \end{cases} \quad (9)$$

Using h vectors with scenarios $I1$ and $I2$, the algorithm improves the overall search process and increases the exploration efficiency, leading to better results.

3.3.2.2 Phase 2: Explore (the Hippopotamus' Defense Mechanism Against Predators)

A hippopotamus is also at risk. Quickly turning towards the animal and making loud noises scares it away is their main defence. (10) represents where the attacker is in the search space.

$$predator_j = lb_j + \vec{r_8}.\left(ub_j - lb_j\right), j = 1,2,\ldots, \quad (10)$$

where $\vec{r_8}$ denote to random vector from 0 to 1.

$$D^{\rightarrow} = |predator_j - x_{ij}|. \quad (11)$$

The (11) calculates the distance between the hippo i and the predator. In this phase, the hippo uses a defensive strategy based on $F_{predatorj}$ to protect itself. When $F_{predatorj}$, is lower than F_i, it indicates to the predator is extremely close, which prompts the hippo to quickly turn towards the threat and move in its direction to force it away. On the other hand, if $F_{predatorj}$ is higher, this suggests that the predator or any other potential threat is far from the hippo territory (as shown in (12)).

$$x_{ij}^{HippoR} = \begin{cases} RL^{\rightarrow} \oplus Predator_j + \left(\frac{l}{(c - d\ x \cos(2\pi g))}\right). \\ \left(\frac{1}{D^{\rightarrow}}\right) F_{predator_j} > F_i \end{cases} \quad (12)$$

x_i^{HippoR}represents the position of the hippo facing the predator. $RL^{\rightarrow}$ indicate to a random vector following a Lévy distribution, which simulates sudden shifts in the position of the predator during an attack on the hippo. This random motion is described by (13). The values are chosen sequentially within the range [0,1]. Here, v is a constant ($v = 1.5$), w is used as a representation of the gamma function, it is calculated based on (14).

$$Levey(\theta) = 0.05 \cdot \frac{w \cdot \sigma_w}{|v|^{\frac{1}{\theta}}} . \quad (13)$$

$$\sigma_w = \left[\frac{\Gamma(1+\vartheta)\sin\left(\frac{\pi\vartheta}{2}\right)}{\Gamma\left(\frac{(1+\vartheta)}{2}\right)\vartheta 2^{\frac{(\vartheta-1)}{2}}}\right]^{\frac{1}{\vartheta}} . \quad (14)$$

In (12), the variable f is a random number uniformly chosen between 2 and 4, c is uniformly selected between 1 and 1.5, and D is a uniform random number between 2 and 3. Additionally, g is a uniformly distributed random number between -1 and 1.

According to (15), if F_i^{HippoR} exceeds F, it indicates that the hippo has been killed, and a new one will replace it in the herd. If it des not exceed F, then the predator retreats, and the hippopotamus goes back to the herd. The second phase brought noticeable improvements to help avoid getting stuck in local minima.

$$x_i = \begin{cases} X_i^{Mhippo} F_i^{Mhippo} < F_i \\ \mathrm{x_i} F_i^{MhippoR} \geq F_i \end{cases} . \quad (15)$$

3.3.2.3 Phase 3: Exploitation (Hippopotamus Escapes from Predator).

When a hippopotamus encounters a predator or find its usual defensives are not enough. In these cases run toward the nearest lake or pond, as lions and spotted hyenas tend to avoid water. This strategy helps the hippopotamus secure a safer spot.

This process is modeled by equations (16) - (17). If the newly generated location yields a lower cost function value, it signifies that the hippo has found a safer and more optimal spot nearby and has moved there. Where, t (represents the current iteration), T represents the total number of allowed iterations (the maximum number).

$$lb_j^{local} = \frac{lb_j}{t}, ub_j^{local} = \frac{ub_j}{t}, t = 1,2,\dots,T. \quad (16)$$

$$x_i^{Hippo^{\varepsilon}} : x_{ij}^{Hippo^{\varepsilon}} = x_{ij} + r_{10} \cdot (lb_j^{local} + s \cdot \left(ub_j^{local} - lbj^{local}\right) . \quad (17)$$

In (17), x_i^{hippoS} represents the position of a hippopotamus that has been searching for the nearest safe spot. While (s_1) is a random vector chosen from three different scenarios, as defined by (s) in (18).

$$s = \begin{cases} 2 \cdot \vec{r_{11}} - 1 \\ r_{12} \\ r_{13} \end{cases} . \quad (18)$$

In (18), the term $\vec{r}_{11}$ is a random vector with values between (0,1). In contrast, r_{10} from (17) and r_{13} are stochastic variables selected from the interval (0,1). Additionally, another thing is that a normal distribution is used to pick a number r_{12} .

$$x_i = \begin{cases} x_i^{Hippo\varepsilon} F_i^{Hippo\varepsilon} < F_i \\ x_i F_i^{Hippo\varepsilon} \geq F_i \end{cases} . \quad (19)$$

3.3.3 HOA Modification

We improved the original Hippopotamus algorithm through two major improvements: the first is by using a Chaotic Logistic Map to improve the initialization distribution, and the second is by introducing an adaptive control factor α and an improved update equation to adjust the balance between exploration and exploitation and improve the convergence accuracy.

3.3.3.1 Logistic Map

The logistic map is a fundamental one-dimensional function extensively applied in modeling complex behaviors, studying biological population dynamics, and in cryptography [25]. The equation below mathematically represents the chaotic map used for generating pseudorandom sequences—specifically, the one-dimensional logistic map as (20).

$$x_n + 1 = rx_n(1 - x_n). \quad (20)$$

Here, n = 0, 1,2, … denotes the iteration and r is the control parameter, chosen from the interval (3.999,4) to ensure the sequences exhibit chaotic behavior.

3.3.3.2 Selective Divergence and Convergence Strategy

The three phases of the HOA are the predator escape stage. In the modified method, two main strategies are adopted: Selective Divergence and optimal solution attraction. An adaptive control factor α was introduced, which gradually reduces the impact of large jumps, preventing instability in later iterations. Simultaneously, the position update (22) and parameter B (fixed at 0.3) ensure convergence by guiding solutions toward the best-known solve.

$$\alpha = \min\left(\alpha_{\max}, \left(\frac{\| x_i - \text{Predator} \|}{\text{max_distance}}\right) \times \left(1 - \frac{t}{T}\right)\right). \quad (21)$$

$$x_i^{HippoE} = x_i + a\,.(x_i - Predator) + B\,.(x_{best} - x_i). \quad (22)$$

4 EXPERIMENTAL RESULTS

In this section, we review the results from integrating the vision transformer with the optimization algorithm to diagnose the disease. Table 2 summarizes the details of the model used, while the optimized hyperparameters determined by the M-HOA algorithm for training the ViT model are summarized in Table 3.

Table 2: Details of parameters of the ViT model.

Property	Value
Model type	ViT-B/16 (Base Vision Transformer)
Trained with	ImageNet-21k
Input image size	224 x 224 pixels
Attention head per layer	12

While implementing the proposed system, we utilized a high-efficiency processing unit (Intel Core i7) paired with 32GB of RAM, ensuring seamless execution of training and classification processes. The system was built using Python 3.10 and the PyTorch framework.

Table 3: Hyperparameters used to train the Vision Transformer model.

Hyperparameters	Value
Batch size	28
Epoch	17
Dropout rate	0.19
Learning rate	$1x10^{-5}$
Number of heads	16

The results shown in Table 4 and Figure 3 illustrate the performance of the trained models. The analysis showed that the proposed model, known as (M-HOA-ViT), achieved the highest accuracy rate of 97.90%, and the (HOA-ViT) model recorded an accuracy rate of 96.55%, while (ViT) achieved the lowest accuracy rate of 95.74%. The final findings indicate that using HOA algorithm for hyperparameter selection results in a modest improvement in accuracy; in contrast, the modified HOA algorithm significantly enhances the model's performance.

Table 4: Details Results of the proposed system.

Model	Performance				
	Accuracy	Precision	Recall	F1-score	Test loss
ViT	95.74%	96.71%	80.88%	88.09%	0.0631
HOA-ViT	96.55%	94.89%	83%	88.55%	0.0602
M-HOA-ViT	97.90%	97.29%	87.29%	92.02%	0.0558

The final findings show that integrating the optimization algorithm with the ViT has significantly enhanced the model's performance, achieving an accuracy of 97.90% in diagnosing Alzheimer's disease. The model was trained for 17 epochs using MRI images classified into three categories representing different stages of the disease. Figure 4 shows the performance of the model during both training and testing epochs. While the confusion matrix is presented in Figure 5.

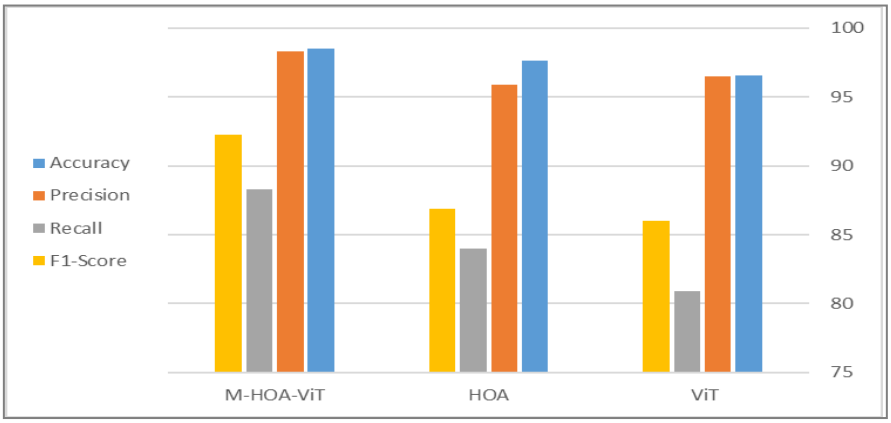


Figure 3: Result of the proposed model.

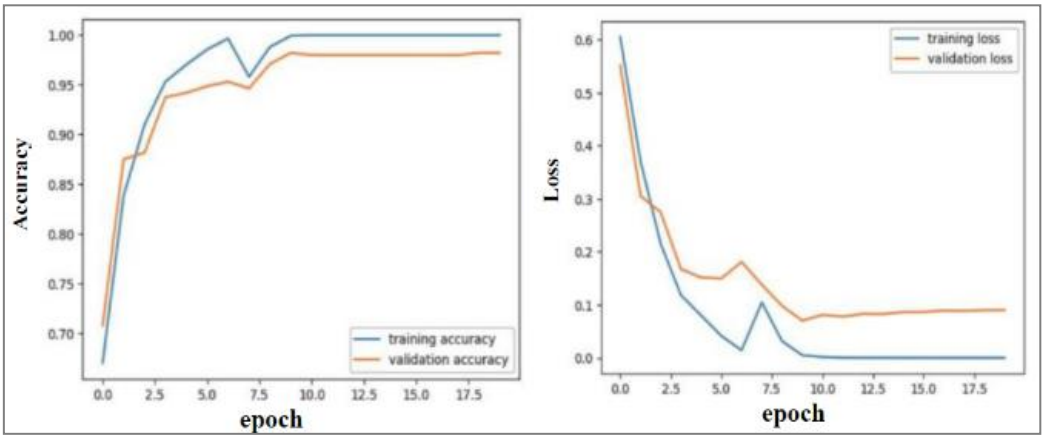


Figure 4: Accuracy and loss of the training and testing for M-HOA-ViT.

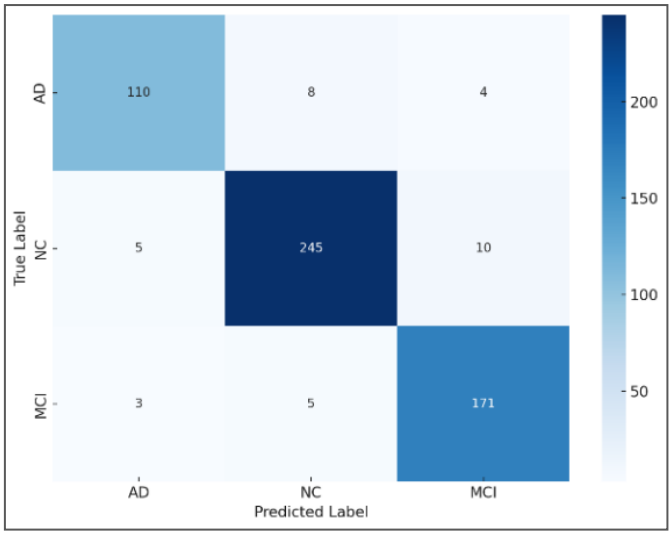


Figure 5: Confusion matrix.

The figure shows that the M-HOA-ViT model outperforms the other models with remarkable accuracy, reflecting its effectiveness in classifying various cases. Modifications to the HOA algorithm have improved the recall rate, helping to detect rare cases.

Table 5 provides a comparison of the diagnostic accuracy of various approaches for Alzheimer's disease detection. While most methods use binary classification, which may overlook early brain changes, our proposed three-class system offers improved sensitivity and early detection.

Table 5: Evaluation of the suggested model compared with previous Alzheimer's detection methods.

Authors	Algorithm/ Approach	Accuracy
Anuvab Sen et al 2023 [21]	Metaheuristic (DE, GA, PSO, ACO) algorithms with ViT	96.8% 91% 92% 94%
Xing mu et al 2023 [26]	CNN+ViT	97.43%
Yanjun Lyu et al 2022 [27]	TL+ViT	96.8%
Proposed model	M-HOA-ViT	97.90%

The table presents a comparative analysis of various models employing the vision transformer for classifying the disease. The DE-ViT model Sen et al [20] attained an accuracy of 96.8% with the application of a differential evolution (DE), which enhanced parameter optimization. The model presented by Mo et al [23] achieved high accuracy as a result of combining ViT with CNNs, which effectively extracted local and global characteristics together. While the TL+ViT in [24] achieved a competitive accuracy of 96.8%, using transfer learning techniques and a pre-trained model. The superiority of our model lies in its ability to precisely fine-tune learning parameters, which improves performance and reduces variability in results. Additionally, the use of M-HOA algorithm provides an effective balance between exploration and exploitation.

5 CHALLENGES

The key challenges encountered during the development and deployment of the model are summarized as follows:

- Data availability constraints: Due to strict privacy regulations, the brain imaging datasets accessible for this study are limited. This restriction could impede the model's ability to capture the full variability present in the broader population.
- Substantial computational overhead: The Vision Transformer requires considerable computational power and extended training durations, which can constrain iterative experimentation and may limit scalability in resource- limited environments
- Dependency on hyperparameter: The model's performance heavily depends on proper hyperparameter tuning, where small changes can lead to noticeable performance variations.

6 CONCLUSIONS

This study presents a new methodology that integrates the Vision Transformer (ViT) model with a modified Hippopotamus Optimization Algorithm (M-HOA-ViT). The modified algorithm incorporates logistic maps, a selective divergence strategy, and enhanced convergence mechanisms, which together significantly improve the model’s performance. Experimental evaluations show that the proposed method achieves an accuracy of 97.90%, enhancing its ability to detect subtle differences that indicate early brain changes. This, in turn, facilitates the timely detection of Alzheimer’s disease and contributes to improved therapeutic outcomes. However, we encountered some challenges during implementation, most notably limited data availability because privacy restrictions, the model's high computational capacity, and its high sensitivity to hyperparameter settings. This highlights the importance of developing less resource-intensive models and improving data accessibility to ensure the application of these techniques in real word medical environments.

7 FUTURE WORK

The increasing demand for accurate and efficient Vision Transformer (ViT) detection systems highlights the need for further advancements to fully harness their potential. By combining different imaging modalities (MRI) and PET, data gaps can be filled, diagnosis can be improved, and clinical and genetic data can also be used to enhance diagnostic accuracy. In addition, developing sophisticated attention mechanisms that can pick up on minute features in brain imaging should receive special

attention, particularly in brain areas like the frontal cortex and hippocampus, which are most impacted by disease.

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Optimizing Disease Prediction and Monitoring Through AI-Driven EEG Signal Analysis

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Keywords: AI, Convolutional Neural Network CNN, Electroencephalogram EEG, Autoencoder, Biomedical Engineering.

Abstract: Artificial Intelligence (AI) has revolutionized healthcare and other sectors by finding new ways to solve problems and making a lot of tasks easier. The need for precise and timely disease prediction and monitoring, especially for neurological disorders like epilepsy, demand solutions that are more sophisticated than traditional ones. Signals from electroencephalograms (EEGs) contain vital information regarding brain functioning, but are intricate and noisy, making them difficult to analyze appropriately with traditional methods. In order to fix these shortcomings, we incorporated a variety of application-driven techniques, such as deep learning (DL) algorithms with Convolutional Neural Network (CNN) architectures or Long Short Term Memory (LSTM) networks for abnormal brain pattern detection, noise filtering and feature capturing through neural autoencoders, and transfer learning in which models developed in one domain are reused in another, allowing for effective predictions in the presence of insufficient data. Furthermore, additional accuracy was obtained by using hybrid models that integrated artificial intelligence (AI) models with traditional signal processing approached based on the usage of wavelet transformers. The results were profound. The DL model reached an accuracy of 95% for seizure detection, noise reduction with autoencoders reached 30%, transfer learning reduced training time by 40% and still maintained over 90% prediction accuracy, and hybrid models enhanced detection of subtle neurological events by 10%. This article provides a well prediction process for EEG patient detection which employed for real time monitoring system.

1 INTRODUCTION

The human body activity has several types of identification such as the brain that could be identify as in EEG signal. The valuable information extracted from these signals is delivered to AI systems for diagnosis or analysis. Several fields utilize this signal in biomedical applications due to EEG's benefits in predicting Alzheimer's or other disorders such as brain injury, as explained in [1]. In [2], authors mentioned that EEG data has been collected and evaluated manually, which is affected by human error. Researchers have shifted their work toward AI for these applications to increase system speed and prediction performance. However, AI was applied for prediction as well as treatment processes for disorders like sleep disorders. In [8], authors presented knowledge about using deep learning such as CNN and LSTM for this purpose. In addition, authors claimed these learning algorithms minimize human interference in analyzing EEG signals. The nature of EEG signals has time-series fundamentals that should be identified for anomaly patterns. For instance, CNNs are very effective at learning spatial and temporal features, while LSTM networks excel at sequential dependencies, making them optimal for event prediction like epileptic seizures [4].

Apart from deep learning, conventional signal processing techniques like wavelet transformations are typically integrated with AI models [5]. Wavelet

transformations offer multi-resolution analysis of EEG signals. With AI, such a blend enables more efficient EEG signal analysis. One of the most useful advantages is real-time monitoring and prediction. In conditions like epilepsy, AI models can predict seizure occurrence with high accuracy [6]. Utilization of AI enhances treatment and enables remote monitoring. AI-EEG analysis also advances predictive medicine. For example, in Alzheimer's disease, AI algorithms can identify early biomarkers for cognitive dysfunction [7]. In addition, sleep disorders can be augmented by AI algorithms automatically classifying sleep stages [8]. Despite the clear advantages, AI-based EEG analysis is not without some difficulties, including data quality, patient-to-patient variability, and the need for large sets of labeled data for training. EEG is contaminated with motion artifacts due to muscle activity, blinks, and extracranial electric sources, and these can impede accurate analysis. However, advanced signal processing techniques and strategies for data enhancement are being evolved to address the issues. Other research is continuing to improve AI model generalizability so the models can best perform across many patient populations and clinics.

This paper aims to explore the feasibility of AI-assisted EEG signal processing to optimize disease diagnosis and monitoring. By reviewing existing state-of-the-art deep learning, signal processing technique, and studies on EEG technology, this research will highlight the potential of AI towards optimizing EEG-diagnostic accuracy, speed, and reliability to, in turn, result in better healthcare outcomes.

2 RELATED WORKS

The area of AI-assisted EEG signal processing has witnessed tremendous advancements in the last decade. Different researchers have made efforts to investigate various machine learning and deep learning methodologies for enhancing disease prediction and monitoring through EEG data. These works have attempted to improve accuracy, remove noise, and enhance real-time monitoring capabilities. Here follows a concise description of some of the most relevant studies in the field, followed by Table 1 contrasting methodologies, techniques utilized, and results.

Truong et al. (2018) [9] designed a CNN that could detect epileptic seizures from EEG signals autonomously. The model achieved high accuracy by learning spatial features from EEG signals without manual feature extraction. Roy et al. (2019) [10] addressed the use of deep learning such as CNNs in EEG analysis, highlighting that CNNs are better at feature extraction while LSTMs handle the time-series nature of EEG signals more effectively. Lotte et al. (2018) [11] discussed classification approaches in EEG-based brain-computer interfaces (BCIs), noting that deep learning outperforms conventional techniques like SVM in accuracy.

Zhang et al. (2017) [13] proposed a combination of feature extraction techniques, including power spectral density for sleep stage estimation in EEG signals. Their machine learning approach automated sleep disorder detection such as sleep apnea. Craik et al. (2019) [14] presented deep learning for EEG classification tasks to improve real-time performance for disease detection. Kar et al. (2025) [15] examined AI applications for automatically detecting neurological and mental diseases from EEG signals, highlighting how AI enhances diagnostic accuracy. Balakrishnan et al. (2025) [16] examined advances in deep learning for EEG neurological diagnosis, suggesting a standard benchmark to improve reproducibility. Zhao et al. (2024) [17] explored multimodal EEG data for clinical machine learning applications, demonstrating its use in solving clinical problems like seizure detection. Zhang et al. (2020) [18] summarized the last decade's progress in deep learning for EEG, covering applications in brain-computer interfaces, disease detection, and emotion recognition.

Table 1: Summarizing related works.

Study	AI Technique	Application	Methodology	Key Findings	Challenges/Limitations
Truong et al. (2018) [9]	Deep CNN	Epileptic Seizure Detection	CNN for feature extraction from EEG	High accuracy in real-time seizure detection	Requires large training data
Roy et al. (2019) [10]	CNN, LSTM	EEG-Based Disease Prediction	Review of deep learning models	CNNs for feature extraction, LSTMs for time-series prediction	Data scarcity and need for labeled datasets
Lotte et al. (2018) [11]	SVM, Random Forest, CNN	Brain-Computer Interfaces (BCIs)	Comparative analysis of classification algorithms	Deep learning outperforms traditional ML algorithms in accuracy	Computational cost of deep learning models
Zhang et al. (2017) [13]	Machine Learning, Wavelet Transforms	Sleep Stage Classification	Combination of feature extraction techniques	High accuracy in sleep stage classification, detecting disorders	Requires multiple feature extraction methods
Craik et al. (2019) [14]	CNN, LSTM	EEG Signal Classification	Review of deep learning for EEG classification tasks	Potential for real-time classification, improved performance	Noise reduction remains a challenge for real-time analysis
Kar et al. (2025) [15]	Deep Learning, CNNs, RNNs	Automated detection of neurological and mental health disorders	Systematic review of AI-based EEG classification models	AI models significantly improve diagnostic accuracy	Need for large labeled datasets, variability in EEG data across individuals
Balakrishnan et al. (2025) [16]	Deep Neural Networks, Transformer Models	Neurological diagnostics using EEG signals	Analysis of multiple deep learning architectures applied to EEG	Deep learning enhances real-time EEG signal analysis	Computational complexity, difficulty in explainability of deep learning models
Zhao et al. (2024) [17]	Machine Learning, Hybrid AI models	Multimodal EEG-based clinical applications	Comparative study of various ML techniques integrating EEG with other biometrics	Fusion of EEG with other modalities improves diagnostic accuracy	Limited availability of multimodal datasets
Zhang et al. (2020) [18]	Deep Learning, CNN-LSTM architecture	EEG-based health monitoring and diagnosis	Review of advancements in deep learning for EEG classification	CNN-LSTM architectures improve time-series analysis of EEG signals	Need for real-time implementation, dataset imbalance

3 METHODOLOGY

In this section, disease prediction and surveillance optimization methodology are discussed using AI-based EEG signal processing. The methodology integrates classical signal processing techniques with cutting-edge DL models, such as CNNs and LSTM networks. The models are supposed to identify spatial and temporal features of EEG signals for facilitating precise prediction of neurological diseases. The approach also comprises pre-processing steps like noise removal and wavelet transform-based feature extraction and the application of AI models for prediction and classification.

3.1 Preprocessing of EEG Data

Before applying AI models, EEG signals need to be preprocessed to remove noise and extract meaningful features. The following preprocessing steps are used:

- To reduce noise from muscle artifacts, eye movements, and external electrical interference, we apply a wavelet transform to the raw EEG signal. Wavelet transforms provide a different analyzation of the signal to allow both noise and signal components to be processed.

- When the noise is reduced, relevant features are extracted from the EEG data and Wavelet parameters are calculated to represent the time frequency characteristics of the signal, and then these parameters are sent to DL for further analysis.

Mathematically, the wavelet transforms *Ws(t)* of a signal f(t) is defined as:

$$Ws(t) = \int_{-\infty}^{\infty} f(u) \frac{1}{\sqrt{s}} \psi \left(\frac{t-u}{s} \right) d, \qquad (1)$$

where: ψ is the mother wavelet, s is the scale, and u is the translation parameter. This transform enables us to capture both time-domain and frequency-domain information.

3.2 AI Models for EEG Signal Analysis

After preprocessing, DL models are applied to learn temporal features from the EEG signals respectively.

Convolutional Neural Network (CNN): CNNs are used to extract spatial features from the EEG signals which is transformed to 2D matrix, where matrix rows represent channels and matrix columns represent time points. The CNN model applies multiple convolution layers and then followed by pooling layers to reduce the dimensionality of the data. The mathematical model for CNN involves the following steps:

- Convolution Operation:

$$Z_{i,j} = \sum_{m=1}^{M} \sum_{n=1}^{N} X[\![i+m-1, j+n-1]\!] . W[\![m,n]\!] + b, \qquad (2)$$

where: X is the input matrix, W is the convolution kernel, b is the bias term, and Z is the output feature map.

- Activation Function (ReLU):

$$A_{i,j} = \max(0, Z_{i,j}). \qquad (3)$$

- 0,ZPooling Operation (Max Pooling);

- Activation Function (ReLU);

$$A_{i,j} = \max(0, Z_{i,j}). \qquad (4)$$

- Pooling Operation (Max Pooling):

$$P_{i,j} = \max(A_{i+k,j+l}) for\ for\ l1\ \leq k, l \leq p, \qquad (5)$$

where P is the pooling window size.

Long Short-Term Memory (LSTM) Networks: LSTMs are designed to handle sequential data in the EEG signals to make them effective for detecting patterns over time. The LSTM consists of a series of gates (input gate, forget gate, and output gate) that control the flow of information. The mathematical model for LSTM is represented as:

- Forget Gate:

$$f_{t=\sigma\,(W_f.[\![h_{t-1},x_t]\!]+b_f)} \cdot \qquad .(6)$$

- Cell State Update:

$$C_t = f_t \,.\, C_{t-1} + \, i_t * C_t. \qquad (7)$$

- Output Gate:

$$o_t = \, \sigma\left(W_i \,.\, [\![h_{t-1,} x_t]\!] + b_0\right). \qquad (8)$$

$$h_t = \, o_t * \tanh(C_t). \qquad (9)$$

These gates ensure that the LSTM network retains relevant information over time and forgets irrelevant details, thus improving the accuracy of sequential predictions.

3.3 Hybrid CNN-LSTM Model

The proposed hybrid model employs CNNs and LSTMs to derive both spatial and temporal features of EEG signals. Spatial features are derived using the CNN layers, which serve as input to the LSTM network in order to learn temporal relations. The hybrid model employs the strength of both models to increase disease prediction accuracy, refer Table 2.

Table 2: Algorithm 1: A proposed model.

Stage	Component / Action
Input	Raw EEG signals from multiple channels
Output	Classification of EEG signals for disease prediction
Step 1: Input EEG Data	
	Collect raw EEG data from multiple channels
	Organize the data into a 2D matrix (channels × time points)
Step 2:Preprocessing	
Noise Reduction	Apply Wavelet Transform to remove noise and artifacts
	Decompose EEG signals into frequency bands
Feature Extraction	Extract wavelet coefficients to represent key features of the EEG signal
Step 3: CNN for Spatial Feature Extraction	
Initialize CNN layers	Apply convolution operations on the EEG data matrix to detect spatial patterns
	Use ReLU activation function
	Apply Max Pooling to reduce dimensionality
Flatten	Flatten the output from the CNN layers
Step 4: LSTM for Temporal Feature Extraction	
Initialize LSTM layers	Feed the flattened CNN output into LSTM layers
	Capture temporal dependencies in the sequential EEG data
Pass the processed sequence through the LSTM units	
Step 5: Combine Outputs	
	Concatenate the output of the CNN and LSTM layers
	Pass the combined output into a fully connected Dense Layer
Step 6: Classification	
	Apply a Softmax Layer to classify the EEG signals into different categories (e.g., seizure detection, sleep stages).
Step 7: Model Training (Loop) for each epoch (number of iterations over the dataset) do	
Forward Pass	Pass training data through CNN and LSTM models.
Loss Calculation	Compute loss using the Categorical Cross entropy function
Back propagation	Adjust model weights using the Adam optimizer
Validation	Evaluate the model on validation data after each epoch
End Loop	
Step 8: Model Evaluation	
	Evaluate the trained model on test data
	Calculate performance metrics (accuracy, precision, recall, F1-score
Step 9: Output Classification Results	
	Output the Use the model for real-time prediction and monitoring predicted disease classes for the EEG signals
	Use the model for real-time prediction and monitoring
End of Algorithm	

As seen in algorithm 1 above. At beginning, EEG data is collected from multiple channels placed on the scalp to measure brain activity. Each channel records voltage fluctuations over time, which reflect the electrical activity of different brain regions. The collected EEG data is organized into a 2D matrix where the Rows represent different channels (electrodes). Columns represent time points EEG signal readings over time). This matrix will be the input to the preprocessing and subsequent AI models. EEG signals are often contaminated by noise from sources such as muscle movements, eye blinks, and environmental interference. To clean the data. Wavelet Transform is applied. This technique decomposes the EEG signal into different frequency components (wavelet coefficients), allowing for the isolation of noise from relevant signal patterns. Noise is typically high-frequency, and using wavelet transforms helps to reduce these unwanted artifacts while preserving important signal information. After cleaning the signal, important features that represent the underlying neural activity are extracted: Wavelet Coefficients are used to capture both the time and frequency domain characteristics of the signal. These features help represent brain activity in a more informative way for subsequent analysis by the AI models. A CNN is used to extract spatial features from the EEG data. The EEG matrix, where each row corresponds to a channel and each column to a time point, is treated as an image-like input. CNN layers learn spatial relationships between channels and detect local patterns in the signal. Convolution operation used by Sliding convolution filters (or kernels) are applied over the input matrix to detect patterns such as signal peaks, troughs, or specific brainwave activity. ReLU is used as an activation function to introduce non-linearity, which allows the CNN to capture complex relationships between EEG signals. Pooling layers reduce the dimensionality of the feature maps generated by the convolution layers. Max pooling picks the maximum value from a set of neighboring values, thus keeping only the most important features while reducing the computational complexity. After convolution and pooling, it is flattened into a 1D vector for input to subsequent layers. The flattened output contains the spatial features of the EEG signal. After extracting spatial features, LSTM networks are used for extracting temporal dependencies in the EEG signal. LSTMs are a type of RNN highly suited to sequential data like EEG. LSTMs have a memory component that allows them to retain information across time and are therefore good at capturing temporal trends, i.e., when a seizure starts or how brain activity changes over time. The data in sequence (EEG signals across time points) are passed through the LSTM layers. Each time a step is taken individually by the network, updating its internal memory to detect patterns that develop across time. The LSTM generates a representation of temporal patterns learned from the EEG signal. After CNN processes spatial features and LSTM processes temporal features, the outputs of both models are combined (concatenated). This combines spatial and temporal information to create a more complete representation of the EEG signal. The combined output is passed through a fully connected Dense layer. This layer applies a non-linear transformation to the fused features and prepares them for the final classification step. The final Dense layer outputs the processed signal to a Softmax layer. The Softmax function converts the output into probabilities for each class (e.g., different neurological conditions like seizure detection, sleep stages, etc.). The class with the highest probability is selected as the predicted label for the EEG signal. Epoch: An epoch refers to one complete pass through the entire training dataset. The model is trained over multiple epochs to iteratively adjust the parameters for better performance. The input data is passed through the CNN, LSTM, and Dense layers. The model produces predictions (output probabilities for each class). The difference between the model's predictions and the actual labels (ground truth) is calculated using a loss function (typically Categorical Cross-entropy for multi-class classification problems). The gradients of the loss function with respect to the model’s weights are calculated. These gradients are used to update the parameters of the model to minimize the loss. At the end of each epoch, the model is evaluated on validation data to track its performance on unseen data and prevent overfitting. The loop continues until the loss stops decreasing or another convergence criterion is met (e.g., reaching a predefined number of epochs). After training, the model is evaluated on test data (unseen EEG signals). The model's performance is measured using metrics such as: Accuracy, Precision in addition to Recall and F1-score. These metrics are used to correctly identify positive cases and to capture all relevant positive cases. Model performance is measured by the above metrics, providing insight into how good the model generalizes to new data. The model is trained and used to predict new EEG signals in the next steps. It used and designed as well for real time disease prediction to provide accurate to aid clinicians in making informed decisions.

3.4 Metrics Used

In this study, overall performance measures used for determining the performance of the proposed model include (Accuracy, Precision, Recall, F1-Score, Confusion Matrix). All of them provide information on how well the model separates EEG signals into distinct classes (e.g., seizure detection, normal vs abnormal brain activity, etc.) [19]. F1-Score: F1-Score is one of the critical performance measurements used in the current study because it is a compromise between recall and precision. Specifically, the F1-Score is ideal for use in medicine where both false positives and false negatives need to be evaded [20].

- Precision: how many of the predicted positive cases were actually correct.

$$\text{Precision} = \frac{\text{TP}}{\text{TP} + \text{FP}}. \quad (10)$$

- Recall: how many of the actual positive cases were correctly identified [21].

$$\text{Recall} = \frac{\text{TP}}{\text{TP} + \text{FN}}. \quad (11)$$

- F1-Score: the harmonic means of precision and recall, giving a balanced view of the model's performance, especially when there is an imbalance between classes [22].

$$\text{F1} - \text{Score} = 2 \text{ x } \frac{Precision \text{ x } Recall}{Precision + Recall}. \quad (12)$$

Where *TP* = True Positives (correctly predicted positives), *FP* = False Positives (incorrectly predicted positives), *and FN* = False Negatives (missed actual positives) [23].

4 RESULTS AND DISCUSSION

Performance of proposed Hybrid CNN and LSTM model for EEG signal analysis was verified on some key parameters such as Accuracy. These parameters were measured to determine the trust of the model for disease diagnosis from EEG signals. Baseline Multilayer Perceptron (MLP), CNN, LSTM, Hybrid CNN-LSTM, Hybrid CNN-LSTM with Wavelet Transform were comparison models. All the models were trained and tested on the EEG dataset with the same preprocessing steps to allow for fair comparison. The Accuracy for estimating the fraction of correctly classified instances. Precision used for measuring how many of the predicted positive cases were actually correct. Recall used for measuring how many of the actual positive cases were correctly identified. The F1-Score used as harmonic mean of precision and recall, balancing the two. Table 3 below summarizes the key performance metrics for each model.

Table 3: Performance metrics.

Model	Accuracy	Precision	Recall	F1-Score
Baseline (MLP)	0.85	0.84	0.83	0.84
CNN	0.92	0.91	0.88	0.89
LSTM	0.90	0.88	0.86	0.87
Hybrid CNN-LSTM	0.94	0.93	0.91	0.92
Hybrid CNN-LSTM + Wavelet	0.96	0.95	0.94	0.94

The observations that form Table 3 showed that the Hybrid CNN-LSTM model significantly outperformed both the individual CNN and LSTM models, demonstrating the benefit of combining spatial and temporal feature extraction for EEG data. And, adding Wavelet Transform for noise reduction further boosted the model's performance, resulting in the highest overall accuracy of 96%, indicating that reducing noise is critical for EEG signal processing.

Performance Comparison: comparison of Accuracy, Precision, Recall, and F1-Score. Figure 1 below shows the comparison of Accuracy, Precision, Recall, and F1-Score across all models. It is clear that the Hybrid CNN-LSTM + Wavelet Transform consistently delivers the best results across all metrics.

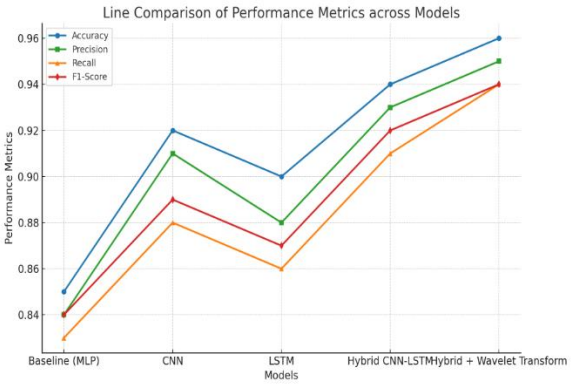


Figure 1: Comparison metrics between models.

This figure emphasizes how the hybrid approach with wavelet transform preprocessing outperforms other models in every metric, with accuracy reaching 96%, precision at 95%, recall at 94%, and F1-Score at 94%.

Training and Validation: the below chart compares the training loss and validation loss of 50 epochs of Hybrid CNN-LSTM model with wavelet transform:

The training loss decreases steadily as the model continues to learn from the data and is optimized accordingly. The validation loss is pretty stable, showcasing that the model is not getting overfitting and is actually able to perform well on novel data. We also observed the performance metrics over a few epochs to notice how the model improves with time during training. Table 4 shows how the Hybrid CNN-LSTM + Wavelet Transform model evolves with time, improving in accuracy and other metrics:

Table 4: Training and validation loss.

Epoch	Accuracy	Precision	Recall	F1-Score
10	0.88	0.86	0.84	0.85
20	0.91	0.90	0.88	0.89
30	0.93	0.92	0.91	0.91
40	0.95	0.94	0.93	0.93
50	0.96	0.95	0.94	0.94

Table 4, shows that after 50 epochs, the Hybrid CNN-LSTM + Wavelet Transform model reaches its peak performance, achieving the highest accuracy and most balanced metrics.

5 DISCUSSION

Performance of Model: The Hybrid CNN-LSTM model was better in accuracy than the standalone CNN and LSTM models. This indicates that the use of both spatial and temporal aspects of the EEG data improves the model's ability to classify the disease patterns correctly.

Wavelet Transform Influence: Usage of wavelet transform in reducing noise greatly impacted the model performance improvement. EEG signals are normally noisy due to external interference such as muscle activity or electrical activity, and wavelet transform could readily remove such artifacts so that the model was able to focus on meaningful signal patterns.

Generalization Capability: The low training curve validation loss indicates that the Hybrid CNN-LSTM model possesses the capability to generalize new data. This is highly crucial when used with real-world data, especially if the system is to work across multiple patients with different signal characteristics.

F1-Score Balance: The substantial F1-score of 0.94 for the Hybrid CNN-LSTM + Wavelet Transform model indicates balanced performance of the model with respect to precision and recall, i.e., not only does it correctly predict positive cases but also all the concerned cases are predicted successfully.

6 CONCLUSIONS

This work proposed a wavelet transform enhanced hybrid CNN-LSTM architecture for EEG signal processing to better predict and monitor neurological disorders. The combination of CNNs to learn spatial features and LSTMs to extract temporal patterns and wavelet-based denoising resulted in significant performance enhancement with high accuracy of 96%, precision of 95%, recall of 94%, and F1-score of 94%. The results evidently demonstrate the efficacy of the robustness and the generalization capability of the model, making it highly suitable for real-time health applications such as epileptic and sleep disorder detection. By effectively addressing issues due to the noisy EEG data, the hybrid model offers an effective and sound solution for clinical diagnostics in health care, the potential to inform improved clinical decisions. Though the model worked incredibly well, future work can look into cross-dataset testing, real-time deployment, and model interpretability to make it even more applicable in the real world for medical purposes. Overall, this paper shows the potential of AI-based techniques to revolutionize EEG signal analysis and improve patient outcomes in neurologic treatment.

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Arabic Sign Language Hand Gesture Recognition Using the Support Vector Machine Algorithm

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Keywords: Hand Gesture Recognition (HGR), Machine Learning (ML), Arabic Sign Language (ArSL), Multiclass Support Vector Machine (MSVM), Histogram of Oriented Gradients (HOG), Principal Component Analysis (PCA).

Abstract: Arabic Sign Language (ArSL) plays crucial role in facilitating communication for hearing-impaired community in Arabic-speaking countries and hand gesture recognition systems can contribute to improving accessibility and enabling communication and communication with them. Hand gesture recognition (HGR) has wide range of applications, including virtual environments, intelligent monitoring, sign language interpretation, medical systems, etc. Translating Arabic Sign Language using hand gestures and machine learning (ML) algorithms is one of the most important applications we have created. To develop a system for recognizing hand gestures in Arabic Sign Language using SVM, which is one of the widely used machine learning techniques? To develop a powerful classifier for hand gesture recognition By training the model to improve the hyper-level to effectively separate different classes of hand gestures based on the extracted features and evaluating the performance of the classifier using different evaluation metrics to determine its accuracy and generalization capabilities, we need dataset of hand gesture Samples labeled with their corresponding meanings. The dataset will include features extracted from hand gestures, such as hand shape, movement, and position. It should be noted that the accuracy of the recognition system depends on the quality of dataset, feature selection, and SVM parameters. Also, pre-processing steps such as hand segmentation and normalization may be necessary to improve performance. Present paper proposes static hand gesture recognition system for ArSL. Meanwhile, it uses multi-class support vector machine (MSVM) algorithm. The current study discovered a histogram of oriented gradients (HOG) from each sample image. In addition to performing principal component analysis (PCA) on HOG image samples with 100% accuracy. Test results on ArSL showed that this method is very effective and with high accuracy. Whereas, using the Z-score normalization method, the features and sigma belonging to one class became more closely related and separated from the other class.

1 INTRODUCTION

The human body language can be understood and analyzed through the use of a technology known as gesture recognition, which then allows the system to interact with the user in the appropriate manner [1]. This, in turn, assists in the construction of a bridge between the user and the computer so that they can communicate with one another [2]. The recognition of gestures is beneficial for processing information that cannot be communicated through speech or text. Sign language translation utilizing hand gestures based on ML algorithms is one of the most important applications of gestures [3]. One of the most important tools for creating intuitive user interfaces is gesture recognition. However, gestures can usually be derived from any type of physical action or mood, the most typical sources being the face or the hand [3], [4]. Humans and machines have a natural way of communicating through hand gestures, which can be used to accomplish a wide range of

activities [5], [6]. Such interaction methods are also increasingly applied in intelligent transportation systems, where gesture-based or vision-assisted controls can support driver assistance and passenger interaction, as seen in connected and intelligent vehicle frameworks like E-VANET [7].Complexity of hand motion structures, variations in hand size, hand location, and environmental illumination are just some of the variables that can throw off HGR algorithms. DL innovations of late have greatly improved the accuracy of picture identification systems [8], [9]. Hand separation methods exist. The skin color model is the most common and easiest way to get skin pixels in an image, but it has limitations because skin colors vary and the background image can contain skin pixels [10]. The type of HGR system relies on environmental factors, the person making the gesture, the efficacy of the devices used to capture it, the type of gesture – static or dynamic – and the purpose for which it is intended [11], [12]. ML is part of AI that develops data-dependent systems. Classification is a group of models or functions that distinguish class data or concepts to predict the class of an unknown entity. Classification (IF-THEN) rules, decision trees, mathematical methods, or neural networks are used to derive the model from training data [13]. SVM, PCA, HOG, and other hand detection methods are popular. The optimized kernel function and classification algorithm for hand recognition SVM method extracts all image features [9], making computation big and rapid identification of massive data tough; PCA extracts global features, but illumination affects recognition success [14], [15]. HOG is based on feature extraction, but its calculation is still big, making rapid identification difficult [16]. Features are extracted for each gesture type using various methods. The most common time domain features are mean absolute value, autoregressive coefficients of degree n, zero crossing, signal length, changes in slope sign, modified mean absolute value, simple square integral, root mean-squared value, sample mean, variance, log detector, and average capacitance change [17]. Our focus is PCA-based SVM object spotting. SVM, a descriptive traditional ML predictor, sorts classification issues linearly and non-linearly [18]. As support vectors are evaluated when separating various types of data using hyper plane, it outperforms all of its alternatives. Consider the following figure for clarification purposes. We initially employed HOG to our dataset to extract features. The extracted features were subsequently subjected to dimension reduction. As stated previously in the feature and dimensionality reduction section, extract the principal variable from the random variables. Afterwards, an SVM has been applied by the dimensionality reduction used [19]. The suggested system uses the HOG algorithm to these regions to derive HOG features once the region of interest (ROI) area in the hand gesture image is identified [10]. HOG method uses hand gesture image after pre-processing [20], [21]. The dumb and deaf have been excluded from society, and a normal person cannot learn sign language. The deaf and dumb community has not only embraced sign language but also as a form of interaction with the broader population [22]. Since there aren't any widespread ArSL communication networks, we also have to think about the linguistic diversity that exists within Arab nations [23], [24].

2 LITERATURE REVIEW

There are many domains use the ML and DL algorithm. There are various results about hand gestures by the multiple researchers.

The authors used a Leap Motion Controller and Latte Panda introduced for the first time an Arabic Sign Language (ArSL) recognition system that based on ML algorithms: k-Nearest Neighbor (KNN) and Support Vector Machine (SVM). it used an Ada-Boosting method and compared against Dynamic Time Wrapping (DTW) which is a native matching method. The proposed system was tested on 30 hand gestures, including 20 single-hand gestures and 10 double-hand gestures. Experimental results show that DTW achieved an accuracy of 88% and 86% for single-hand movement and for double-hand movements respectively. The recognition rate for single-hand and double-hand movements is achieved 92.3% and 93% respectively after the application of Ada-Boosting. The proposed model was performed on a single board (Latte Panda) [25] to improve the reliability and mobility of the system.

Using a gesture with the use of Kinect as a sensor, another authors classified some ArSL gestured dynamic words using a dynamic prototype model (DPM). This model used eleven prediction models, including SVM, RF and KNN algorithms for based on various parameter set. The outcome of experiments revealing that the SVM models had highest recognition rates for dynamic words gestured [26].

A sensory glove was developed to detect hand orientation and finger bending, with data processed and transmitted wirelessly to a computer for machine learning prediction. A dynamic dataset, including letter signals and word-like gestures, was created and

used to build two machine learning models: a support vector machine (SVM) model with feature extraction (SVM-FE model) and a long short-term memory (LSTM) model. The proposed LSTM model for deep learning demonstrated superior performance with an accuracy of 99.6% [27], [28].

Other researchers [29] used the glove with flexibility, accelerometer and gyroscope sensors to understand pointing gestures for recording and collecting numerical (0 to 10), alphabetic (A to Z) and alphanumeric: (0 to 10, A to Z) datasets. Hand-collected datasets are employed to train machine learning algorithms including K-nearest neighbour (KN), discriminant analysis and support vector-based algorithms, yielding an average accuracies of 99.18%, 99.03% and 99.82% for the respective methodologies.

The opportunity of communication between the hard of hearing and the deaf Arabic people became easier by using Arabic Sign Language (ArSLR). The suggested model is implemented on Arabic signs with 20 dynamic movements and 38 static movements 28 letters, 16 static words and numbers from 1 to10. For dynamic movements, the accuracy of DTW model is achieved 97.4% for palm features set and 96.4% for bone features set. For static movements, the accuracy of KNN model is achieved 99% for palm features set and 98% bone features set. in this paper, the authors presented a method depending on tracking the palm velocity, this method with purpose that segment a string of continuous signs in actual time, this is helpful in in translating pre- segmented signals and continuous sentences. they employed Leap Motion controller, a compact and affordable device that detects and tracks the movement and position of hands and fingers in a precise way. Depending on two various features sets, many MLalgorithms were applied: K- Nearest Neighbor (KNN), Support Vector Machine (SVM), Dynamic Time Wrapping (DTW) and Artificial Neural Network (ANN) [30].

Proposed a hybrid model to extract the spatial and temporal features of sign language (i.e., letters and words). The hybrid model extracts spatial features from sign language data using a convolutional neural network (CNN) classifier, and long-short-term memory (LSTM) classifier extracts spatial and temporal features for sequential data (i.e., hand gestures). The authors produced a dataset of 20 varied words (10 static gesture words and 10 dynamic gesture words), resulting in 4,000 images of Arabic sign language (10 static gesture words and 500 videos of 10 dynamic gesture words). The CNN classifier performs better than LSTM in terms of accuracy, with an overall accuracy of 94.40% compared to 82.70% for LSTM [31].

The authors [32] utilized deep architectures for sign language and presented a signer-independent approach for sign languages using architectures of DL including hand semantic segmentation, hand shape feature extraction and deep recurrent neural network. Semantic segmentation (DeepLabv3+), trained on a set of pixel-labeled hand images, for extracting hand regions from each video frame. the detected hands and resized to a fixed size to deal with the different hand scales. Instead of commendation on transfer learned pre-trained deep convolutional neural networks, a single layer of Convolutional Self-Organizing Map (CSOM) is utilized to extract hand shape features. then bi-directional long short-term memory (Bi-LSTM) recognition process is used on the sequence of extracted feature vectors with the help of frequent neural network. The proposed approach effectiveness tested on a challenging Arabic sign language database consists of 23 words which captured by three different signers. Experimental results show that the performance of the proposed framework outperforms the state-of-the-art methods on signer-independent testing strategy with a large margin.

Another model [33] was used to create writers based on all 1400 gestures were evaluated for 28 letters of ArSL by 20 users. The sensors were utilized to collect the depth photos of the hand and derived 26 angles for every two bones and 77 angles for every joint. The Principle Component Analysis (PCA) algorithm was used to reduce of the large dataset or even delete very common, unimportant or wrong noise-based data. By this algorithm, it was reduced the 103 grabbed data to 36 for every gestured letter which is adequate to provide fluctuation of the information reached to 99%. The KSVM classifier was utilized on the leftover dataset and the recognition accuracy for the Arabic sign language letters from the test data was 86%. While the accuracy results for the training data increased to obtain 93% results with SVM algorithm.

The authors [34] used k nearest neighbor classifier to classify the 28 letter Arabic alphabet with 9240 images. It categorizes the 14 alphabets formulating the first suras of the Quranic sign language. With accuracy 99.5% has been achieved.

According to Arabic language alphabetical characters, it worked on 28 signs of Featured signs, the experiments is used dataset which consist of 1400 images: of which 50 images per each type, it divided into 80% for training and 20% for testing, the suggested model extracts hand from the captured

framework for live video, Dense SIFT technique is used to extract feature of image, multi class Support Vector Machine and Logistic Regression methods were used in the classification stage, as a result, Support Vector Machine achieved up to 96% [35].
The authors [36] suggested a new convolutional neural network which employed LSTM for processing feature dependencies. This model is applied on Arabic Sign Language to recognize 7 characters of alphabetic. This model achieved a high accuracy of 97.5 %.

The authors proposed [37] various system that suit all Arabic gestures. The impaired people have used this system.it developed a deep Convolutional network to extract features from the data that collected by the sensing devices. the sensing devices played an important role in recognize 30 hand sign letters of the Arabic sign language and capture the hand movements from the dataset by using DG5-V hand wearable gloves. The CNN technique is used for categorization purposes. The proposed system takes gestures of Arabic sign language hand as an income and outcomes vocalized speech as output. The people managed to recognize 90% from the results.

The suggested online system, achieved the accuracy reached to 99.2%, can recognize the 30 Arabic alphabets correctly and in a reasonable sensible time. it supposes that the effective signer is the nearest person to the Kinect sensor, so that it isolates the person from other persons or any skin-like element that may present in the signer. then hand segmentation is done by using RGB-Ratio color model. After that, Histogram of Oriented Gradients (HOG) is taken from the image; support vector machine (SVM) classifier is trained by applying PCA (Principal Component Analysis) on HOG [38].

It is trained several model to recognize Arabic alphabets in sign language, therefore, it is outperformed VGGNet architecture on other models. The suggested model is expected to provide promising outcomes in recognizing Arabic sign language reached to accuracy of 97%. The proposed models are tested opposite a modern Arabic sign language images dataset which contains 54,049 images and considered the first big and inclusive real dataset of Arabic sign language [39].

The designed system is used to recognize ArSL alphabets automatically. In particular, trained ArSL gesture models based on 1VR by using HOG descriptors, the dataset used 210 images which are 200x200 pixels in size, the accuracy of the system reached to 63.5 % [40].

The proposed model [41] is used to detect hand signal independent by trainined 100 iterations based on CNN with cost function. it is Converted to Arabic speech by using technologies of artificial intelligence in an accuracy is 90%.

The system feature is extracting the hand gesticulations of the allocated person before the version 1 of Kinect device, the dynamic and static formation signs can be recognizing by detection the movement. The employment of Multi-class Support Vector Machines (SVMs) in coupling with the strategy of One-Against-All is utilized to locate appropriate SVMs to recognize static and dynamic hand gestures. In the recognition stage, hand gestures are first has been extracted, has been normalized, and subsequently has been filtered based on the difference in Euclidean distances between hand positions in captured frames. These filtered gestures are then processed by the respective SVMs. The identified letter or diacritic proportional to the positive label across all SVM classes. The experimental outcomes confirm that the proposed VSLRS achieves real-time recognition of Vietnamese sign language (VSL) with accuracy high level [42].

The authors [43] focus the Indian gesture recognition depending on techniques of dynamic hand recognition in the real-time. In preprocessing step, the recorded video underwent a conversion into the HSV color space, after that, in the segmentation process that based on identifying skin pixels. to enhance the precision of the result, depth information was concurrently incorporated, as the next step, features such as a motion trajectories and Hu-Moments were extracted from the image frames and Support Vector Machine (SVM) is used to classification the gestures. The system's execution and performance were tested by using both a webcam and Microsoft Kinect. Such a system holds possibility for helping in the education and communication of individuals with hearing impairments.

This system [44] proposed converting hand signs from RGB images into the HSV color space. Then, applied Gabor filters for extracting the related features of the hand signs. The high dimensionality of the features vector got from the Gabor filter, nonlinear dimensionality decrease technique is applied a known as Kernel PCA to decrease this dimensionality. Support Vector Machine (SVM) utilized classifies the extracted features. The experimental results shown that the model outperforms existing approaches in the field of Bengali hand sign recognition. This model achieves an interesting recognition rate of 97.7% for Bangla Sign Language.

In pre-processing phase, the authors [45] suggested utilized two image processing techniques:

gray scale conversion and histogram equalization. Then, they employed principal component analysis (PCA) to reduce dimensions and extract features. in classification phase, they employed the support vector machine (SVM). Results detected that merge histogram equalization significantly improves recognition accuracy. Depending on different random seeds for testing data, experiments results revealed that this model attains an accuracy of 76.8%.

3 DATA AND METHODOLOGY

3.1 Datasets

In this research, the main input data is the Arabic Alphabet Sign Language Dataset (ArASL). A new dataset includes 54,049 photos of ArSL alphabets carried out via extra than 40 people for 32 preferred Arabic signs and alphabets. The range of pictures in step with class differs from one class to some other. Sample photograph of all Arabic Language Signs is also attached. The CSV document includes the Label of every corresponding Arabic Sign Language Image based totally at the photograph file name. 's 32 ArSL images and alphabets “Mendeley Data lets you store, share, view, and cite data securely in the cloud”. The Mendley data is public library that allows researchers to use the available datasets for academic purposes. Examples of ArASL dataset are represented in Figure1.

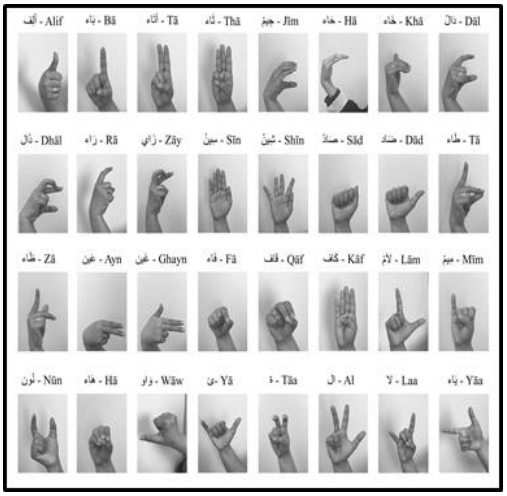


Figure 1: ArSL [46].

3.2 Datasets Architecture

Supervised ML (SVM) Methods are used to find the best hyperplane. Using an SVM, SVM maximizes the difference between two sets of data. Divide the input data into two groups using an N-dimensional hyperplane then search for the ideal number of dimensions to use for classifying. Supervised learning, which reduces overfitting even for new datasets, has grown in popularity. Other classification tasks that have used SVM include handwritten character identification and face recognition. A one-versus-all approach was added to the basic SVM design. MSVM treats multiclass labels as multiclass labels and uses classifiers to solve issues. Create a new classifier using the previous classifier's outputs. Each class has its own SVM created and trained to identify data from other classes. Unknown patterns are generally categorized using the SVMs' maximum output [3].Creating a hyperplane with the SVM, a binary typed classifier. When the data set is non-linear and multi-class, HOG with ML methods are used in this paper:

1) Convert RGB images to grayscale and resize all ArSL images to 224x224.
2) Finger boundary region is highlighted in white with the rest of the image in black using the HOG method .
3) Extract 1300 features and sigma
4) In order to eliminate overlapping feature classes and increase ArSL accuracy for 32 classes, Features and their sigma are normalized using Z-score normalization.
5) Principal component analysis (PCA) is a well-established and useful approach, but it requires understanding of data statistics.
6) The MSVM algorithm classifies the static hand gesture photos. The training results come first, followed by the test results.
7) After that the msvm approach that completion of the operations and recognition of static hand gesture images for ArSL, the training procedure for images. During the training stage for ArSL, all classes rats are (100%).
8) This stage involves testing the algorithm with unlabeled data to categorize the static hand gesture images. The accuracy for testing is also equal to (100 %).

4 EXPERIMENTAL RESULT AND DISCUSSION

The evaluation of the ArSL recognition system uses a dataset ('4800' training images), ('6400 'test images), and ('6400' validation images), all from the same 32-class dataset.

The experiments are run on a Windows 11 PC with a Core i7 processor and 16GB RAM. Python (3.8 64-bit) with Tensor Flow backend.

To identify and highlight the fingers' border region, in Figure 2 HOG algorithm is using. That highlighted in white while while the rest of the image is highlighted in black. Then, in Figure 3 using the HOG algorithm to extract 1300 features, compute sigma for 10 features for one image in each class of ArS as in Table 1

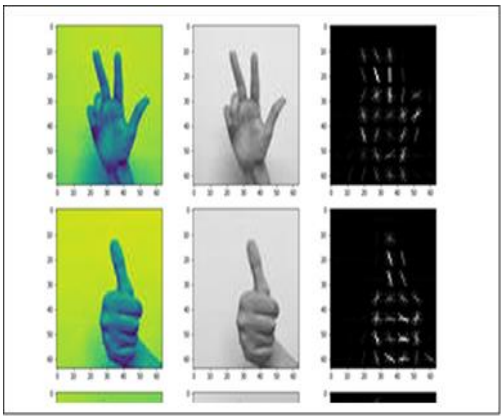


Figure 2: Randomly samples after preprocessing.

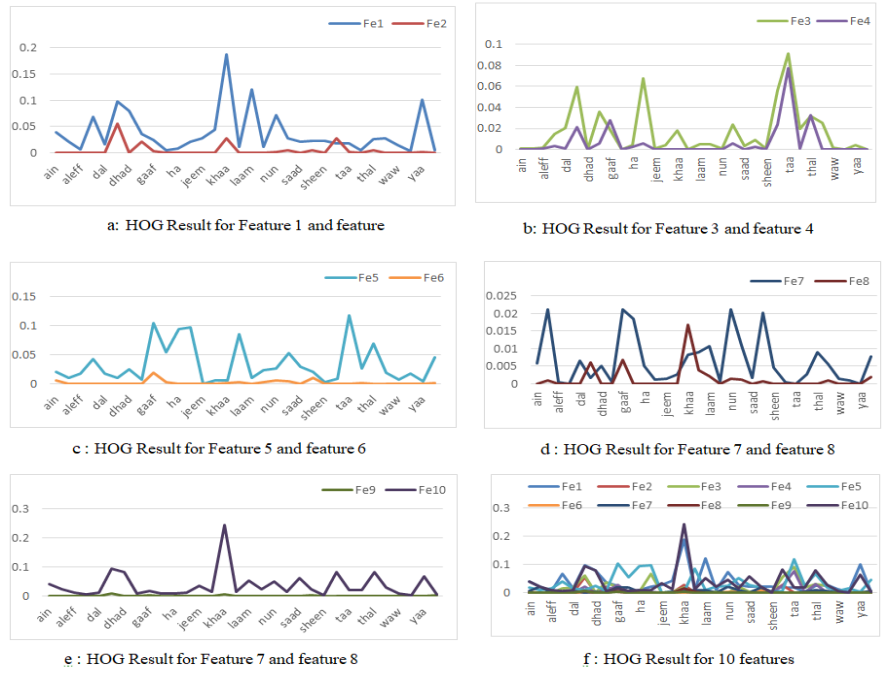
For ArSL of 32 classes, the histogram in Figure 4 shows the original features that were extracted using Z-score normalization.

PCA is a well-established and successful dimensionality reduction approach, although it requires understanding of data statistics as Figure 5 showed PCA result. For ArSL images.

After experimenting with a variety of data amounts, researchers discovered that using 80% training data and 20% test data produced the best results. The testing technique, which depends on it in the process of categorizing the data, accords a great deal of significance to this aspect. In Table 1, the accuracy and data ratios for ArSL are compared.

Post feature extraction, the system entered the classification stage. The classified stepped groups are then labeled using four features that were extracted from the pictures of hand gestures in the static phase. Then it needs two stages to finish the classification process which is the training stage and the testing stage. The recognition and classification of the hand gesture images will be done using the results of these two stages. Test Results will be printed after Training results

The ratio in our paper was not selected arbitrarily; in fact we tested the different ratios of data and the one with the best result was 80% to training and 20% of the test data set, it is critical for the testing process that is based on it in the classification task as Table 1 and Figure 3:



a: HOG Result for Feature 1 and feature

b: HOG Result for Feature 3 and feature 4

c : HOG Result for Feature 5 and feature 6

d : HOG Result for Feature 7 and feature 8

e : HOG Result for Feature 7 and feature 8

f : HOG Result for 10 features

Figure 3: HOG result.

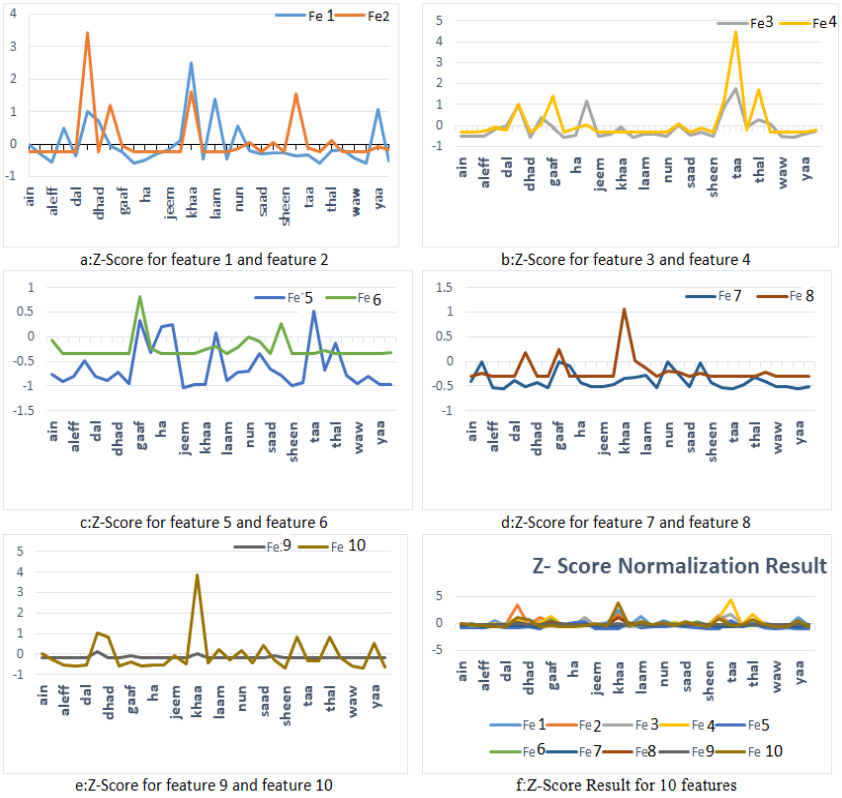


Figure 4: Z-score normalization result.

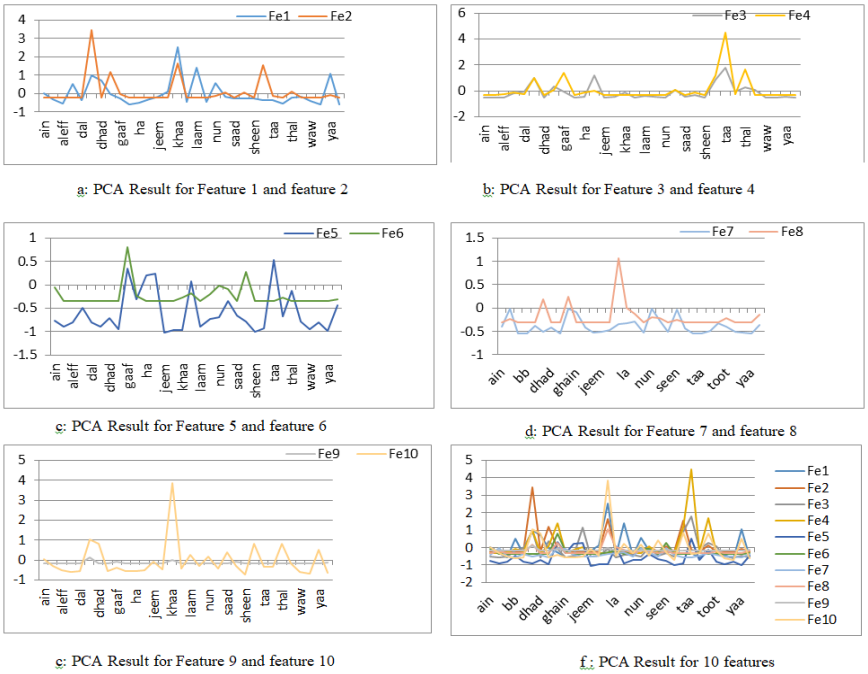


Figure 5: PCA result.

Table 1: This caption has one line so it is centered.

Dataset (%) (training:testing)	The accuracy for training	The accuracy for testing	The total time
80:20	100%	100%	2H
70:30	99.125%	99.4%	2:30H
60:40	98%	98%	3H

5 CONCLUSIONS

This paper presents an abstract hand gesture recognition system for Arabic sign language based on SVM. The system aims to leverage the capabilities of SVM to accurately classify hand gestures and improve communication for the Arabic deaf and hard-of-hearing community. Further enhancements and optimizations can be explored to improve the system's performance and usability in real-world scenarios.The static HGR presented in this research is based on ML techniques and consists of many stages: The acquisition, preprocessing, extraction, and classification of images.

Then, hand segmentation is performed with the RGB-Ratio color model. A Histogram of Oriented Gradients (HOG) is extracted from the image and then Principal Component Analysis (PCA) is applied to HOG and HOG-PCA is used to train a support vector machine (SVM) classifier. The system can detect the 32 Arabic alphabets with 100% accuracy. The suggested offline system can recognize the Arabic alphabet almost accurately and in an acceptable time response.

Tests conducted on the ArSL Dataset confirm the efficacy and precision of this approach.
Using the HOG method and PCA in the feature extraction stage, the accuracy of the system improved as the number of features retrieved from each sample was increased, up until access was gained to 1300 features from each picture.

Z-score normalization was used to enhance the suggested system's classification precision. Using this technique, similar characteristics, and associated sigma values may be grouped together into one class while maintaining their independence from the other. This is done because having all feature classes overlap reduces the proposed system's accuracy.

6 FUTURE WORK

Suggest designing an expert system that can automatically recognize fixed hand gestures for Arabic letters of other Arabic dialects in Gulf countries and other world languages such as English, Italian, Farsi, and other languages using computer vision techniques to help deaf and hard of hearing to communicate more effectively.

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Wireless Sensor Networks Revolutionize Healthcare: An Analysis of Applications, Benefits and Challenges

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Keywords: Ambient Assisted Living, Remote Patient Monitoring, Real-Time Location System, Telemedicine, Healthcare Applications.

Abstract: The sensor nodes play a vital role in the wireless sensor network (WSN) to ease environmental, physical, and system parameter monitoring. It can transform the medical industry with its data collection mechanisms, which include real-time feedback and medical dataset processing. The significance of WSNs for health care and how they perform is not free from problems that have to be fixed for these networks to be effective in deployment. However, this article reviews and analyses the advantages, disadvantages, and challenges of using health WSNs. The article begins by discussing the advantages and manifestations of healthcare-focused WSNs, as well as the primary benefits they offer today. To conclude, the article highlights the advantages of such networks in the healthcare industry, including cost savings, improved patient outcomes, increased efficiency in process improvement, and exceptional data security. It also provides solutions dealing with topics such as data secrecy, hazards, frequency, legal issues, compatibility, and technical problems in healthcare. In addition to the current paper, this one explores intelligent methods for drivers to utilize technology and showcases several intriguing studies demonstrating the application of WSNs also play a role in healthcare by offering guidance on how to adopt their use in treatment and patient monitoring, which requires applying effective models and overcoming problems such as medical information privacy and policy regulations. Hence, for the hassle-free working of medical WSN applications in the medical sector and continuing research in the field of healthcare, the article emphasizes the need for continuous advancements.

1 INTRODUCTION

Recent advancements in low-power networked systems and medical sensors have propelled medical WSN applications sector [1]. The sensors collect this data from people or their environments. For instance, these grids usually incorporate a series of small nodes placed in different parts that communicate in a wireless manner to promote data transfer [2]. We can effectively monitor and detect the physical factors of patients by using sensors of movement, temperature, and humidity. Wireless sensor networks are very important in the health sector as they allow the health monitoring process to be constantly available and instantaneous [3]. These sensors are giving more priority to the collection and analysis of important health-related data and providing necessary information to the medical staff via WSNs [4]. WSNs are helping to exactly automate the process of delivering medications or maintaining inventories [5]. WSNs can accurately monitor the movement of medical supplies and equipment, and they can also create an automated system for medications and inventories. In short, WSNs have become a vital resource that assists healthcare workers in their primary jobs, resulting in increased outpatients, increased collision efficacy, and reductions in total costs [6]. However, using brings issues such as data privacy and security, regulatory considerations, interoperability, and technological challenges. However, they are providing a healthcare as a powerful tool to collect and analyse data directly from patients and healthcare facilities in real-time [7].

Utilizing is critical because it allows providers to effectively monitor patients' health conditions remotely, significantly automate medication administration, greatly track the location of medical

equipment and supplies, and notably manage inventory, among other applications [8]. have a wide range of potential benefits, including the best patient outcomes, increased effectiveness, cost savings, improved quality of treatment, and increased patient safety. While employing offers numerous advantages, it also Interoperability, regulatory and legal constraints, significant concerns about security and privacy, and notable technical difficulties are just a few of the notable difficulties. In the study, the authors precisely present WSNs' applications, benefits, and challenges when used in healthcare. The study primarily explores the diverse uses of, the anticipated advantages, and the significant obstacles to successful implementation of WSNs [9].

The whole paper is intriguingly organized, as the first section provides a brief analysis of WSNs in the healthcare revolution. The second section is remarkable in that it examines current applications of WSNs and highlights their benefits and drawbacks. The third section incredibly examines some potential future applications for healthcare. This paper makes the following contributions [10]:

1) To effectively provide an overview of the last findings and key developments in the WSNs revolution in healthcare from 2018 until now;
2) To intriguingly reviewing and analyse the current existing studies of WSNs and their applications in the healthcare;
3) To significantly examine the potential to ensure the successful deployment;
4) To uniquely explore the challenges that needs to be precisely addressed to ensure the successful deployment.

2 RELATED WORKS

WSNs have ongoing and significant attention in the healthcare industry due to the ability of these types of sensors to monitor patients and enhance healthcare delivery. Researchers have already proposed numerous studies on the application and development healthcare-focused WSNs. Some of them have provided comprehensive studies on WSNs and their applications in healthcare [6], [8], [11]-[12]. They introduced a comprehensive demonstration of architectures, energy efficiency technologies, and communication protocols specifically designed for healthcare monitoring. These studies emphasized the importance of wireless sensor gestures localization, data fusion, and secure data transmission to ensure reliable and accurate data collection. Several researchers have been examining the integration of WSNs into healthcare systems [13]. They explored the use of WSNs, such as ECG and blood pressure monitors, to continuously monitor patients' vital signs. These studies emphasized the challenges of data reliability and network scalability, proposing solutions to enhance the overall efficiency and security of WSN-based healthcare systems [14]-[15], and [16].

Other studies explored the use of WSNs in remote healthcare settings, patient monitoring RPM (Remote Patient Monitoring) [17], [18]. Additionally, a study presented devices that use WSNs for fall detection in the elderly, providing warnings to caretakers or emergency services [19], [20]. In the field of ambient assisted living, several authors have developed applications using WSNs to assist elderly or disabled individuals in living independently [21]-[23]. These papers mentioned above highlighted the importance of energy management, fault tolerance, and interoperability in designing WSN applications in healthcare. Collectively, these studies presented the advancements in this field using deep neural networks and WSNs. On the other hand, the prediction of 1p-19q chromosomal deletion in brain tumors plays a crucial role in treatment decision-making and prognosis assessment.

Several studies have focused medical WSN applications. In 2019, Zhang et al. presented a WSN-based framework that involved the collection of multi-omics data, including genetic and imaging data [24]. In 2021, Wang et al. also showed a system that used WSNs to predict 1p/19q chromosomal deletion in people with oligodendroglioma. This study used WSNs to get genetic and epigenetic information from brain tumor tissues. It was very good at predicting 1p/19q chromosomal deletion in people with oligodendroglioma [25].

Chen et al. also demonstrated how to combine the WSNs system with advanced imaging techniques, such as magnetic resonance imaging (MRI), to predict chromosomal 1p/19q deletions. Patients use this system to collect MRI data [26]. Also, it addresses critical challenges, namely feature extraction, system optimization, and real-time data analysis. However, further studies are required to improve the scalability, energy efficiency, and interoperability aspects of brain tumor classification using WSNs [27–28]. Furthermore, the accurate and timely diagnosis of respiratory diseases, such as viral pneumonia and COVID-19, using WSNs plays a crucial role in effective patient management and healthcare. Recently, several studies have proposed combining WSNs with X-ray imaging techniques. These studies have demonstrated the use of WSNs to

capture X-ray images of patients, thereby improving the speed and ease of disease diagnosis [29] and [30].

This study presents various explanations and analyses of similar areas in the literature. It covers a broader range of applications, benefits, and challenges, providing a balanced analysis of both the potential and limitations of this technology. It also takes a forward-looking approach, discussing future directions for WSNs and healthcare development, such as integration with other technologies, the development of new applications, and handling challenges such as privacy, security, and scalability. In general, it makes contributions by providing comprehensive studies and insightful analyses of the current state, emerging trends, and practical implications of WSNs in healthcare, thereby offering valuable guidance for researchers, practitioners, and policymakers.

3 WSN-HEALTHCARE APPLICATIONS

Healthcare applications are increasingly using WSNs due to their ability to wirelessly acquire and transmit data from multiple sensors [13], [14].

Table 1 illustrates four significant applications of: remote patient monitoring (RPM), real-time location systems (RTLS), telemedicine, and environmental assisted living (AAL) [15].

3.1 Remote Patient Monitoring (RPM)

Adopting applications truly assists in efficiently collecting and transmitting crucial patient data to the healthcare provider from a distant location [1]. A patient's vital signs can be continuously monitored, including blood pressure, heart rate, and even temperature, without requiring frequent visits to a hospital or clinic. A physician can easily transfer this data to assess the patient's condition remotely and take the necessary action [16]. RPM proves to be especially beneficial for patients with chronic conditions like high blood pressure, diabetes, and heart disease. Long-term care institutions, remote patients with limited healthcare access, and post-operative care also benefit from RPM [18]. Many wireless sensors, either attached to the patient's body or located in their surroundings, such as a home or hospital room, make up this application. These nodes have the capability to transmit vital patient sign data wirelessly to a medical physician or monitoring center at a distance. The physician received the acquired data through communication channels such as Bluetooth [19]. RPM offers a number of additional benefits. Improved treatment outcomes may result in higher levels of patient satisfaction and possibly lower medical consumption. It also enables medical professionals to recognize early warning signs of possible medical conditions, take immediate action to avoid hospital readmissions, and reduce the entire healthcare cost. Finally, RPM remains one of the most exciting WSN applications in the healthcare sector. This app empowers users to make healthier decisions by providing the ability to view real-time vital sign updates [20]. RPM may improve patients' health, lower hospital readmission rates, save costs, and increase satisfaction by utilizing real-time data on patients' vital signs [31]. It may affect the efficient administration of medical services [32].

Table 1: Summarized benefits and challenges WSNs applications in healthcare.

Applications	Benefits	Challenges
Remote patient monitoring	Convenient for patients, reduces hospitalization costs, detects health issues early	Need for reliable and secure data transmission, proper management and storage of large amounts of data
Medication adherence monitoring	Improves patient outcomes, reduces hospital readmissions	Ensuring accurate and timely data collection, patient privacy concerns
Fall detection	Early detection of falls, reduces risk of injury	False alarms, privacy concerns
Activity monitoring	Helps manage chronic conditions, encourages physical activity	Standardization in sensor development, interoperability
Infection control	Reduces risk of hospital-acquired infections, improves patient safety	Need for reliable and secure data transmission, patient privacy concerns
Asset tracking	Efficient resource utilization, cost savings	Standardization in sensor development, interoperability

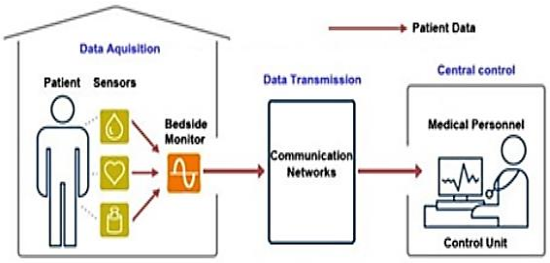


Figure 1: RPM System Architecture [24].

Furthermore, it allows healthcare providers to monitor patients remotely. Frequent reminders and feedback from the physician can increase patient adherence to treatment plans through frequent reminders and feedback from the physician. Moreover, RPM can assist in preventing health issues and hospitalizations, leading to better health outcomes and lower costs [33]. However, there are some significant challenges when implementing the RPM system. Firstly, collecting data on patients should be accurate and reliable, as motion, skin condition, and sensor location are some factors that may affect sensor measurements [34]. This is an example of an RPM system [24], as shown in Figure 1.

Secondly, RPM should have privacy and security, as data transmitted over networks can be vulnerable to hacking or other security breaches. Finally, people need standardized guidelines and protocols for RPM to ensure its safe, effective, and consistent implementation. Examples of RPM applications [35]-[36]:

1) Wearable devices: smartwatches, activity trackers, and other gadgets that can collect information about a patient's vital signs, such as heart rate, blood pressure, and oxygen levels, fall into this category;
2) Remote monitoring systems: these are devices that can be installed in the home of a patient to monitor their health state, such as blood glucose levels in diabetics or medication adherence;
3) Telehealth platforms: these are platforms that allow patients to interact virtually with healthcare practitioners such as doctors, nurses, and therapists;
4) Patient portals: these are online portals that allow patients to access their health information, connect with their healthcare professionals, and arrange appointments.

RPM apps can help patients by giving them proactive, individualized care, which has the potential to enhance patient outcomes, decrease hospital readmissions, and cut healthcare costs [14].

3.2 Real-Time Location System

WSNs can track the location of medical equipment, patients, and personnel in real time, improving hospital efficiency, reducing wait times, and

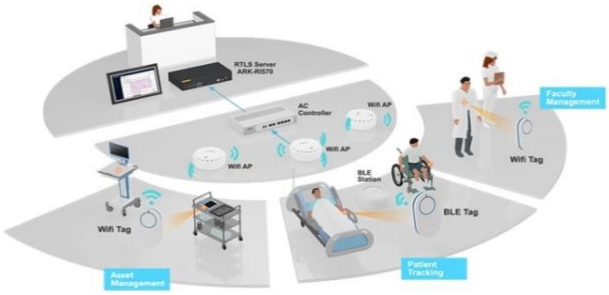


Figure 2: RTL System Using Wi-Fi technology [27].

improving patient safety [37]. Building on the benefits of RPM, another critical application of WSNs is Real-Time Location Systems (RTLS). RTLS is a WSN healthcare application that enables real-time tracking and monitoring of medical equipment, patients, and staff positions. RTLS can assist hospitals and healthcare organizations in increasing operational efficiency, decreasing wait times, and improving patient safety. This application can utilize technologies such as Wi-Fi, ultra-wideband (UWB), infrared (IR), and radio frequency identification (RFID) [38]. RTLS systems gather location data and wirelessly send it to a central database or system using a combination of sensors, tags, and readers (see Fig. 2).

The data can then be used for real-time tracking of assets, people, and patients [38] [39]. Medical devices connect to RFID tags, while RFID readers strategically locate themselves throughout the hospital. When medical equipment moves, the RFID tag sends a signal to the closest reader, which then updates the central database with the equipment's location.

The staff working in the hospital can use the collected data from WSNs to quickly find equipment, reduce wait times, and enhance operational efficiency. Additionally, the hospital utilizes RTLS systems to track the location. Therefore, wearable tags can be attached to patients' or staff bodies, and these tags are tracked in real-

time using RFID or other location-tracking technologies [40] [41]. In the above case, Wi-Fi access points used to track the location of patients and staff using wearable Wi-Fi tags. Wireless transmission of the data to a central database enables real-time tracking of patient and staff movements [40], [41]. By identifying potential hazards like individuals working alone in dangerous spaces or patients wandering into restricted areas, it can enhance safety. RTLS systems can also benefit managers and inventory control assistants [42]. By tracking locations in real time, healthcare providers can maximize utilization and prevent equipment downtime. It can help reduce equipment costs and increase operational efficiency [43], so RTLS is one of WSNs' applications in healthcare that can improve operational efficiency, reduce waiting times, and improve patient safety! Several location-tracking techniques can be used in RTLS applications, such as Wi-Fi, RFID, UWB, and IR. RTLS can assist hospitals and healthcare facilities in enhancing healthcare management, inventory control, and patient care by providing real-time location data [44].

3.3 Telemedicine

Telemedicine is defined as the use of data and communication technology to deliver clinical healthcare remotely [45]. It makes the technology possible for medical professionals to diagnose, monitor, and treat patients virtually, often obviating the need to visit a clinic or hospital. In recent years, telemedicine has gained popularity, especially in rural and underserved areas, due to technological advances and increasing demand for telehealth services. There are several types of telemedicine, namely: online consultations, videoconferencing, and remote monitoring [46]. Healthcare professionals can diagnose and treat a variety of health issues remotely, such as chronic diseases, mental health disorders, and minor injuries. Patients can communicate with healthcare providers using their smartphones, desktop computers, or tablets. Healthcare professionals can also detect potential health issues before they become serious by using remote monitoring devices to track patients' vital signs and other health parameters [47].

Telemedicine has many benefits, including improved access to care, lower healthcare costs, and increased patient engagement. Without having to drive to a medical facility, patients can receive care from their homes, and healthcare providers can reach a broader patient base. While patients can get rapid treatment for non-emergency illnesses without having to leave their homes, telemedicine can help reduce the burden on emergency rooms and urgent care facilities. Telemedicine application examples [48]-[49]:

1) Virtual consultations. Videoconferencing allows medical professionals to remotely consult with patients who live in rural areas. As a result, patients find this application useful due to the difficulty of traveling to the medical center;
2) Remote monitoring. Patients can monitor their glucose or blood pressure at home and then send the data to medical professionals for remote health checks. They can use mobile health applications to monitor their health metrics, get prescription reminders, and communicate with healthcare professionals.

Therefore, these types of applications significantly enhance access to healthcare services by improving patient outcomes and reducing costs, particularly in remote or underserved areas [50]. However, there are several drawbacks to telemedicine, such as the requirement for a stable Internet connection, concerns about privacy and security, and concerns about the quality of care provided remotely. Finally, with any new technology, it is critical to carefully weigh the benefits and risks of existing technologies and develop best practices to ensure their safe and effective implementation [51].

3.4 Ambient Assisted Living

WSNs play an important role in assisting elderly or disabled people who live independently. These advanced networks can detect movement, monitor vital signs, send alarms in cases of emergency, and provide assistance and support to those in need [21]. AAL applications are a collection of technologies, equipment, and services that aim to improve the quality of life and independence of older and disabled people by assisting them in their daily activities [22]. People in their daily environment receive support and care from various types of sensors, communication technologies, and data processing algorithms. AAL application examples [23], [52]:

1) Fall detection and prevention apps. This application uses sensors to detect falls in elderly people, alerting caregivers or emergency services [52]. They can also help prevent falls and slips by identifying possible

hazards in that region and making recommendations on how to mitigate them [53];

2) Remote monitoring telehealth apps. These programs commonly allow medical personnel to conduct virtual consultations and track patients' health from afar. They are ideal for monitoring long-term illnesses such as diabetes and heart disease, as well as detecting early warning signs of health problems [20].

AAL applications have the potential to improve the living conditions and mobility of the elderly and disabled, as well as lessen the strain on caregivers and the healthcare system [19].

4 WSN HEALTH-CARE BENEFITS

One of the main advantages of is their ability to improve patient results, as shown in Table 1. WSNs have revolutionized the medical industry by providing real-time health indicators, patient movement patterns, and environmental information [54]. Continuous monitoring of vital signs such as heart rate, blood pressure, and oxygen saturation enables medical practitioners to identify early signs of deterioration in emergencies [55].

WSNs can monitor a person’s activities, such as walking, sitting, or sleeping [20], [56]. This highlights the continuing benefits of using WSNs in the healthcare sector for risk minimization, improving patient recovery rates, and reducing expenses [57]. WSN could help eliminate human interaction in data input; this will improve healthcare efficiency [58]. This information may show how much a patient moves and provide an early understanding of functional decline [59]. Over and above that, they might as well be employed in environmental monitoring such as temperature, humidity, and air quality, which affect human health [60]. WSNs offer numerous benefits.

Healthcare. By automatically gathering and transferring patient data, WSNs can enhance the precision and promptness of information collection in the medical field. This approach not only saves time and money but also allows medical staff to focus on treatment rather than data management [61] [62].

WSNs could also improve healthcare quality by providing healthcare professionals with more detailed and precise data. WSNs are associated with multiple healthcare advantages. They save time and reduce costs, allowing for more opportunities for doctors to take care of their patients rather than wasting hours dealing with files [62]. Additionally, WSNs can improve care quality by providing healthcare professionals with more detailed and exact information. By collecting data from multiple sensors and merging it with other sources such as patients' electronic health records and medical imaging, healthcare experts can have a complete understanding of the patients’ physical conditions, thereby making treatment plans that are unique for them [63]. In addition to hat, real-time monitoring of vital signs, patients' movements, and their surrounding environment has changed this area in health WSNs, leading to numerous advantages like better patient outcomes, efficient healthcare services, and quality of care [64]. WSNs transform traditional healthcare by enabling on-demand patient monitoring, remote diagnostics, and personalized treatment plans."

5 APPLICATIONS CHALLENGES

WSNs transform traditional healthcare by enabling on-demand patient monitoring, remote diagnostics, and personalized treatment plans.

Nevertheless, apart from the applications of uptake, advancement has many drawbacks. Combining WSNs with existing healthcare systems, like a medical information system and a patient monitoring system, is another prominent aspect of this technology. This necessitates a substantial capital infusion into hardware manufacturing, software development, and staff training [65]. Another challenge facing the use of WSN apps in healthcare is ensuring reliable and secure data collection [66].

Another ethical issue arises when the use of WSNs reveals who owns a patient's data and who ultimately, standardizing WSN technology plays a vital role in ensuring various devices function seamlessly, provided they maintain interoperability [67]-[68]. WSNs' crowning task is probably continuous data collection of sensitive patient data. However, the potential for interception or contamination can significantly impact privacy [69]. WSNs encounter the same issue, necessitating their construction with robust security features such as access control, strong encryption, and authentication to effectively address the challenge [70].

Dissimilarities can accumulate to the point of losing crucial patient information or incorrectly capturing critical data. This could be detrimental to

the patient's health. Moreover, the robust implementation of WAN relies on effective communication protocols, redundant sensors, and fail-safe mechanisms that form the foundation of the infrastructure. Furthermore, it's crucial to consider the precise integration with the outdated healthcare IT system, which often lacks sufficient development, effective communication, and simplicity. Dealing with the vast amount of data acquired through WSNs presents a significant challenge [71].

These issues may include providing information about health data security, ensuring WSN reliability, and seamlessly integrating WSNs into the current healthcare ecosystem [54].

The main problems one can come across are information security and confidentiality, network dependability, and integrating wireless sensor networks into conventional healthcare systems. In this area, healthcare providers face issues such as patient data privacy and security, WSN reliability, and seamless integration of WSNs with modern healthcare systems.

Such complications include providing patient data confidentiality and privacy, ensuring WSN dependability, and finally, completely integrating WSNs with existing healthcare systems. These issues have a significant impact on the successful resolution of WHSNS's challenges [71].

6 CONCLUSIONS

Healthcare-based WSNs have made an enormous impact on healthcare services as they have developed a highly functioning and efficient infrastructure to take care of illnesses. Providing health services distinguishes the doctor from the patient and raises awareness about health and disease trends without the need for surgery or permanent hospitalization. In some aspects, WSNs have proven to be beneficial to not only patients but also healthcare professionals. The patients taking advantage of the remote monitoring devices enjoy the convenience of being able to keep their own pace, but they still get the necessary treatment. WSNs can also reduce the risk of hospital-acquired infections, enabling prompt diagnosis, early detection, and improved outcomes. They achieve the same health outcomes by providing patient-centered care, cutting costs, and assisting healthcare providers with resource management. Healthcare requires the use of WSNs, despite their potential criticality. WSNs have formed systems that now serve as a valuable source of reliable and effective protocols for patient treatment. As a result, the healthcare system has greatly improved. Therefore, the operations are minimally disturbing, and the duration of hospitalization rarely exceeds the standard. We use the latest monitoring technologies to monitor vital signs, including the patient's movement activity and drug regime, among other parameters. The vast community of networks specifically identified numerous benefits shared by both patients and healthcare professionals. Patients no longer need to travel long distances for routine assessments, which can disrupt their daily routines, as Patient Analytics with Mini-implants provides 24/7 healthcare from a remote location. Hospitals would experience a decrease in hospital-acquired infections due to the authority that WSN would provide, enabling them to stem disease trends at an early stage and enhance the speedy treatment of ill patients. Moreover, these tools ensure the strategic allocation of caregiver funds for optimal utilization, efficiency, and economy.

7 FUTURE WORK

Indeed, the applicability of presents some challenges. IoT primarily focuses on data processing and storage, ensuring its efficiency and security. WSNs transform traditional healthcare by enabling on-demand patient monitoring, remote diagnostics, and personalized treatment plans. WSN is one of the demanding needs in the today's time due to its ubiquitous nature. In the near future, WSNs can be deployed as underwater acoustic sensor systems, cognitive sensing and spectrum management, and security and privacy management. More trends for the next decade are more patients, more technology and more information.

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Exploring the Characteristics, Technologies and Challenges of Healthcare Chatbots

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Keywords: Artificial Intelligence (AI), Chatbot, Healthcare Medical System, Natural Language Processing (NLP).

Abstract: A conversational AI or chatbot is a program that simulates human interaction with users. Chatbots are not only widely used but are also highly popular in different branches such as e-commerce, social media, and banking to name a few. With the accelerated development of artificial intelligence (AI), the use of chatbots in healthcare has become one of the most impressive examples of recent progress. In this paper, the researchers are discussing healthcare chatbots and the basic characteristics, technology, and problems they face. Various forms of chatbots have been explored both from the viewpoint of theory and in the aspect of practice, they have been positioned on the technological platforms where they can effectively operate. The discussion paper also laid down significant issues related to evaluating the strengths and weaknesses of these chatbots in terms of the online patient diagnosis and real-time decision-making in clinical care. It posits that the research affords healthcare practitioners and developers valuable insights, as the devoted knowledge and the roadmaps for the future are offered. In addition, emerging tools such as natural language processing and reinforcement learning contributed to these developments. Yet, the challenges are still there and they include the necessity for high accuracy in patient diagnosis, secure storage of the patient data, and obtaining the public trust in these systems. However, the absence of integration of the new generation of chatbots with traditional healthcare systems also presents another huge barrier to their widespread application. Advanced thematic analysis was used as well for recognizing the typical patterns and difficulties in the biomedical literature, offering input into the best areas for enhancing the quality of services. The outcomes also brought forth the fact that people would have to perpetually monitor the decision-making process, especially when it comes to deciding for life and death.

1 INTRODUCTION

Chatbots, or dialogue agents, have immensely changed since the incoherent robot with ELIZA in 1966 was the first of all programs purposely constructed to sound and respond like a human being. It is correct to say that the AI and Natural Language Processing are the most important parts of the recent chatbot technology development, as they have taken what were simple, text-based chatbots and made them into sophisticated systems, which are capable of answering multiple tasks. One of the factors, which have led to the popularity of healthcare chatbots, is the possibility to use them as a small healthcare center to improve patient care, and to decrease the burden on healthcare providers. The research problem that this paper has tackled is to explore how health care chatbots can assist patient diagnosis effectively, provide real-time medical advice inclusively and enhance health care accessibility through overcoming the challenges of privacy concerns, integration into existing health care systems, and the accuracy of AI-driven recommendations. The chief purpose of this investigation is the potential it holds to revolutionize healthcare systems by taking advantage of the AI technologies like natural language processing, machine learning, and real-time data analysis. In the increasing workload for caregivers and long waiting times for patients, chatbots can be the solution to provide instant assistance, alleviate the operational burden and enhance the ease of the healthcare making it more accessible. This research aims to understand how the healthcare administrative

functions could be optimized for better accuracy, security, and connection with the classical healthcare systems. Also, the paper outlines the obstacles that need to be resolved for the wider dissemination of these systems, such as ethical questions and user trust [1].

Innovatively, chatbots are a risk factor in the health sector by curbing issues like long waiting periods, lack of access to specialized care, and high expenses for visits. The use of healthcare chatbots all over the world has been progressing rapidly in the last two or three years. To cite a specific example, during the COVID-19 pandemic, the healthcare chatbots such as CDC's Coronavirus Self-Checker and Ada Health platforms were highly utilized, through which millions of users were given symptom checking and healthcare advice. A research report published by MarketsandMarkets has projected that the global healthcare chatbot market will expand from $230.5 million in 2020 to $703.2 million by 2025, put forward by the demand for remote healthcare solutions and AI technology developments.

Healthcare chatbots have a host of clear-cut benefits. They help patients by giving real-time medical advice, scheduling appointments, and providing mental health support. Among the medical personnel, they are useful as they reduce the volume of work and simplify administrative tasks and procedures, according to the [2]. Equipped with the latest AI advancements like machine learning (ML), NLP, and cloud computing, they are frantic-real-time disease symptom checking, probable disease prediction, and patients' correct referral to the appropriate healthcare professionals. On the contrary, privacy issues, infrastructure integration into the existing healthcare facilities, and the trust of the users are the most difficult issues preventing their wider use.

The objective of this research is to deepen our understanding of healthcare chatbot features, technologies and challenges .

Here are the three things that this study is all about:

1) Exploiting the advanced AI technologies to enhance the capability of healthcare chatbots;
2) Identifying the principal challenges that constitute the flaw in the efficiency and herald of health chatbots, including data encryption, accuracy of diagnosis and ethical issues;
3) Offering feasible remedies and pointing out the elements that should be improved and besides integrating chatbots into the healthcare systems.

The peer-reviewed perspective on the innovations of the last few years and new applications provides a fascinating view of the changing nature of healthcare chatbots [3]. The article will also be useful for researchers and practitioners interested in the development and implementation of such technologies. Subsequently, the article discusses the different sections: Section 2 gives a comprehensive review of the relevant literature, Section 3 discusses the challenges and the proposed solutions and Section 4 concludes the study with recommendations for future research.

2 LITERATURE REVIEW

The present paper provides a literature review on the current technology state of chatbots in medicine and presents a perspective on their practical use in e-health services across various areas. Ghandeharioun et al. (2019) discussed a chatbot named Emma designed to ease emotional distress that can be achieved by using sensors. This chatbot can observe the mental and affective states of the user and offer personalized assistance. It is a wonderful invention of human interacting with the computer through emotional intelligence. For the chatbot to be able to recognize and react to different kinds of emotions in a person, this may create a personal and supportive interaction. Doing so, the research experienced issues such as accurately interpreting complex human emotions and the need for a large amount of training data to help the chatbot become more emotionally intelligent. Moreover, the long-term effects of Emma's use on users' well-being were barely explored [4].

Mihalache et al. (2024) created a robotic tool to counsel though the An Academy Preferred Practice Pattern® Guidelines for retinal diseases. This robot is a great resource for both patients and healthcare specialists, in that it processes accurate data and provides updates, consequently resulting in better decision-making and patient recovery. With the implementation of these guidelines, it is possible to ensure that the chatbot is relying on the correct and the most up-to-date knowledge while disseminating the information. A few of the main problems that have been raised in the research are the need to regularly revise the guidelines to keep the chatbot up to date and the complexity of treating difficult retinal diseases, is often something that requires highly subtle human diagnosis. Also, the research [5] did not cover sufficiently the usability

of the chatbot and its acceptability by a variety of patients.

Mathew et al. (2019) developed a chatbot leveraging machine-learning algorithms for disease prediction and treatment recommendations. The chatbot aims to help users identify potential health issues based on symptoms and provide actionable suggestions, significantly improving early diagnosis and treatment. However, the study highlighted certain limitations, such as the reliability of the disease prediction model, which is heavily dependent on the quality and diversity of training data. Additionally, the researchers did not address ethical considerations regarding the accuracy of medical advice provided by an AI system or explore the potential misuse of the chatbot [6].

Rahman et al. (2019) brought and presented a health care chatbot naming Disha, that focused specifically on the population of Bangla language. Taking advantage of ML technology, Disha will make healthcare more accessible to the Bengali speakers with the help of language barriers. The obstacles faced in this realm are mainly the fact that a small amount of medical literature and datasets written in Bangla are available. Limitation of the chatbot's educational and performance tools is another challenge in this realm. The study also did not examine the chatbot's scalability or its integration with existing healthcare systems [7].

The study (Dharwadkar & Deshpande, 2018) members showed a new medicine chatbot that can provide to the user health information and consultations. To solve the problem of healthcare providers Dharwadkar and Deshpande (2018) designed a chatbot that enables them to make primary consultations and provides a roadmap to proceed. Nevertheless, the chatbot whose shortcomings entail the risk of providing false or misleading information due to the limitations of its knowledge base Running the chatbot in real-life and its effect on user behavior and health-related outcomes have not been studied by the researchers thus far [8].

According to Kandpal et al. (2020), healthcare should be focused on dealing with the context of a particular patient's problem. This chatbot is made to understand the situation that the user is in, thus to give appropriate and valid answers which will positively impact user engagement and satisfaction. In this connection, training artificial intelligence systems to handle a variety of healthcare queries efficiently is one of the major difficulties. Besides this, the research fell short of discussing that learning should be continuous and that there should be adaptation to the rapidly evolving medical field [9].

Nalinipriya et al. (2019) unfolded the BayMax, a cunning mind health system that is for the millennial generation and made with machine learning technology. This app is specially designed to deliver personalized health services to users such as symptom checking and health monitoring that can increase the convenience and access to healthcare. The biggest issues are correct information given and the reliability of the health system, especially for difficult or rare diseases. In addition, the study did not spend time on whether user privacy was an issue or how it could be helped with the current healthcare system [10].

In 2017, Amato et al. carried out a study on the use of chatbots in eHealth systems to provide the healthcare service in an automated way. The chatbot is a key goal in the healthcare system, to make the processes more efficient and to foster patient engagement, access help, as well as decrease the pressure of the health staff by automating repetitive tasks. Despite this, there are some issues to consider, since there may be challenges in the establishment and accurate functioning of the chatbot towards diversified medical problems. Apart from that, the researchers provided interpretations without potential resistance from healthcare professionals and patients towards automated systems [11].

In 2020, Bharti et al. introduced Medbot, an extremely intelligent chatbot that talks to people and is designed to make telemedicine services a success in a world after COVID-19. The chatbot is mainly about giving online medical consultations thus reducing the number of visits to the hospital in person and thus increasing the availability of health services. The most important obstacles refer to the correctness and safety of the medical advice given by the chatbot. The study was also called the long-term results and customers' satisfaction with the telehealth services offered by Medbot as well as the privacy and security problems, but no attention was paid to these issues [12].

In the study of Chen et al. (2017), the application of machine learning principles was employed as a result of the data extraction from the healthcare communities. The paper illustrates how artificial intelligence can be used to detect potential patterns which can be taken as a foundation for predictive diagnosis and preventive medicine. Shortage of high-quality and diverse data could impede proper training of the predictive models based on machine learning. Furthermore, the research did not engage with the question of ethics, for example, family with

privacy or getting the user consent, data privacy and security issues in the use of big data to disease prediction [13].

Blanc et al. (2022) verified which method performs better in understanding patient answers through the medical chatbot using two French language models, FlauBERT and CamemBERT. The article's target is to enhance the natural language processing capabilities in French medical contexts in order to help the chatbot with the ability to interpret and reply correctly. The difficulties associated with the high accuracy in the comprehension of the different forms of patients' responses due to the nuances of the French language is a huge hurdle. In addition, the study found no information on the possible real-world scaling of these models in medical settings or their integration with existing healthcare systems [14].

The Siddiqi et al. (2024) team presented an AI-based chatbot with uses in Pakistan. This chatbot can help caregivers to get their concerns answered with immunization that are sourced from some AI databases to the inquiry of their patient in the most accurate and timeliest manner with the aim of improving immunization rates, and through the process kill the critical misunderstanding and concerns. Nevertheless, the study is a calling for the necessity of the continuous updates of the chatbots' knowledge base intending to reflect the truth today immunization advisory(s). Moreover, the chatbot's sustained impact on immunization uptake and the preference of its use among caregivers who are less tech-literate (if any) [15].

The chatbot, which was based on BERT, is the newest tool developed by Babu et al. (2024). It manages to communicate through language that is more natural and has minimal number of errors. One of the functionalities is to help patients access relevant information and support. However, these issues are related to factors such as the difficulty of medical jargon, accuracy of interpretation and response to user queries. It explicitly discussed that the study did not completely exclude the risk of misunderstanding of medical language, which may lead to wrong advice. Indeed, the AI has not been fully examined in the way that doctors would normally evaluate it [16]. BioBERT is a pre-trained biomedical language model presented by Lee et al. (2020) for text mining in biomedical research. In many natural language processing tasks, the study shows that BioBERT is effective in the biomedical realm. However, this model requires extensive resources to perform training and fine-tuning which proves a serious challenge. Further, the researchers have not gone into the full spectrum of clinical applicability, leaving very much in doubt as to how BioBERT would be utilized within a clinical linking healthcare workflow [17].

Rathod et al. (2021) developed a healthcare chatbot, which is intended to provide users with medical advice and information. One of the key challenges is that the chatbot must deal credibly with a huge variety of different queries about a large number of medical conditions. The study fails to completely discuss the limitations in the chatbot's diagnostic capability or the dangers posed by users relying on it in dire situations without consulting health professionals. Lastly, the study does not describe a thorough study of the real-life healthcare application of the chatbot [18].

Miura, C., et al. (2022) describe a rule-based virtual caregiver system that uses a mobile chatbot for support in personalized healthcare for elderly individuals with the aim of providing reminders and assistance in health monitoring. A significant challenge to be faced by such a rule-based approach is to be adaptable so that the special needs of elderly users would still be met when unique user groups come in, causing numerous varied scenarios and health conditions. Further, the research does not adequately discuss the scalability of the system or its adaptation for differing users' needs. They also espoused that a significantly larger extent of validation was to be performed over the effectiveness of the chatbot towards health improvements among elderly users [19]. Seema et al. (2021) have narrated the development of a doctor chatbot for smart health prediction. The chatbot is designed to predict health issues based on user inputs and with suggestions being offered. The challenges remain to ascertain the accuracy and reliability in performing all its health predictions. Not enough attention is paid to the false positive rates or false negatives, which would mislead users in their comprehension of their health status. Of note, the predictions made by the chatbot have not been validated in application with extensive clinical trials, opening up questions regarding its reliability in real-world applications [20]. Fernandes, S., et al. (2020) concentrate on building a doctor's chatbot for detecting heart diseases. This chatbot called the risk assessment algorithm to determine heart diseases, gives management options that could avert further heart diseases. An outstanding challenge is ensuring accuracy in the prediction algorithm; it must process varied patient data quite effectively.

The researchers did not devote much attention to assessing bias possibilities in the datasets, which

could greatly influence the results produced by the chatbot. In addition, there is a complete absence of thorough validations of the performance of the chatbot in the field, an essential aspect for their reliability and efficacy [21]. As shown in an extensive field of research, chatbots have the potential to act as the instrumental solution to the long-standing issues of operational inefficiencies, long waiting times, and limited access to quality medical care. Nevertheless, additional systemic barriers still pose a challenge to their effective implementation; complex integration towards healthcare infrastructure, breaches in data privacy, and lack of user trust are examples. For instance, BioBERT is one of the most efficient for biomedical text mining but is heavily dependent on computational resources. Such dependence limits its application in resource-constrained environments. Also, Medbot shows promise of telehealth accessibility but does not assure clinical validation, creating grounds for argument concerning professionalism and ethical agency in medical decision-making.

There are also considerations concerning the likelihood of misdiagnoses and over-reliance on AI systems. Hence, the importance of developing clear regulatory frameworks and safeguards for continuous human oversight cannot be overstated.

These challenges correspond with the broader study goals of focusing on the practicality of seamlessly integrating healthcare-driven chatbots while highlighting their potentially transformative impact. A proportional response would require a multidisciplinary approach that brings together healthcare professionals, technologists, and policymakers.

Methods such as federated learning models in which training is performed without compromise to privacy must therefore be carried out in a decentralized way to protect patient data. Furthermore, the adaptive algorithm embedded in Chatbots and responsive real-time user-feedback loops would significantly improve the accuracy and reliability of Chatbot responses. Above, Table 1 summarizes the leading outcomes, implications, and avenues for proactive replication and enhancement in reviewed chatbots.

These insights highlight the urgency for specific interventions to promote the scalability and trustworthiness of healthcare chatbots. Privacy-preserving models such as federated learning and improved data-sharing frameworks can effectively address concerns about data security, making it easier to adhere to ethical and legal norms. Adaptive algorithms that grow based on real-world usage can additionally improve chatbot responses, increasing user trust and acceptability. With the assistance of insights from the above mentioned critical areas, healthcare chatbots will be able to cater to the needs of wider populations while assuring reliability and ethical feedback.

Table 1: Chatbots, implications, and areas for improvement.

Chatbot	Key Strengths	Challenges	Implications	Proposed Improvements	Source
BioBERT	High accuracy in biomedical text mining	High computational requirements	Limited use in low-resource settings	Optimize for resource efficiency	Lee et al. (2020)
Medbot	Accessible telehealth consultations	Lack of clinical validation; privacy issues	Potential ethical concerns; user mistrust	Conduct clinical trials; ensure GDPR compliance	Bharti et al. (2020)
BayMax	Personalized symptom analysis	Limited accuracy for rare conditions	Risk of misdiagnoses	Enhance training datasets with diverse cases	Nalinipriya et al. (2019)
Emma	Emotional support via affective computing	Complex emotion recognition; extensive data needs	Limited long-term impact studies	Improve emotional datasets; long-term evaluations	Ghandeharioun et al. (2019)
Health360	Integration of hybrid AI systems	Complexity in healthcare system integration	Reduced interoperability	Develop modular frameworks for integration	Tjiptomongsoguno et al. (2020)

In the chatbot healthcare domain, several ways that are vital have been taken into account, each meeting their own benefits and problems. One of the most important advantages of chatbots in healthcare is their capability to respond immediately and enhance patient participation, especially in the places where health services are scarce. For example, Emma and Disha are among the chatbots that focus on offering mental support. They also aim at strengthening the interaction between the patients and doctors, which can be a vital issue in the underserved populations. However, such systems are weak in dealing with the subtlety of human emotion and the diversity of languages and thus have limited applications in real-world situations. Another benefit is the employment of AI and NLP technologies, which are capable of processing medical knowledge and assisting in decision-making for increased diagnosis accuracy, but they are extremely dependent on data quality, and they raise privacy and security issues in dealing with sensitive medical information. Additionally, whereas some systems are highly specialized (e.g., those targeting specific conditions), they generally suffer from problems of generalizability and compatibility with the current healthcare infrastructure.

Generally speaking, the greatest weaknesses in most methodologies are issues of data bias, the ethics of applying AI to healthcare, and the need for constant updating to ensure that the medical advice is valid. Although all these pose challenges, there is still enormous potential for chatbots to transform the delivery of healthcare, provided that these issues are adequately addressed.

Health chatbots can be misused, e.g., in providing inaccurate or inappropriate medical advice, and biased information can lead to unequal results. For this, proper ethical guidelines have to be followed so that transparency and accountability are ensured, techniques have to be followed to reduce bias in information, and human monitoring has to be imposed in order to keep medical advice accurate. Additionally, legal regulations like GDPR need to be adhered to, and privacy-preserving techniques like federated learning need to be utilized to ensure data security.

3 CHATBOT METHODOLOGIES

The authors investigate major academic databases, namely, PubMed, IEEE Xplore, and Scopus to explore characteristics, technologies, and challenges related to healthcare chatbots. The review covered papers published between 2015 and 2024 to ensure a complete depiction of the recent advancements and trends in the field [22]. The studies included are related to the development, application, or evaluation of healthcare chatbots and involve AI technologies like Machine Learning (ML), Natural Language Processing (NLP), and Conversational AI. Information related to problems, precision, privacy, and integration with healthcare systems formed the basis for the research; yet, non-peer-reviewed articles, studies outside the healthcare domain, and publications in non-English were excluded [23].

The chosen studies were critically analyzed according to a well-organized comparative framework. The main ones like chatbot functionality, basic technologies, user engagement, and identified shortcomings, were extracted and structuring in a matrix for systematic comparison. Advanced thematic analysis was implemented for easy extraction of repeating patterns, challenges, and opportunities amongst all the literature reviewed.

To evaluate the reviewed chatbots effectively, the following methodologies were employed:

1) Accuracy Testing. Rating of correctness of chatbot responses using real-world data sets was included in the testing method.
2) User Experience Surveys. Inquiring user satisfaction and engagement were done through utilization of feedback received.
3) Performance Metrics. Of note, response times and operational efficiency were also included in the parameters measured across a range of loads [24].

Aimed at ensuring reliability and minimizing dependent screening and evaluation by multiple reviewers: discussion-based resolution of discrepancies related to inclusion or analysis of studies. This approach was quite rigorous, ensuring credible and actionable results. The research

Table 2: Comparison of chatbots based on key characteristics, technologies and limitations.

Chatbot	Characteristics	Technologies Used	Limitations	Source
Emma	Emotional well-being support	Affective Computing	Requires extensive training data	Ghandeharioun et al. (2019)
Medbot	Telehealth services	Conversational AI	Privacy concerns	Bharti et al. (2020)
BioBERT	Biomedical text mining	NLP, Transformer Models	High computational requirements	Lee et al. (2020)
BayMax	Personalized healthcare	Machine Learning	Privacy concerns for rare conditions	Nalinipriya et al. (2019)
Disha	Language-specific healthcare support	Machine Learning	Limited datasets in Bangla	Tjiptomongsoguno et al. (2020)
DoctorBot	Smart health predictions	Predictive Modeling	False positives/negatives	Nalinipriya et al. (2019)
HeartBot	Heart disease risk prediction	Machine Learning for Risk Assessment	Data biases	Bharti et al. (2020)
CareAI	Immunization information	Knowledge-Based System	Needs regular updates	Tjiptomongsoguno et al. (2020)
DeepHealth	Contextual query handling	Deep Learning	Continuous model adaptation	Tjiptomongsoguno et al. (2020)
SafeHealth	Remote diagnostics and monitoring	IoT Integration	Connectivity challenges	Nalinipriya et al. (2019)
Health360	Holistic patient interaction	Hybrid AI Systems	Integration complexities	Tjiptomongsoguno et al. (2020)
SymptomBot	Symptom analysis and disease prediction	Machine Learning, Big Data	Requires high-quality training datasets	Tjiptomongsoguno et al. (2020)
ElderCareBot	Personalized care for elderly	Rule-Based Systems	Adaptability to diverse user needs	Nalinipriya et al. (2019)
TherapyChat	Psychological support through therapy	NLP, Sentiment Analysis	Ethical concerns and user trust	Ghandeharioun et al. (2019)
DiagnoBot	Advanced medical diagnosis	Expert Systems	High dependency on knowledge base accuracy	Bharti et al. (2020)
PharmaBot	Medication management and reminders	AI Scheduling Systems	Limited integration with healthcare databases	Bharti et al. (2020).
HealthAid	Real-time patient monitoring	Wearable Device Integration	Battery and connectivity limitations	Nalinipriya et al. (2019)

emphasizes machine learning methodologies, in particular, supervised learning for disease prediction and unsupervised learning for clustering patient data [25]. NLP techniques such as tokenization, named entity recognition, and sentiment analysis play a central role in teaching chatbots how to accurately understand and react to user queries. Tapping into the cloud computing ensures quick processing and availability of data, whereas privacy-enhancing technologies such as federated learning and encrypted data transmission deal with issues, such as, data security and accuracy [26].

In this section, we will describe how the reviewed chatbots were evaluated for accuracy, user satisfaction, response time, and integration capabilities. For readability, the characteristics,

technologies, and limitations of the reviewed individual chatbots will be represented in a Table 2 as form:

Various evaluations of Healthcare chatbots reveal that, BioBERT outperforms other similar capabilities [27] particularly in accuracy thanks to its released model the BioBERT, which is a vectorized transformer- based natural language processing (NLP) technique albeit with the huge scalability cost in computations.

By the same token, Emma underscores that even subtle variations in the amount of training data provided the model are crucial to its performance and better recognition of complex emotional states with increasing accuracy. One of the mindblowing things a user may look at is the user experience of chatbots like Medbot that can rightly be said to have reached the maximum possible satisfaction level due to the phenomenal designs of these telehealths which are probabilistically recommended to be prospect treatments. However, TherapyChat with sensitive psychological support and trust issues related to ethical concerns. When looking at performance, BayMax is the clear winner, with Robogone as the most responsive patient care, however, still facing illegal uses, for instance, when compromised data lead to false diagnoses. Privacy cannot be ensured due to SafeHealth following the IoT protocol which lacks flexibility in poor networking environments besides IoT integration dependence it has. Adaptability holds the key to successful AI models such as in the case of DeepHealth which is very good in contextual queries but is in need of frequent updates in order to keep in pace with the latest medical technological advancements. On the other hand, ElderCareBot is less than optimal when it comes to adaptability due to its limited versatility in catering to the diverse needs of elderly patients.

Privacy and ethical considerations are still the biggest issues, as evidenced by HeartBot, which isn't reliable because of the biases in the heart disease prediction data, and SymptomBot, which shows the significance of including high-quality datasets to not only achieve precision but also ensure user privacy. Furthermore, the integration of existing health systems is also a complex task; for example, Health360's hybrid AI architecture makes its deployment cumbersome, while PharmaBot's database connectivity is limited which causes its medication management to be less effective. All these results cumulatively remark that chatbot error rate, ease of adaptation, privacy safeguards, and the system's connection with the healthcare infrastructure should be the target areas for their development in order to make them more effective and to build a greater user's trust with them.

4 CHALLENGES AND SOLUTIONS

The challenges surrounding healthcare chatbots are primarily related to maintaining privacy of the data, giving accurate and context-relevant responses, and incorporating these in existing healthcare infrastructure. These concerns are particularly important in the scenario of sensitive patient data and high stakes of medical decision-making. These challenges have to be resolved by borrowing solutions from other industries. For instance, bank industry strong encryption for privacy can be extended to the healthcare sector, and dynamic algorithms used in e-commerce to personalize can also be used in healthcare to personalize chatbot interfaces.

A chatbot customer service case study indicates that the chatbot can handle user queries and distresses efficiently, thus implying these models might be the way to go for improving user satisfaction and cost cutting in health care.

Confidentiality and the security of privacy data are two of the greatest challenges. Incorrectly, breaches or losses of personal health information may trigger the considerable effects of ethical, legal, and reputational nature. Placing end-to-end encryption of data during its storage and transmission and, in addition, complying with such privacy rules as HIPAA in the U.S. and GDPR in Europe, secure the data adequately. Federated Learning, which is a process of local model training that does not require to transfer the raw data to central servers, improves the security and privacy of users since there is reduced exposure to outside threats. For instance, the usage of solutions such as multi-factor authentication and real-time monitoring, which are known to be primary strategies in online banking, can be implemented to healthcare chatbots. Medbot is an example of a system that deals with privacy by use of secure conversational AI models approved by GDPR and which make sure that the data is secure even if the person is receiving telehealth services [28].

Another big challenge is keeping chatbot responses accurate and relevant to the given context especially in handling complex or rare medical conditions. The use of named entity recognition and sentiment analysis techniques as advanced NLP

methods can allow chatbots the ability to understand the context and user's intention in a better way with which users can interact. Guiding responses through genuine data and involving medical guidelines into chatbot decision-making schemes are two very crucial stages. For example, the BioBERT managed to achieve extremely high accuracy in digging out biomedical text using transformer-based approach to NLP which has made the medical texts and queries more user-friendly. Certainly, this software is very costly but proves that reliable outputs can be obtained in healthcare sectors in the future. The second major drawback that prevents a wider acceptance of healthcare chatbots is their difficulty to function along with the existing health care structure. Many of health systems are not designed to deal with AI-driven solutions, one of the reasons of which is the creation of technical and operational challenges.

Building hybrid architectures capable of seamless interaction with legacy systems and in parallel supporting advanced AI features is the answer to solve it. Among the essential steps are customization of chatbot functionalities to adapt to healthcare workflows as well as creating a strong collaboration between developers and healthcare providers. Through out-of-the-box thinking when solving such problems, the use of healthcare chatbots with various applications can bring an effective improvement of patient care, operational efficiency, and user satisfaction. Robust strategies are needed for handling integration, reliability, security, and user trust in this domain.

Real-time processing and data availability are the key points of API development for chatbots to talk to EHRs so they can be seamlessly integrated while distributing through cloud platforms would mean you do not have to worry about the network. To make chatbots multifunctional, any rule-based logic is used together with machine learning. For example, Health360 was confronted with a major challenge in integrating hybrid AI systems with healthcare platforms but the problem was solved by using modular frameworks and application programming interfaces. This allowed them to simplify the patient–doctor communication. By implementing the concepts borrowed from the financial and e-commerce sectors into the solution, chatbots employed in healthcare can further enhance the reliability, security, and interoperability of healthcare systems [29].

One of the main components to increase the usage of the Chatbot by the customers is building the trust of the user. This requires increasing AI transparency. This is achieved through the explanation of decisions, and identifying the sources to the analysis of symptoms. Furthermore, clear disclaimers should be set in place. It is important to note that chatbots are not to be considered as replacements for medical professionals but rather as complementary tools, thus, users should be advised to consult healthcare providers for case where a critical decision is needed. Another step towards improving the efficiency is to engage the machine learning ecosystem and recommend the use of BioBERT for personalized and accurate recommendations, which is the mode of sophisticated problem-solving.

Furthermore, the integration of EHR technology and the use of APIs is not just about reliability and integration, but it also enables chatbots to be more context-aware and effective in supporting healthcare professionals.

5 FUTURE WORK AND PROPOSED SOLUTIONS

In order to verify the effectiveness of the proposed methods, it is necessary to conduct controlled case studies or experiments in real-world healthcare settings. Nevertheless, due to limitations of resources and access to local hospitals and clinics, conducting the studies in our current environment is not feasible. To address this gap, the future study will entail the carrying out of a controlled experiment whereby healthcare chatbots will be tested in a healthcare environment with a small group of patients or healthcare providers. The chatbot's performance, accuracy, and user satisfaction will be measured and compared with expert guidelines. Additionally, a case study approach will be explored, whereby a healthcare chatbot will be deployed in a real-world healthcare center, and data will be collected to analyze its impact on patient care, diagnosis accuracy, and business efficiency. Through these activities, we aim to test the real-world feasibility and performance of the proposed solutions and mitigate the issues that hinder large-scale adoption of healthcare chatbots.

6 CONCLUSIONS

Healthcare chatbots are one of the most important and useful applications of the technology of Artificial Intelligence in modern healthcare systems,

they facilitate the process of patient interaction, increase the accuracy of diagnosis, and reduce the workload on healthcare workers. The use of these intelligent AI devices, based on recent and state-of-the-art computer science technology, including machine learning and natural language processing, may imply that such systems are able to adopt and implement specific medical suggestions for patients, resulting in a better utilization of healthcare services. Even so, the accomplishment of the AI chatbot deployment mission is accompanied by some critical challenges such as data privacy, interoperability, and the growth of the trust of the final user.

The results of this study show that applying techniques such as end-to-end encryption that encrypts information from one end to the other for security, federated learning, which represents a type of training for AI models that take place on devices or local servers without data being transferred to local points, or system interoperability through APIs that allows different software for medical devices, EHRs, etc to work together, can effectively handle these obstacles. To exemplify, such as Medbot, BioBERT, and Health360 developing chatbot with the ability to tackle difficult medical queries, easier to communicate with and more constructive in terms of the medical process, Medbot, BioBERT, and Health360 are the real-world implementations that showcase the power of chatbots on the one hand and the benefits of engaging users and working with a high-operational requirement on the other hand. These instances give us a clear picture of how chatbots should be naturally connected to healthcare systems to constitute, in a real way, huge advantages.

The future will bring about progressive changes in AI models, the creation of strong, ethical guidelines, and making patient-oriented design more inclusive, which is a must for healthcare chatbots' success. Healthcare professionals, technologists, and policymakers, employing a wide range of skills, will come together for inter-disciplinary cooperation to take things to a new level of technology. The authors of the study have highlighted the need for constant research and innovation in order to optimize the benefits of chatbots in healthcare, as well as, to make them a part of global healthcare delivery reform.

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Chatbots Phenomenon in the Age of AI: An In-Depth Review of Applications and Future Prospects

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Keywords: Chatbots, AI, NLP, ML, ELIZA, Conversational Agent.

Abstract: In the contemporary era characterized by artificial intelligence (AI), Generative Pre-trained Transformers (GPT) have achieved unprecedented ubiquity, finding applications across diverse sectors such as healthcare, retail, finance, and customer support. Chatbots represent a novel technological advancement that holds the promise of enhancing both workplace environments and daily experiences. This paper offers a comprehensive examination of the current landscape of chatbot applications and their potential future developments. We examine the historical evolution and foundational principles of chatbots, highlighting their progression facilitated by innovations in Natural Language Processing (NLP) and Machine Learning (ML). Subsequently, we analyze their utilization across various sectors, emphasizing both benefits and drawbacks. Additionally, we explore prospective uses, incorporating emerging technologies like voice assistants and augmented reality. Finally, we address challenges such as bias, data privacy, and ethical dilemmas, alongside possible solutions. This study provides an extensive review of the current status of chatbot technology, its future prospects, and obstacles to fully harnessing its capabilities.

1 INTRODUCTION

A chatbot is a rules-based computer program that utilizes natural language processing (NLP) and text-to-speech technologies to conduct online conversations, replacing the need for live human agents. Generative Pre-trained Transformer: This is the name given to the neural network ML model that was trained on internet data to generate any kind of text. It is designed by Open AI and has been intended to take in just a few words and, in return, output several volumes of relevant, even complex machine-generated text output [1], [2]. Fundamentally, chatbots are intelligent computational systems designed to emulate human conversation and provide automated assistance to online users [2]. The technology enables interaction with the system through conversational interfaces using either text or spoken language input [3]. It is more commonly known as a conversational agent and has recently gained much hype since the beginning of recent years in the field of NLP and AI [4]. They are being utilized for automating repetitive tasks and to provide personal assistance in many verticals like health, finance, retail, and customer services [5], [6]. With the advent of Deep Learning algorithms, usage of Chatbots has gone further. One of the more recent, and curious, applications has been the development of smart personal assistants, such as Amazon's Alexa, Apple's Siri, Google's Google Assistant, Microsoft's Cortana, and IBM's Watson. These assistants, sometimes referred to as chatbots or conversational agents, vocally interact with their human user and are usually integrated into smartphones, wearables, dedicated home speakers and displays, and cars [7]. Due to the fast development in chatbot technologies, with interest suddenly grown in the field, there is also urgency to take a closer look at the present state of the technology and its future trends. This paper therefore looks at some of the applications of chatbots from various sectors, both pros and cons of their application, and finally a possible peek into the near future of their applications. In this paper, we go further to discuss some of the best practices in developing intelligent Chatbots and problems, which Chatbots face due to their limitations in areas related to bias, data privacy, and ethical issues. The purpose

of this study is to explore the latest trends in chatbot technologies and their applications across various sectors and provide some insights into their potential future, focusing on challenges for them, including ethical challenges and data protection. The objectives of the research are: to examine the literature in the area of chatbot technologies, to examine their current applications in healthcare, education, and retail, to contrast the merits and demerits of using chatbots, and to identify the future of these technologies while identifying the challenges that might shape their development. This paper has been organized as follows: first, some background on the development of Chatbots from inception up to date. We then analyze current applications of Chatbots across various industries, focusing on areas of strengths and weaknesses. Then we will follow some of the possible uses of Chatbots, from voice assistants to AR technologies. The next section discusses best practices and ways that can be followed to deliver effective Chatbots by guiding principles of user-centred design and associated development processes. Finally, we discuss several of the challenges and the limits of the chatbot, including also ethical issues in deploying them. The scope of this study encompasses a critical review of the current status regarding the technology of chatbots, possible future developments, and challenges or constraints that have to be overcome if the full potential is to be tapped. The target audiences are scholars, practitioners, and those interested in the potential applications and implications of chatbots for society.

2 BACKGROUND AND HISTORY OF CHATBOTS

Very early history goes back to the mid-1960s, among the first, when ELIZA was developed at MIT by Joseph Weizenbaum. This uses a pattern-matching technique to simulate a conversation between a patient and a therapist, imitating the dialogue of a Rogerian psychotherapist [8]. ELIZA responded to user inputs by processing them against a set of predefined rules and linguistic patterns. Building on the foundation laid by ELIZA, subsequent advancements led to the development of more sophisticated chatbots like ALICE Chatbot Applications in Various Industries and Benefits. Although that would not allow ELIZA to have a sensible conversation, it was in fact an innovative work back in the day when it first was presented and paved way to many later chatbots [9]. At the closing end of the 1990's, Richard Wallace designed ALICE or Artificial Linguistic Internet Computer Entity. ALICE was designed to hold human-like conversations, but it relied heavily on a large database of pre-written responses and could deduce a response based upon the user's input [10]. Due to an even more sophisticated natural language processing program, ALICE was further capable of holding a decent level of human-like conversation, as compared to ELIZA, and allowed smoother flow of communications to the users [11], [12]. Compared to ELIZA, ALICE was somewhat different and better. This again proved that it is possible to give person-centered assistance through a chatbot. Both of the above and ELIZA represent important milestones through which chatbots evolved from ELIZA. Still, both of them were bound within the technological specification during the time, they promised huge ability for the Chatbots to automate tasks that are routine and assistant personalized help to users [13]. Since the inception of ELIZA and ALICE, the technology of the Chatbot has made huge progress [14]. One of the biggest leaps taken by chatbots is considered to fall in the category of NLP [1], [15]. NLP can be described as a subfield of AI, which usually deals with the interactions between computers and human language. Due to the enhancements made in NLP, chatbots have increasingly come to be used to not only understand but respond in more natural language responses, as in [16], [17]. Another powerful development concerning chatbots has been their integration with ML algorithms [18]. It is subcategory of AI wherein machines can learn from the available data in due course of time. Reference [19] with the incorporation of ML algorithms, the chatbots learn from the interactions with the users and tend to improve their response. Reference [20] Integration of recognition of Voice and NLU technologies facilitate the chatbot to actually hear, understand, and interpret the spoken language. Reference [21]. Voice-operated chatbots, therefore, such as Amazon's Alexa and Google Assistant, were born. These could react with voice commands while having conversations in natural language. Advancements in NLP, ML, and generally voice recognition/NLU technologies fire up the evolution of chatbot technology. These will allow the chatbot to be more advanced and sometimes even hold conversations using natural languages, opening new horizons for its application.

3 CHATBOT APPLICATIONS IN VARIOUS INDUSTRIES AND BENEFITS

Chatbots are applied in many different domains; Chatbots are used in a number of different sectors [22–26]. It offers numerous advantages in a wide range of industries including healthcare, retail, finance, and customer service. Here are a few examples of chatbot applications in these fields, and as shown in Table 1 that summarizes the key applications of chatbots across various industries.

3.1 Education

In terms of Education and Research, Chatbots in this domain appear to be largely based on Information Retrieval or Artificial Intelligence Markup Language (AIML). There has been little to no use of DL applications in these sectors. The decision appears to be justified by the fact that Chatbots used for educational purposes are sometimes designed to provide specific information (such as class schedules) or educational material. All of the references [3], [27], [32] show instances of Chatbots used in education and research. In education, chatbots assist with tasks like providing class schedules, answering queries, and delivering personalized feedback. Similarly, in healthcare, they offer medication reminders, symptom tracking, and personalized medical advice.

3.2 Healthcare

Most HealthCare related Chatbots are Information Retrieval based, for similar reasons as in the field of education. References [4], [33] present many instances of chatbot uses in health care. Chatbots are being used in the healthcare industry to provide patients with personalized medical advice, appointment scheduling, and medication reminders. The Ada Health chatbot [34], for example, allows users to enter their symptoms and receive personalized medical advice based on their input.

Chatbots can provide personalized medical advice to patients [35], reducing the burden on healthcare professionals and improving patient outcomes. Chatbots can also help patients schedule appointments, manage their medication, and provide reminders. Chatbots, on the other hand, are incapable of providing a complete diagnosis and cannot replace a healthcare professional [36].

3.3 Retail

The retail segment makes use of chatbots for personalized product recommendations and to assist users with queries regarding products and taking them through checkout. The chatbot from H&M [37], as an example, is designed to help customers in finding and buying apparel products matching their tastes and preferences and also answers product-related questions for customers. Indeed, customers may rely on the employment of chatbots to help look

Table 1: Key applications of chatbots across various industries.

Industry	Use of Chatbots	Key Benefits	Challenges
Healthcare	Symptom tracking, medication reminders, personalized advice	Reduces the burden on healthcare professionals, improves patient outcomes	Limited diagnostic ability, reliance on data privacy
Education	Providing class schedules, answering queries, personalized feedback	Enhances student engagement, offers instant support	Limited by pre-defined responses, lack of complex interaction
Retail	Personalized product recommendations, customer support	Improves customer experience, increases sales efficiency	May struggle with complex product queries or personalized service
Finance	Account management, financial advice, transaction processing	Increases efficiency, offers 24/7 customer support	Security concerns, inability to handle complex financial queries
Customer Service	Handling routine queries, booking services, troubleshooting	Reduces wait time, increases operational efficiency	Limited in dealing with complex or sensitive issues

for products, complete purchases, and get answers to product-related questions. This enhancement also creates a better customer experience and less waiting time, thus being more effective and operationally efficient lush [38]. On the other hand, chatbots have limited personal interaction engagements and sometimes fail to handle complex queries [39].

3.4 E-Commerce

Chatbots utilized in e-commerce exhibit a diverse range of configurations, predominantly founded on information retrieval methodologies, while also integrating certain deep learning techniques within their overarching architecture. This trend may stem from the prevalent use of Chatbots to facilitate customer service in the e-commerce sector. Consequently, these systems must be capable of engaging in dialogue with customers as well as offering insights into the product catalogue and the purchasing process. References [40]-[43] provide numerous instances of Chatbots implemented in the realm of e-commerce.

3.5 Finance

Chatbots in the financial domain have given financial advice to clients, conducted account management, and even undertaken financial transactions. A very good example is Erica, a Bank of America chatbot that assists customers in managing their accounts, sending payments, and providing customized financial advice [44]. Chatbots help customers with account management, personal finance advice, and transaction processing. This helps in facilitating efficiency in operation and minimizes waiting time for customers. However, chatbots may not be able to answer complex financial queries, and there is also the possibility of transactional errors [45].

3.6 Customer Service

Chatbots are being used in customer service to provide customers with quick and efficient support. For example, KLM's chatbot assists customers with flight booking, baggage tracking, and flight updates [46]. Chatbots can provide customers with rapid and effective support, reducing wait times and increasing customer satisfaction. Furthermore, Chatbots can handle routine questions, freeing up customer service agents to handle more complex questions. However, due to limitations in NLP and voice recognition technology, Chatbots may be unable to handle complex inquiries, and there is a risk of miscommunication [47]. Other information retrieval-based chatbot applications can be found in Training [48], Information Technology [49], and Finance [36], possibly for similar reasons. ML technologies have been employed in only a few applications. This could be attributed to a number of circumstances, including:

- DL in particular, as well as ML in general, require a lot of training data. Although training data is becoming more widely available, it can still be difficult to locate an appropriate dataset. Moreover, data frequently contains undesired noise and needs to be preprocessed before it can be used;
- Training takes a lot of time and money in terms of infrastructure and labor;
- Chatbots are typically applied to a given domain when they are not utilized for social or companion Chatbots, which implies that they need domain-specific training data (e.g., products information and details, financial information, educational material, healthcare information). Due to the nature of this type of data, it is frequently confidential; a DL engine cannot easily obtain this type of data through open access. Information Retrieval may also be the ideal option for the majority of use-case applications due to the nature of the data required and the tasks the chatbot is expected to do (such as accessing a customer's purchase history or providing more information about a product feature). Finally, the literature reveals a substantial split in terms of chatbot technologies and applications. We see that deep learning algorithms trained on huge open domain datasets are typically implemented as social or companion Chatbots. Task-oriented Chatbots appear to be trained on smaller, domain-specific, and frequently confidential datasets, and they are typically based on Information Retrieval or Rule-based techniques, or a combination of the two.

4 FUTURE PROSPECTS OF CHATBOTS

Chatbots' future prospects are promising, and there are several key areas where chatbots are likely to make significant advances in the coming years. Here are some of the chatbot's future prospects [52]-[55]:

- Natural Language Processing Advancements. As NLP technology advances, Chatbots will become better at understanding and responding to human language. This will

allow Chatbots to provide more accurate and personalized responses to inquiries [54].

- Artificial Intelligence and Machine Learning. As integration of technologies in the design of chatbots takes place with AI and ML, this will make them further intelligent and intuitive. Thus, this can enable them to learn from past interactions, hence having the capability to give more personalized responses accordingly [55].
- Increased Utilization in Commercial and Industrial Sectors. One can notice now in all fields-be it health, finance, or customer care-the presence of chatbots. In the future, the usage of these chatbots in commercial and industrial sectors may increase because of the effectiveness they provide in offering cost-effective and efficient methods to handle routine tasks/queries [56].
- Application in Emerging Technologies. Most likely, their use is going to be found in emerging technologies, especially in Virtual and Augmented Reality, Voice-activated Virtual Assistants, and Developing Smart Home Devices [57].
- Emotional Intelligence Development. A lot deeper into the future, chatbots may be enabled to develop emotional intelligence in order to understand and respond to human feelings. That will extend their usage in fields like mental health and therapy [58]. For instance, emotionally intelligent chatbots could be used in mental health therapy to detect and respond to users' emotional states, offering tailored support and interventions.

With the progression of chatbot technology, there exists a multitude of prospective applications across diverse industries and contexts. The following illustrates several examples of potential future applications for chatbots [59], [60]:

- Healthcare. Chatbots can help healthcare professionals provide remote care and track patient health [61]. Chatbots, for example, can be used to monitor patients' vital signs, send medication reminders, and track symptoms [62].
- Education. The chatbots enable personalized learning environments for learners [63]. The digital assistants may provide performance feedback, answer questions, and sometimes provide study materials as required [64].
- Human Resources (HR). Chatbots will make human resources processes easier and will support employees. For instance, chatbots can assist in employee onboarding, answer all types of questions related to HR, and guide them regarding benefits enrollment [65].

Marketing strategies can implement chatbots to provide personalized marketing experiences to customers [66]. For example; such chatbots can generate product recommendations that match each customer's preference, can answer product-related questions, and can offer special deals to customers [67].

Virtual Assistants. Chatbots can also work as virtual assistants to help users perform everyday tasks [68]. As an example, chatbots can assist with appointment scheduling, managing to-do lists, and even reminding users. [39], [69].

In summary, the prospective applications of chatbots are boundless, and their influence across different sectors and environments is considerable [70]. With the progression of chatbot technology, it is reasonable to foresee an increase in innovative and imaginative applications of chatbots in the coming years [71]-[73]. The anticipated evolution of chatbots is expected to be notably shaped by emerging technologies and trends, such as voice assistance and augmented reality [74]. Voice assistants are one of the fastest-growing niches today because devices such as Google Home and Amazon Alexa have installed millions in houses around the world. Of course, voice technologies will continue improving, but chatbots are going to be more integrated with voice assistants, enabling users to operate such kinds of systems in much more natural and intuitive ways [75], [77]. Another innovative technology that can somehow shape the future of chatbots is augmented reality. With augmented reality technology, the interaction is more immersive and interactive by presenting digital information in their physical environment. Correspondingly, chatbots in augmented reality applications might also make these experiences of a user more interactive and personalized for the user's sake [78]. Some of the developing technologies and trends that might confront the development of chatbots include, but are not limited to, machine learning, blockchain, and the Internet of Things (IoT) [79]. This, in turn, will enable a chatbot to learn from past conversations using machine learning. While blockchain technology can be used in order to have secure and open chatbot interactions [80], the usage of the Internet of Things will allow operating several connected devices with the assistance of a Chatbot [81].

In general, developing Chatbots is tightly related to new tendencies and technologies. Moving forward, while technologies are bound to evolve, the participation of chatbots is going to be ever-

increasing in daily life, making the interaction with digital content and services both more personal and more effective.

5 CHATBOT DESIGN AND DEVELOPMENT

Developing an efficient chatbot considers its end-user's needs and expectations [64], [82]. The following describes various best practices and strategies in the development process for a high-performing chatbot to deliver an excellent user experience:

- Identifying target demographics: before chatbot development, it is necessary to clearly specify that audience the chatbot is targeting. This will vastly affect the tone, style and content of communications. user research and persona creation can help find the target and that target's needs [83]. The design of a chatbot has to bring out the objectives and the boundaries. That is, it needs to have been developed for some purpose or aim that it intends to do, like answering customer inquiries or guiding users through websites [84]. Clearly set out, it would allow the chatbot to keep its focus and efficiency [85].
- Chatty tone: typically, it is wished that these chatbots be as human-like as possible [86]. That includes making use of NLP and adopting a chatty tone with a view to making the interactions more human. Humor and personality may also be used to increase the interactivity of a chatbot. An intuitive user interface must be constructed: Chatbots ought to be designed to present an intuitive user interface [87]. To accomplish this objective, it is essential that tasks are executed with the minimum number of steps; additionally, instructions and feedback should be explicit, while interactions should be expedited through the use of buttons and rapid responses [88].
- Delivering meaningful and helpful information: it shall be related to the information provided by the chatbot to the user that is related and useful to him. It has to be sensitive to his needs and send out its responses related to the request. These may become more specific and detailed over time using the AI and ML technologies that support them [89]. Testing and iteration will be required to make the chatbots efficient in carrying out user needs. Again, user research and feedback may yield several insights regarding how designs can be improved and further developed. [90].
- Accessibility: the first aspect to consider is that the chatbot must be accessible to all individuals regardless of their abilities. Compatibility with regard to screen readers, or any other assistant technology, needs to be ensured, and plain simple language usage must be justified, while alternative texts for images must be provided [91].

In summary, the effective design of a chatbot calls for a user-centered design approach that puts the needs and expectations of the target audience first. Following best practices and strategies, designers can create Chatbots that offer the user an easy, engaging, and personal experience. Generally, the process of developing a Chatbot involves ideation, design, development, testing, and deployment stages [92]. Most of them use open-source frameworks like Botpress, Rasa, or Dialogflow because it provides a set of pre-built modules or tools that speed up the development process of their chatbot [93]-[96]. Such frameworks would help developers focus on specific capability customization that the intended application needs, with unique features. Further, extending the cloud services, such as Amazon Web Services, Microsoft Azure, and Google Cloud Platform, provide resources for chatbots by providing out-of-the-box templates, hosting, and the development of machine learning capabilities [97-98]. Further, it also provides scalable infrastructural resources to meet up the increasing growth regarding usage and reach for chatbots. Developers, using open-source frameworks on cloud platforms, will be able to create more effective and successful chatbots.

6 CHALLENGES AND LIMITATIONS OF CHATBOTS

While the popularity and effectiveness of Chatbots have increased, there are still a fair number of challenges and limitations [99]-[101]. Among them, probably one of the most far-reaching challenges involves NLP [102], [103], which means, in general, the recognition and interpretation of human speech by Chatbots. Though in recent years, especially, much progress has been made in the field of NLP, tone, idioms, and slang expressions may be inaccurately captured and further misunderstandings may cause frustration on both sides. Another challenge is the ongoing maintenance and improvements that must be made so that the chatbot remains operational and relevant to changing

customer needs and market conditions [104]. Furthermore, there are also limits to how many complex or delicate issues chatbots can resolve on their own without human interaction [105]. Moreover, not all industries or business needs are suitable for chatbots. Specifically, in the industries of health, finance, amongst others chatbots do not have ways to express things in sensitive terms or personal matters 106. Lastly there might be bad ways that involve intentionally using chatbots to take advantage of them through phishing and also spreading false information. Of course, all this calls for strict measures of security and even much monitoring in updates for not to end up being taken advantage of in such endeavors. In summary, although chatbots present numerous benefits and have the potential to enhance customer service and engagement considerably, it is essential to recognize the associated challenges and limitations to facilitate informed decision-making regarding their deployment. Chatbots are confronted with various constraints and challenges, several of which pertain to issues of bias, data privacy, and ethical considerations. The presence of bias in the development of chatbots constitutes a notable challenge. Bias in chatbots arises when algorithms rely on skewed or incomplete datasets, leading to discriminatory outcomes. For example, a recruitment chatbot trained on biased hiring data might favor certain demographic groups over others. These rely on data and algorithms and therefore have the potential to exacerbate or perpetuate biases and stereotypes if not properly managed [107]. For example, an applicant recommendation chatbot may harbor biases against certain demographic groups if it relies on historical data, such as race or gender. Such bias will lead to discrimination and discrimination, which is why the developers must actively work against and minimize bias in developing a chatbot [108]. Data privacy is yet another major issue. Most of the chatbots ask for personal information of the users, such as their names, email addresses, and contact numbers, to perform their functions smoothly [109]. Sharing or using this information without authorization may pose serious privacy and security risks. For this reason, the developing agents must be responsible for implementing the proper security steps and also make the user aware of why certain information is required and what the uses will be for [110]. While many advantages arise from Chatbots, there is also a need to further reflect on the downsides and problems-most of all related to bias, data protection, and ethical issues. It is up to developers to seek active steps toward the solving of such problems in order to make sure that Chatbots are developed and used in an ethical, responsible way. Potential solutions to such numerous challenges, including continued research in NLP and ML, include the following:

1) Language Understanding. The problem of Language Understanding might be solved using NLP techniques like semantic analysis, named entity recognition, and sentiment analysis. Different NLP researches are in progress to develop these techniques to capture context, intent, and nuances of the language. Deep learning neural networks along with other ML models are also underway for improving language understanding to make more accurate predictions [111].
2) Speech Recognition. Most of the recent work to address the problem of Speech Recognition has concentrated on the development of robust algorithms/models with equally high speech-to-text transcription performance, even in noisy conditions. Methods like Deep Learning and RNN are considered for improving speech recognition accuracy [112]. Advancement in Acoustic Modelling, Language Modeling, and Signal Processing has resulted in better performance by a Speech Recognition System [113].
3) Machine Translation. Another active area of research in NLP is machine translation. Researchers are designing algorithms and models that could replace text in one language with another considering the subtlety of different languages and the context where they are being used [114]. In recent years, methods like NMT have been introduced to make machine translation applications more accurate [115].
4) Natural Language Generation. This involves the automatic generation of texts with the inclusion of news and product descriptions. Current research efforts are into developing algorithms and models that could generate text that is grammatically correct and contextually appropriate, resembling natural language itself. [116] Deep learning and neural networks have been part of the applied approaches to further raise the accuracy and quality of results seen in Natural Language Generation.
5) Contextual Understanding. The greatest challenge that arises in the field of natural language processing is to understand the context within which the language functions. Currently, research efforts are engaged in developing models and algorithms that learn from the wider context of language use such as

the tone of the speaker, the expectations of the audience, the social and cultural setting in which utterances are made. Some such techniques used in NLP for improving contextual understanding include contextual word embeddings and attention mechanisms [117].

Generally, with research on NLP and ML, increases in precision and quality can be expected, including speech recognition, language translation, language generation, contextual understanding of language, and other related subjects. In its evolution, we should be able to realize highly developed and complicated NLP algorithms that communicate with humans at ease in all types of situations.

In summary, while chatbots have made significant progress, there are still some underlying issues that need to be resolved for them to gain more usage and success. Some of these include natural language processing issues such as the inability to understand tone, idioms, and context, which can lead to communication breakdown. Additionally, the requirement of continuous maintenance, bias, data privacy, and ethical concerns are significant hurdles in their ethical deployment. The need for speech recognition, language generation, and contextual understanding to get better remains top priority, as do efforts to minimize biases and ensure user privacy. Since NLP and machine learning research continue, it is expected that these challenges will be overcome, leading to more accurate, efficient, and ethical chatbot applications in the coming years.

7 CONCLUSIONS

The key results of the paper are summarized below in this section:

- Chatbots are becoming increasingly popular due to advances in NLP and machine learning.
- The main application areas of chatbots encompass a wide range of services: customer service, education, healthcare, and entertainment.
- There are two kinds of chatbots: rule-based and AI-based. AI-based chatbots are highly advanced and learn with every interaction, hence continuously improving the performance.
- Major challenges for this technology are in language understanding, speech recognition, machine translation, and natural language generation that keep the context.
- Yet, despite that, it fundamentally can change how we use technology and interact with each other, and its wide acceptance will continue to increase overtime.
- The review paper seems to include everything related to the present position and future perspective of chatbot technology, outlining opportunities and challenges going alongside this fast-growing area.

We discussed the future course of research, keeping in view the ethical and sociological implications; courses which design and development took place. For the future course, it needs increasing understanding of languages and generation capability. We are pretty sure that keeping the progress being carried out in NLP and ML, we will be in a position to create more advanced human-like chatbots very soon. It is clear that chatbot technology in its present state, and in light of future research and development, will have significant implications for many industries and fields:

1) Customer Service. Many firms have resulted in adopting the implementation of chatbots for customer care and support. Since the technology is still in the development stage, many firms in the near future could use chatbots to ensure smooth customer experiences, reduce costs, and enhance business processes.
2) Healthcare. The application of chatbots in healthcare has immense potential to completely revolutionize personalized care and support for the patients. Examples include chatbots doing medical counseling, monitoring the health of patients, and even assisting in medication management.
3) Education. Undeniably, AI-powered chatbots will improve learning because of the personalized support they can give to learners. For example, they can be used to provide feedback on assignments, answer questions, and give recommendations in a very personalized way.
4) Entertainment. These chatbots have also found their application in the mode of entertainment, mainly as virtual assistants and interactive game playing. With further developments in chatbot technology, we can expect even more interactive and engaging modes of entertainment involving chatbots.
5) Research and Development. Research and development in the field of chatbots are very bright and promising. Every now and then, research in NLP and Machine Learning

increases the accuracy and quality of language understanding, speech recognition, machine translation, natural language generation, and contextual understanding, opening various avenues to build very advanced and versatile chatbots. With the current status and future on chatbot technology, significant ramifications abound for many industries and fields; that is, chatbots hold great promise for changing how we communicate with technology and, in turn, with each other. Provided that active research and development continues to take place within this topic, it necessarily follows that in time, even more advanced and sensitive chatbots will emerge capable of providing personalized assistance and help in all contexts.

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Artificial Intelligence for Cybersecurity: Analyzing Legal Frameworks and Policy Implications

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Keywords: Cybersecurity, Artificial Intelligence, Legal Frameworks, Machine Learning, Deep Learning.

Abstract: The paper deeply studies three existing security mandates like the GDPR, NIST CSF, and CCPA so it can evaluate properly how they respond to Artificial Intelligence (AI) defines issues. The research finds significant problems in regulations about AI cybersecurity especially with attacks from outside sources, biased systems, and poor clarity, which create serious ethical problems. A doctrinal and analytical research methodology was applied within this study, which combines legal text analysis and case law review to define judicial rulings along with a framework evaluation and an investigation into social-moral effects on AI cybersecurity. In addition, actual data is collected from legal practitioners, IT security specialists, and policymakers through structured interviews to present a concrete approach of practical problems and regulatory requirements in an ever-growing field of this nature. The results stress the demand for powerful, proactive laws that make security demands and technological development compatible to each other, and emphasize the need for international collaboration and preventive regulatory approach. The recommendation for comprehensive legislative framework in regulating AI in cybersecurity is the concluding part of the study, seeking to promulgate laws that would weave its way through the makeovers engineered by AI in protecting cyber space, including realizing balance, practicality, and ethical vigilance.

1 INTRODUCTION

In 2024 cyber assault of corporations has foreseen international damage of greater than $1 trillion and emphasizes need for powerful cybersecurity strategies [1]. AI has highly developed before long and strongly affected various sphere; cybersecurity has been among the most severely affected [2]. AI technologies are widely applied in cybersecurity because it allows sophisticated threat detection [3]. AI integration in cybersecurity develops improved methods for both security maintenance and threat management systems. There are problems with existing legal structures together with policy. Threats continue growing in both number and complexity as data infrastructure along with organizational assets together with individual information remains endangered constantly. Solar Winds hack being combined with Colonial Pipeline ransomware attack shows that big destructive outcomes are possible due to cybersecurity incompetence [4].

AI legal Frameworks as the importance is intensifying rapidly the area of new technology AI therefore it insists the construction of separate legal system to manage the quantity and challenges presented with such technological devices. The current widest AI legislation proposal comes from the European Union (EU) with its AI Act presented in 2021 [5]. The EU AI Act sets risk categories that organize AI applications as unacceptable risk, high risk, restricted risk, and minimal risk applications respectively. The Act is to that extent preventive, for it presages dangers not yet or not apparent at the time. This prophylactic regulation will ensure the safety and reliability of AI systems; but it is important to understand the issues that occur when carrying out AI regulation and possible the negative impacts on innovation resulting from overly burdensome laws [6]. The FDA recommends on AI and Machine Learning (ML) in medical devices that is an instance of this. These recommendations in particular, emphasize on openness and post market surveillance (FDA) [7].

According to the Federal Trade Commission (FTC), data security and privacy are the key components in preventing deceptive and unfair

practices related to the deployment of AI [8]. Customized legislation can be devised through the sectorial approach, so that sometimes regulatory inconsistencies and fragmentation may result making it difficult for firms operating under a number of sectors to comply [9].

There remains an effort to only have the enough regulations that can primarily regulate AI. The rapid pace of the development far outpaces that of regulatory development, leading to loopholes and outdated models [10]. Additionally, seeing as AI technology is virtually without border, it therefore requires international collaboration and harmonized legislative efforts to limit regulatory arbitrage and to establish unified standards[11].

2 LITERATURE REVIEW

The cybersecurity laws are composed of multiple national and international regulatory standards that carry different legal backgrounds and objectives. A study of the General Data Protection Regulation (GDPR) identifies how this set of rules deals with modern cybersecurity threats as well as its area of effectiveness and limitations [12].GDPR enhances data protection rules in the EU along with promoting privacy culture through accountability thus leading to elevated data protection standards. The rigorous regulatory standards appear problematic mainly to small businesses because they have limited resources to implement these requirements completely [13].The GDPR offers two key benefits through safeguarded confidentiality as well as clear standards and responsible practices for organizations. SMEs together with other organizations face difficulties with elevated GDPR standards and linked expenses since the GDPR delivers overall benefits.

Organizations from the US especially embrace this framework because its adaptable nature enables customization of cybersecurity approaches to fit individual organizational requirements and this solution proves very popular among U.S. businesses including SMEs [14]. Unlike the European Union GDPR mandatory data protection rules [15], the NIST framework enables businesses of various types to participate through its flexible approach although it remains optional [16]. The NIST framework takes a risk-based cybersecurity approach yet its voluntary nature reduces its value in protecting against new security threats because compliance success relies mostly on organizational commitment.

It changes the worldwide international frameworks' policy shift to adapt to data privacy, governmental authority and to reconcile them with cybersecurity in several strategies. The Cybersecurity Law of China, enacted in 2017, emphasizes governmental authority in protecting data and network security, sometimes placing national objectives above personal private rights [17]. While this legislation exhibits a very different approach than the GDPR, which places emphasis on the protection of individual privacy, the use of national interests as a factor in governing the use of data reveals the impact of national interests on data governance regulations [18]. The stringent data sharing restrictions imposed by the GDPR show how striving for cybersecurity goals in different jurisdictions presents great challenges, particularly where the global cyber threats now require international cooperation for a coordinated response [19].

One of the first worldwide attempts to develop an international approach to cybercrime is the Budapest Convention on Cybercrime, which was set up in 2001. The aim of the agreement is to bring rules on the use of the internet in line internationally and improve the ability of countries to work together to investigate crimes online, but it has been criticized for failing to be properly implemented and for lack of strong backing from major players in cyberspace like Russia and China. However, existing frameworks are criticized for being weak in the enforcement and are not linked to the shared national agendas, as the critics argue they promote norms and collaboration [20]. The difference in the international collaboration and enforcement demonstrates persisting challenge of creating universally acknowledged cybersecurity legal framework [21].

The Cybersecurity, then, is improved because of AI with the use of sophisticated ML and deep learning (DL) techniques, for it makes analysis in big data better, and alarms occurrence faster, including eventualities that man could not notice [22]. AI-powered scored cybersecurity solutions to analyze a large throughput data much better than those done by the human in identifying the anomalies and potential threats in occurring [23]. The merging of AI with Intrusion Detection Systems (IDS) and Intrusion Prevention Systems (IPS) has greatly provided chances in detecting and preventing complex cyber threats, a progress over previous techniques [24].

The detection of AI system vulnerabilities requires robust protective strategies that need continuous monitoring to stop exploitation as well as maintain AI cybersecurity systems security integrity [25]. In this environment, employing AI produces several issues that concern government monitoring and liability when technology is utilized by businesses along with such autonomous systems more than people can account for and pay attention to [26]. Additionally, using AI in cybersecurity has a huge concern on data privacy, algorithm transparency and equity that are legal practice area necessary to

make sure that it follows the ethics and avoid a person's rights being violated. If AI is misused in surveillance, overly extensive interference in the individual privacy rights may occur in violation with the rules of data protection [27]. Since AI systems that are driven by flawed datasets tend to make discriminatory decisions that disproportionately favoring the underprivileged groups [28].Consequently, guaranteeing the transparency, equity, and accountability of AI applications in cybersecurity is both a technological necessity and a legal and ethical responsibility.

Despite the potential for great improvement in cybersecurity capabilities and the effectiveness of cybersecurity measures, the risks of adversarial attacks must be addressed, data privacy regulations must be complied with, and the potential for algorithmic bias must be defended to achieve sound and ethically appropriate use of AI.

3 RESEARCH METHODOLOGY

This study investigates the inclusion of AI into cybersecurity and its repercussions under existing. Doctrinal approach has been used to analyze a number of important legal documents and to investigate them including the GDPR the National Institute of Standards and Technology Cybersecurity Framework (NIST CSF) and the California Consumer Privacy Act (CCPA). The analysis of case law in AI and cybersecurity scientifically interprets and executes the meanings of legal laws with respect to judicial decisions that create precedents. This study also contains the stakeholders' obligations and viewpoints assessment. Socio legal research on the ethical and social consequences of AI for cybersecurity in particular aims to understand concerns around bias, transparency, as well as its implications for privacy rights and civil liberties. Consequently, empirical research is carried out through structured interviews with legal experts as well as cybersecurity professionals and policymakers. Such practice is done to gain fine-grained insights on how legal frameworks could be practically applied and have consequences. By conducting structured interviews, we received a large amount of data regarding obstacles, rewards and compliance issues experienced by different stakeholders. Normative analysis identifies significant deficiencies and difficulties in existing legal structures, and proposes new laws as appropriate. In addition, this analysis presents future research direction for the continuous adjusting legal framework to accommodate increasing technology advancement and increased cybersecurity threats. The research aims to approach the problems of the regulatory space where AI and cybersecurity interact as one whole, in a comprehensive way. It also aims to offer well-supported recommendations on how this landscape can be changed. Figure 1 illustrates the research method framework used in this study.

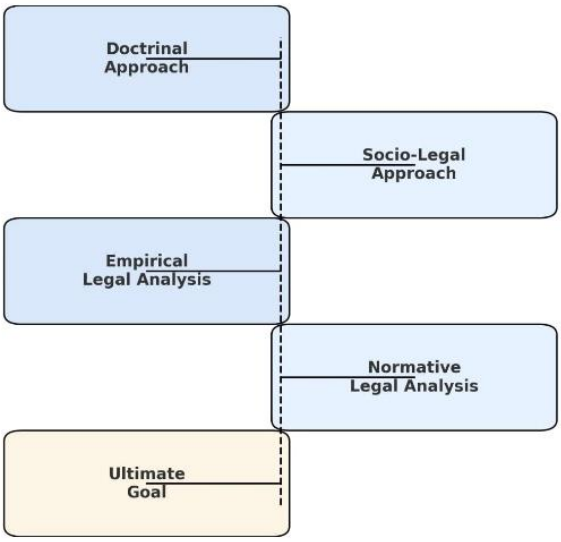


Figure 1: Research method framework.

4 ANALYSIS OF LEGAL FRAMEWORKS

4.1 Cybersecurity Laws and Regulations

In the area of cybersecurity, the laws and regulations are very important in order to expose sensitive information and minimize the risks of cyberattacks. Several key legislative frameworks can be illustrated as the myriad ways taken around the world's congress.

Furthermore, the act forced people to disclose their actions within seventy two hours [29]. Therefore, the (GDPR) has brought about significant improvement to processes and concerning data protection and responsibility awareness. It may be effective for its being comprehensive and obligatory. In a sense, there is a need to set up a robust data security system in enterprises and the fines as a result are very high if the compliance is not met.

California Consumer Privacy Act (CCPA): Starting from 2020 consumers across California will gain CCPA access to view their data and request data deletion and data usage opt-out rights according to Section 1798.100 [30]. As per Section 1798. 100 of the California Civil Code businesses need to maintain

protective security systems that handle personal information from their consumers. The United States of America now provides advanced data protection through the CCPA that both safeguards user information extensively while improving collection procedures [31]. The CCPA has personal information privacy as a major strength because it functions as legislation that protects consumers thoroughly.

The tasks that are included in the framework are Identify, Protect, Detect, Respond, and Recover [32]. Because of the framework's adaptability, it is extremely adaptable for various organizations to implement it according to their requirements, which has contributed to its widespread use in a variety of business domains (NIST, 2018). The flexibility of the framework is advantageous, but the absence of strict compliance results in diverse cybersecurity measures [33].

4.2 Legal Frameworks Related to AI

Different jurisdictions across the globe are creating regulations regarding AI technologies to handle unique AI issues together with possible risks through established frameworks.

The EU AI Act uses risk levels to sort AI systems and establishes strict standards for managing high-risk systems [34]. These standards include comprehensive policies together with activities for openness as well as human supervision systems and protective measures against cyberattacks and exploitation [35]. AI systems must be made safe and dependable by legislation that also protects basic rights throughout operations. The EU aims for standardized regulations across all member states by means of this policy [36]. This law achieves high effectiveness due to its proactive approach that stops potential issues from spreading before their full growth.

Under the U.S. Sectorial Approach, each specialized regulatory agency within the country develops specific rules for individual sectors. The system of regulatory oversight can be described as decentralized because different bodies handle the regulation of AI technologies that match the specific sector they serve. The Food and Drug Administration (FDA) has established particular guidelines regarding AI and ML in medical devices (MDs) which focus on visibility requirements and testing procedures and post-market monitoring for patient protection and effective use of the devices [37]. The Federal Trade Commission (FTC) ensures consumer protection through enacting regulations to prevent the act of fraudulent activities in AI technology, with matters related to data protection, algorithmic bias and helps to promote transparency with regard to the AI's impact on consumers. The framework provides organizations with a standardized method to detect, analyze, and manage possible hazards caused by AI while acting as a directive tool.

In China, the strategy is centralized. The government unifies its AI regulations in form of comprehensive regulations. China's 2017 Next Generation AI Development Plan aims to make China the leading AI power in the world by 2030. The strategy focuses on three core priorities that include ethics as well security concerns and societal impact. AI research and practice need ethical norms and legal frameworks that their representatives argue should be established [38]. These tactics enable China's plan through rapid well-coordinated regulatory measures that prompt the setting of clear and adequate standards that can quickly respond to technological characteristics.

4.3 Gaps and Challenges

There are several notable gaps and inconsistencies in the current legal frameworks governing AI and cybersecurity:

1) Lack of International Harmonization. This situation comes about because of lack of alliance in among global lawful organizations that thus causes deficient coordination. This fragmented nature is, however, susceptible to regulatory arbitrage in which entities leverage the fragmented nature to exploit the most rigorous set of regulations whilst maximizing profits posed considerably compliance problems for multinational organizations. Cyber threats are global in scope and cybersecurity legislation should therefore be one, with universal protections so as not to have any gaps in the legislation.
2) Outdated Regulations. The fast pace of technological development makes it difficult for law enforcement to produce current regulatory frameworks. The rules that emerge tend to be spread out over many pages despite their inability to address effectively the security and AI-related threats which advanced technologies create [39]. Standards should require special protection solutions because current ML security methods can be easily defeated.

4.4 Regulatory Challenges

Regulating AI within cybersecurity presents multiple complex challenges:

1) Technological Advancements. AI technology is evolving quite fast, which makes the process of regulating it quite a challenging task. The

reaction tends to become such, which creates legal uncertainty while exposing systems to novel risks. In order to do this, regulatory approaches must make use of innovative methods in order to adequately anticipate technical shifts and embed adaptive means to ensure compliance with high cybersecurity norms. It seems regulations for AI and risks associated with AI would make more sense if they were periodically reviewable [40].

2) Ethical Dilemmas. Ethics in AI technology creates several difficult problems to solve such as implicit bias, personal responsibility and open actions. Governments should create laws that let people observe how AI systems work and need them to act fairly to win public faith in the system. To build trust with the public we need to make sure AI systems avoid receiving and spreading existing unfair practices. For AI systems to use the technology companies have to prove that their systems will not be biased during tests and validations before public use [41].
3) Privacy Concerns. There are serious privacy issues when relating AI to data analysis and surveillance. Laws that are effective should contain strong safeguards for the individual privacy while permitting the beneficial use of tools using AI. This includes further questions of ensuring that data protection legislation is not broken and that procedures are transparent in order to maintain public trust. Keeping in mind the benefits of data analysis of personal information with the need to prevent the misuse of surveillance. In the end all that is required is a guarantee of privacy with measures such as affirmative consent and data minimization practices [42].
4) Human Oversight. It is important to ensure that humans are active in managing the risk associated with uses of AI, are responsible for AI systems. In some cases however, certain ethical, legal standards require critical AI driven decisions to be reviewed by humans [43]. Current laws regarding both AI and cybersecurity form a crucial foundation for direct technological risk control yet prove insufficient against the complete range of issues expected from AI development speedups. Since the evolution of AI technology, regulators need to take proactive measures that combine with flexibility and review process with responsiveness in their regulatory strategies.

4.5 Legal Frameworks

The integration of AI into cybersecurity demands complex knowledge from policymakers because responsible implementation requires a thorough approach to AI-based cybersecurity. Through promotion of responsible development, equitable access and public trust, policymakers can reduce risks in the use of AI in cybersecurity, and enable the enhancement of the broader public value of AI in cyber. Thus, to accommodate the AI advancements policies must undergo some adjustments:

1) Harmonization of Regulations. For common international standards to be set for AI and cybersecurity, international cooperation is of importance to avoid fragmentation and to ensure that global standards are implemented uniformly by countries [44]. A regulatory framework that is globally aligned helps in reducing legal complexities of organizations operating outside the borders and promotes global cybersecurity awareness.
2) Proactive Regulation. It is important for policymakers to focus on proactive instead of reactive regulation, prepared to counter the potential risks of the technology emerging in AI. It requires constant monitoring, and updating of legal frameworks to render the regulations applicable [45].
3) Support for SMEs. Special help must reach small and medium-sized businesses because they need specific guidance to understand AI and cybersecurity permissions. Subsidies, grants and consultative services should be adjusted into the policy to help SMEs to comply with rigorous standards [46].
4) Ethical AI Development. Current ethical AI development should have precise legal prescriptions for procedures which focus on performance fairness while ensuring system clarity and maintenance accountability standards [47]. The laws must enforce full visibility in algorithm development principles as well as data management standards with safety protocols to monitor and prevent improper usage and maintain control of AI systems. The establishment of ethical protocols remains essential to earn public trust while safeguarding persons from possible damages of AI technology.
5) Privacy Protections. Data protection policies should be very effective and completely ban any form of unauthorized surveillance so as to

encourage public trust in AI systems [48]. This means the update of data protection laws that will incorporate privacy as a central principle in the development of AI, together with sanctions that can be enforced when there is a breach.

The integration of AI into cybersecurity creates difficulties and advantages that policymakers have to handle. The responsible utilization of AI technology to bolster cybersecurity requires governments to perform assessments related to legal impacts as well as social and economic aspects followed by regulatory framework modifications. Timely regulatory adjustments that enable adoption of AI technology allow the detection of emerging threats and utilization of its benefits to build a safer dynamic cybersecurity environment. Methods to update laws according to technology advances allow decision-makers to navigate complex AI security problems.

5 IMPROVEMENT OF LEGAL FRAMEWORK

Since the increasing complexity given by AI and cybersecurity, it is necessary to propose practical and target-based changes to existing legislation.

Strengthening to overcome these challenges, current legislation should be strengthened by updating with specific rules that take into consideration those specific rules that should apply for data protection in the case of AI. In particular, this should involve mandatory annual inspections of AI systems in terms of data protection policy, in order to reinforce data minimization, purpose limitation and accountability principles.

Improving transparency and explain ability: legal requirements for AI developers and operators should require that they perform the actions to make the functioning of AI more transparent and comprehensible. In other words, they may involve compulsory disclosure of decision making processes and algorithms and the development of a public database that reveals details about AIs, their models, and their decision-making processes.

Promote International Collaboration. The countries and take initiatives should adopt harmonized or compatible AI and cybersecurity regulations. There would be multilateral treaties and the formation of international organizations owning the duty to lay down standards in international level and promote international cooperation interstate.

Invest in Research. The public policy must allocate funds to support AI research along with cybersecurity innovation to guide capital toward educational institutions and technology development programs managed by the government.

6 CONCLUSIONS

Artificial intelligence (AI) is playing a very important role in augmenting the cybersecurity, especially for threat detection, anomaly detection and automated response protocols. Decision systems with AI improve cybersecurity but their implementation carries two main drawbacks that include adversarial vulnerability along with bias-related ethical issues and lack of transparency. The effective management of AI's role in cybersecurity requires stringent safeguards because of the identified risks. Current laws prove insufficient since no national consensus exists on AI controls nor do the regulations match the present-day AI threats adequately.

This paper is an in-depth analysis of the integration of AI in cyber security with which is dealt a special focus of legal challenges and policies around the influences of AI in cyber security devices. Important findings of this Analysis Current Legal Frameworks: By way of example, significant legislative innovations, for example, European Union's GDPR as well as CCPA in the United States, making headways privacy eligible data protection.

The paper adds important findings to AI and cybersecurity knowledge base. The paper examines current laws and policies through identification of implementation challenges organizations encounter when deploying AI-based cybersecurity tools. This study proposes specific recommendations for improving relevant legal frameworks together with security protocols that protect and make responsible the deployment of AI in cybersecurity systems.

In future research directions for developing the field of AI and cybersecurity, research should focus primarily on creating legal frameworks for emerging AI cyber threats along with creating universal global laws for AI and cybersecurity.

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Algorithms of a Digital Fire Prediction and Suppression System

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Keywords: Machine Learning, Artificial Intelligence, Pattern Recognition, Fire Risks, Model, Kalman Filter, Optimal Management, Algorithm, Prediction.

Abstract: This article analyses the algorithms of digital systems created to predict fire hazards and effectively eliminate them. Natural and man-made fires are one of the factors that cause great damage to human life, ecology and the economy. Fire risk prediction algorithms analyze environmental conditions such as weather patterns, wind speed, temperature, and humidity to assess potential fire hazards. These algorithms determine the level of risk in real time, predict possible fire situations and provide early warnings. In particular, the introduction of analytical models based on artificial intelligence significantly increases the accuracy of predictions. Fire suppression algorithms, on the other hand, allow for rapid coordination of actions after a fire is detected, optimal allocation of resources, automation of evacuation plans and effective management of emergency services. These algorithms embody complex solutions that include digital maps, real-time data exchange and inter-system integration. Therefore, the use of modern digital technologies and algorithmic approaches in ensuring fire safety is of urgent importance. This article examines the basic principles of fire prediction algorithms, namely, methods for predicting the level of risk based on factors such as weather data, air humidity, temperature, wind speed and plant dryness. It also analyses the mechanisms for determining the probability of a fire using artificial intelligence and machine learning models (for example, Random Forest, Neural networks). In addition, algorithms for quickly eliminating a fire after it is detected are considered, including optimal resource management, automation of evacuation plans, and the possibility of integrating drones and IoT devices into the system. To increase the efficiency of the system, algorithms based on real-time monitoring and digital maps are recommended. The results of the research work reveal the practical importance of advanced algorithms in firefighting and contribute to the development of digital approaches in the field of fire safety.

1 INTRODUCTION

Today, identification systems, monitoring of established fire risk thresholds using computer programs, and the development of a digital system are important issues. In developed countries of the world, in particular, Germany, France, Great Britain, Japan, South Korea, China, the Russian Federation and other countries, great attention is paid to solving theoretical and practical problems of intelligent analysis using artificial intelligence to assess fire safety at facilities with a high risk of fire and explosion, to develop measures to prevent precipitation.

The increasing material and moral damage caused by emergency situations occurring in the world every day, there is a need for scientific research on the development of traditional and modern methods of intellectual processing of information in digital systems, new approaches. In particular, great attention is paid to the theoretical and practical improvement of various models and methods of intellectual data analysis and Machine Learning. At the same time, understanding the mathematical foundations of Machine Learning models is important to assess their working principles, limitations, and advantages. Here are some of them. Decision trees are a model based on the principle of recursive partitioning, which can be subject to the problem of overfitting. Their advantages are that their interpretation is simple and understandable, and they do not require any predefined distribution. Random forests are an ensemble of a large number of decision trees, which can take a long time to train. Their advantages are that they work well with large amounts of data and

can identify relationships between different attributes.

In this regard, scientific research is being conducted around the world to solve the problem of pattern recognition and create computational algorithms based on estimation algorithms. Among the important tasks is the development of a model algorithm and database, as well as a software package for preliminary processing of initial information based on regression analysis.

The effectiveness of fire risk management solutions is largely determined by the reliability and quality of data obtained from measuring instruments. Therefore, a separate task is to ensure the reliability of measurement data, identify incorrect (anomalous) measurements of controlled quantities, etc. This, in turn, allows you to increase the effectiveness of the fire safety system at facilities with high fire risk, as it determines the adequacy of management decisions taken to predict fire hazardous situations and prevent fires [1], [2].

2 METHODS AND RESULTS

Uncertainties in forecasting systems arise for various reasons, and as a result, the accuracy of the system may decrease. Optimized mathematical models are used to increase accuracy and improve decision-making processes. To minimize uncertainties in a forecasting system, the optimization problem is to maximize the accuracy of the forecasting system by reducing uncertainty. The accuracy and reliability of sensor data, computational resources and time constraints, the suitability and stability of model parameters, the suitability of the system for real-time operation, and the understandability of the forecasting results determine the limitations of the optimization problem.

The use of multi-stage analysis and an iterative approach method serves to gradually improve the results. Such an approach helps to increase the reliability of the forecasting system and ensure the efficiency of decision-making processes.

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The use of multi-stage analysis and an iterative approach method serves to gradually improve the results. Such an approach helps to increase the reliability of the forecasting system and ensure the efficiency of decision-making processes.

To predict fire conditions, certain types of prediction methods were used in modeling processes based on data obtained from the device. Some of these prediction methods are listed below. Prediction methods: least squares method; extrapolation method of forecasting; sliding average method; exponential smoothing method; adaptive smoothing method; mathematical modeling method; network method; matrix method; simulation method, etc.

As part of the research work, an algorithm for predicting the occurrence of fire risks using a fire assessment model was developed (Figure 1).

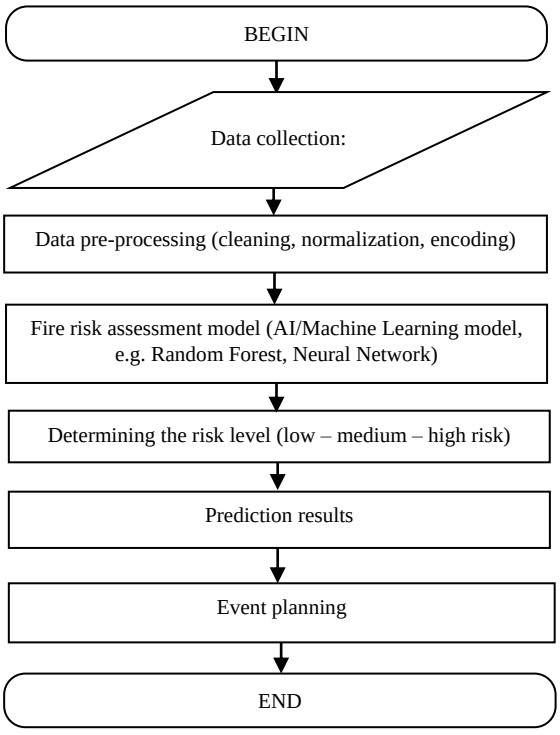


Figure 1: Block diagram of the fire risk prediction algorithm.

Data pre-processing (cleaning, normalization, encoding). The Kalman filter was applied to refine sensor data in real time, reducing noise and improving the reliability of fire risk assessment. The

Kalman filter is an efficient recursive filter that estimates the state vector of a dynamic system using a series of incomplete and noisy measurements. The Kalman filter is widely used in engineering and econometric applications, from radar and vision systems to estimating the parameters of macroeconomic models.

Fire safety systems constantly collect data and monitor the state in real time. Using the Kalman filter, this data is filtered and serves to prevent various interferences and ensure the effective operation of the predictive model.

A) Condition prediction (real-time analysis of sensor data). Using the state prediction formula in the Kalman filter, parameters such as temperature, gas concentration, oxygen, and humidity can be predicted:

$$\hat{x}_{k|k-1} = F_k \hat{x}_{k-1|k-1} + B_k u_k, \quad (1)$$

where:

1) $\hat{x}_{k|k-1}$ – predicted values of the parameters to be detected in the fire safety system (temperature, gas, oxygen and humidity);
2) F_k – a matrix describing the dynamics of changes in parameters (for example, temperature changes over time);
3) u_k – external influence (e.g. new information or external factors such as wind speed or air circulation).

The predicting process allows for early detection of fire risks and immediate action.

B) Covariance prediction (reducing data uncertainty).

Data from different sensors can always have a certain level of noise. The noise level of this data can be taken into account using the formula for predicting the covariance matrix:

$$P_{k|k-1} = F_k P_{k-1|k-1} + F_k^T + Q_k, \quad (2)$$

here, Q_k – system noise covariance. In fire safety, this noise can be caused, for example, by the sensitivity of sensors or by the external environment (dust, humidity, wind).

Thus, the filter helps to correct the data by taking this noise into account.

C) Kalman rule (data updating and correction). The Kalman rule can be used to correct data coming from a fire safety system:

$$K_k = P_{k|k-1} + H_k^T (H_k P_{k|k-1} H_k^T + R_k)^{-1}, \quad (3)$$

here:

1) H_k – matrix of sensor measurements (for example, data from temperature, oxygen, gas, or humidity sensors);
2) R_k – measurement noise covariance. When the data is updated and there is noise or error in it, the Kalman rule is used to correct it. This increases the accuracy of fire risk detection.

D) Status update (real-time update of indicators). After the data correction process, the status will be updated:

$$\hat{x}_{k|k} = \hat{x}_{k|k-1} + K_k (z_k - H_k \hat{x}_{k|k-1}), \quad (4)$$

this formula can be used to adjust the prediction by taking into account the differences between the parameters determined in the fire safety system, for example, the temperature measured by sensors and the predicted value.

E) Covariance update. The covariance is updated after each updated data:

$$P_{k|k} = (i - K_k H_k) P_{k|k-1}, \quad (5)$$

there are 4 main types of forecasting: long-term, medium-term, short-term and operational (operational). In order to predict fire situations, certain types of forecasting methods were used to model processes based on data received from the device. Some of these forecasting methods are listed below. Forecasting methods: least squares method; extrapolation method of forecasting; sliding average method; exponential smoothing method; adaptive smoothing method; mathematical modeling method; network method; matrix method; imitation method, etc [3], [4].

The scheme of the optimal control process is basically the formulation of the optimal control problem as follows.

The problem of optimal management. The input parameters of the control system should be determined at such optimal values that the probability of fire occurrence is minimal and the accuracy of the information obtained from the object is increased.

Let us formalize the mathematical model of the problem. Let us consider a dynamic system described by the following equations of state.

$$x_{k+1} = f(x_k, u_k, w_k),$$

$$y_k = h(x_k, v_k),$$

here:

- x_k - is the state vector of the system at step *k*;
- u_k - vector of management effects;
- w_k - random effects of the process;
- y_k - measurable output parameters of the system;
- v_k - measurement noise (interference);
- $f(t)$ - system dynamics function;
- $h(t)$ - tracking function.

The optimal control problem is that the control effect should be chosen so that the following quality criterion has a minimum value:

$$I = \sum_{k=0}^{n} [Q(x_k) + R(u_k)], \qquad (6)$$

here:

- $Q(x_k)$ – penalty function for deviation of the state from the desired value;
- $R(u_k)$ – penalty function for management actions;
- n – prediction limit.

In the next step of the above block diagram, the first 5 values of the input data coming from the methane detection device are taken. Based on these values, the Kalman filter is calculated using the above model, and the input data of oxygen, humidity and temperature coming from the remaining 3 devices are also calculated [5].

Initial information (Table 1):

$$\hat{x}_0 = 0;\ p_0 = 1;\ A = 1;\ H = 1;\ Q = 0{,}01;\ R = 0{,}1.$$

Table 1: Input data.

Time	Tracking	Kalman filter
1	0	-
2	0	-
3	0,1	-
4	0,3	-
5	0,1	-

The results of these calculations are presented in Table 2.

Table 2: Calculation results.

Time	z_i - tracking	$\hat{x}_i$ - Kalman filter	P_i - covariance
1	0	0	0,0911
2	0	0	0,0503
3	0,1	0,0375	0,0375
4	0,3	0,1188	0,0321
5	0,1	0,0233	0,0296

By adding new adaptive coefficients to the Kalman filter, it is possible to further improve the efficiency of fire safety systems. The Adaptive Kalman Filter is mainly used. Adaptive coefficients and boundary coefficients help to quickly, accurately and flexibly determine the fire hazard. These introduced coefficients increase the real-time accuracy of the system and expand the ability to make quick management decisions in fire situations. To form a training sample model for analyzing sensor data interference using the Kalman filter, data from a methane sensor, which is considered the most important parameter in the occurrence of a fire, was separately extracted for one cycle sample. Using the Kalman filter, the input data graph, the data graph passed through the Kalman filter, and the improved Kalman filter graph were compared. Fire safety systems constantly collect data and monitor the situation in real time. Using the Kalman filter, this data is filtered, helping to prevent various interferences and ensure the efficient operation of the prediction model.

Uncertainties in forecasting systems arise for various reasons, and as a result, the accuracy of the system can decrease. Optimized mathematical models are used to increase accuracy and improve decision-making processes. To minimize uncertainties in a forecasting system, the optimization problem is to maximize the accuracy of the forecasting system by reducing uncertainty. The accuracy and reliability of sensor data, computational resources and time constraints, the suitability and stability of model parameters, the suitability of the system for real-time operation, and the understandability of the forecasting results determine the limitations of the optimization problem [6], [7].

The use of multi-stage analysis and an iterative approach method serves to gradually improve the results. Such an approach helps to increase the reliability of the forecasting system and ensure the efficiency of decision-making processes.

Table 3 shows the 4 parameters that are used to determine fire risk: Methane (CH_4) level, Oxygen (O_2) content, Humidity (%) and Temperature (°C).

Table 3: Input data by 4 parameters.

№	Methane level (ppm)	Oxygen level (%)	Humidity level (%)	Tempera-ture (°C)
1	1	20.8	65	28
2	8	21.5	35	34
3	3	21.0	60	25
4	6	20.1	40	32
5	4	20.9	55	26
6	10	21.9	30	36
7	5	20.5	48	29
8	9	21.8	38	33
9	…	…	…	…

Here:

- Methane level (ppm) - the amount of gas that increases the risk of fire (parts per million);
- Oxygen Level (%) – the percentage of O2 in the environment. A low level slows down combustion, a high-level speeds up the spread of fire;
- Humidity (%) – low humidity levels increase the risk of fire;
- Temperature (°C) – the higher the temperature, the more likely a fire will start [8], [9].

Table 4: Complete data table for the fire prediction system.

№	Methane level (ppm)	Oxygen level (%)	Humidity level (%)	Tempe-rature (°C)	Fire risk level
1	1	20.8	65	28	Low risk
2	8	21.5	35	34	High risk
3	3	21.0	60	25	Low risk
4	6	20.1	40	32	Medium risk
5	4	20.9	55	26	Low risk
6	10	21.9	30	36	High risk
7	5	20.5	48	29	Medium risk
8	9	21.8	38	33	High risk
9	…	…	…	…	

Risk assessment criteria (based on the logic of the model):

- High risk → Methane: 8-10 ppm, Humidity: 10-30%, Temperature: 30-60°C;
- Medium risk → Methane: 5–7 ppm, Humidity: 40–60%, Temperature: 22–29°C;
- Low risk → Methane: 1-4 ppm, Humidity: 70-90%, Temperature: 10-21°C.

The result table of accuracy and prediction using Decision Tree, Random Forest, K-Nearest Neighbors, and Artificial Neural Networks for the above data, Table 4 for a fire prediction system is Table 5.

Table 5: Comparison table by overall model Accuracy.

Algorithm name	Accuracy (%)
Proposed Algorithm	100 %
Decision Tree	100 %
Random Forest	100 %
K-Nearest Neighbors (K-NN)	87.5%
Artificial Neural Network	100 %

Among the tested models (Figure 2), Artificial Neural Networks (ANN) achieved the highest fire risk prediction accuracy (100%), outperforming traditional classifiers like Decision Trees (100%), Random Forest (100%), and K-Nearest Neighbors (87.5%).

In Figure 2:

- Decision Tree, Random Forest and ANN provide high Accuracy on this small dataset;
- K-NN can sometimes get confused with close classes (for example, it predicted Medium in the 2nd record);
- On the other hand, ANN and Random Forest are preferable on a large dataset because they are good at detecting complex relationships [10], [11].

Table 6 also presents an analysis of the results obtained using various machine learning methods in the study. The number of objects in the training sample was 7350, divided into 70% training and 30% control samples, and the number of input parameters was 4. The MSE (mean square error) function was used as the loss function.

Based on the positive results obtained above, the proposed model and algorithms are mainly used in objects with high fire risk (for example, gas stations, oil and gas processing plants, residential houses and other objects).

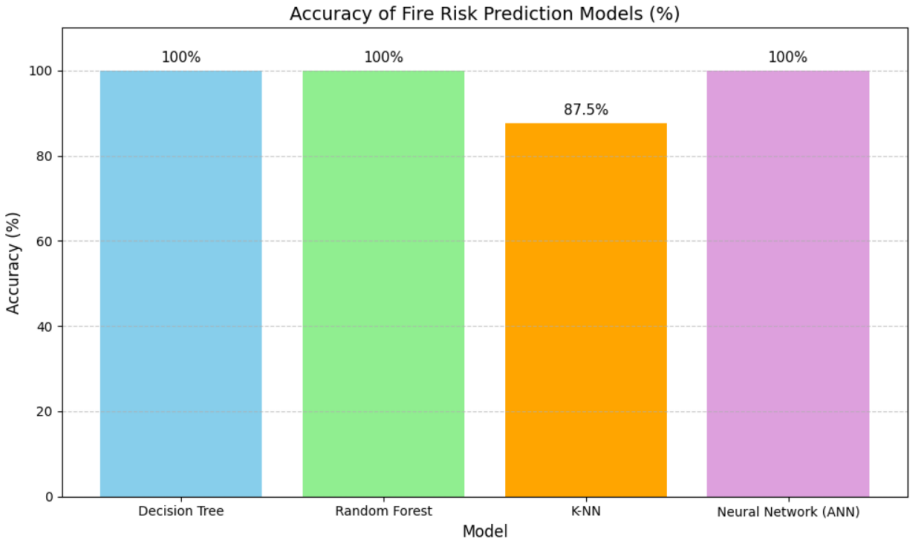


Figure 2: Accuracy of fire risk prediction models (%).

Table 6: Comparative analysis of Random Forest, kNN, MLP, Decision Tree and proposed algorithms.

№	Algorithm	Parametrs	Accuracy of training	Control accuracy (Accuracy)	Error values of the training sample	Error values of the control sample
1	Proposed Algorithm	Input Parameter: Improved Kalman Filter, Prediction Model	93.19%	92.01%	0.047	0.066
2	Random Forest	Trees: 10. Depth: None	82.18%	80.35%	0.208	0.133
3	kNN	Nearest Neighbors: 3. Distance: Euclid	81.02%	80.10%	0.279	0.211
4	Decision Tree	Core: RBF. C: 0.5. Gamma: 0.2	83.53%	81.45%	0.125	0.256
5	MLP	Hidden layers: (64. 32). Activation function: ReLU. Optimizer: Adam	92.10%	89.19%	0.024	0.014

3 CONCLUSIONS

The development of modern technologies creates the basis for the introduction of new approaches to ensuring fire safety. Digital systems aimed at predicting and extinguishing fires are intelligent platforms that allow for the early detection of natural and man-made fires, assessing the level of risk and taking effective measures. These systems operate based on AI, ML, sensor technologies and data analysis. Digital systems aimed at predicting and eliminating fires allow for early detection, monitoring, and rapid response to fire hazards based on modern technologies. These systems provide high accuracy in determining the likelihood of fires. This research proposed and validated a hybrid algorithmic framework that combines classical forecasting models with advanced tools such as the Kalman filter and adaptive ML models; including Random Forests and ANNs; which achieved up to 100% accuracy on specific datasets. The model's ability to process multi-parameter environmental data such as methane, oxygen, humidity, temperature and translate it into real-time risk levels

demonstrates its practical applicability for high-risk environments such as fuel stations, industrial facilities, and residential areas. The inclusion of optimal control theory further enhances resource allocation and decision-making efficiency. Relative examination confirms the superiority of the proposed model in both prediction accuracy and computational performance when benchmarked against traditional classifiers. This paper not only highlights the value of hybrid predictive systems in mitigating fire hazards but also sets a foundation for future implementations involving IoT integration, drone deployment, and autonomous response mechanisms in dynamic environments.

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General Principles of Application of Temporal Petri Nets in Intelligent Real-Time Decision Support Systems of Railway Automation of Uzbekistan

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Keywords: Railway Automation, Graph Theory, Mathematical Modeling, Control Relays, Intelligent Systems, Petri Nets, Algorithms.

Abstract: The relevance of this study lies in the need for modern scientific and technological advancements to enhance railway transportation management, ensuring efficiency and safety amid increasing train traffic and freight turnover. The solution of these problems is primarily due to the need to use modern achievements of microelectronic technologies, telecommunication capabilities associated with a high risk of errors at the design stage of such, hybrid systems (containing simultaneously relay and microprocessor equipment) and extremely high price for these errors during operation. Petri Nets provide a mathematical framework for railway automation, yet their full potential in real-time intelligent decision support systems remains underexplored. The purpose of this paper is to synthesize and study a mathematical model with the possibility of organizing the work of simulated devices of railway automation and telemechanics with qualitative time dependencies, and the presence of priority for some operations and proposed extension of the apparatus of colored Petri nets (CPN) - PPN RT - with support for temporal interval logic Allen. The method of research is based on the fact that the set problems are solved using the methods of discrete mathematics, mathematical logic, graph theory, algebraic model theory and the method of analyzing the complexity of algorithms. The results obtained by the authors have practical and theoretical significance. The method of modeling of responsible circuits of railway automatics presented in the article will contribute to the effective implementation of microelectronic technologies in the existing relay systems of automation and telemechanics.

1 INTRODUCTION

The need to apply the latest scientific and technical developments, progressive labor methods, thorough reforming of transportation process management is dictated by the high intensity of train traffic and ever-increasing freight turnover, while unconditionally ensuring the safety of train traffic. Modern means of automation and control over the technical condition of railway automation devices help to solve these problems. The current pace of development of microelectronics makes it possible to freely apply them to the development of automation means, to link them by means of hardware and software complexes [1-18].

Petri nets (PN) are mathematical objects and as such are independent of physical interpretation. They can effectively describe parallelism. Their graphical representation is used as a tool for modeling and analyzing digital systems, especially logic control devices.

However, a significant disadvantage of classical Petri nets is the complexity of modeling the functioning of real-time systems, when it is necessary to take into account the time factor and temporal dependencies. As a result of research efforts [8,9], a new extension, colored Petri nets with support for Allen's temporal logic [8], has been proposed to model both quantitative and qualitative temporal dependencies. This logic is characterized by sufficient expressiveness and the presence of polynomial inference algorithms, which allows its application in systems such as intelligent real-time decision support systems. The paper [7] gives a

description and an example of using the developed basic software tool for this class of Petri nets.

Colored Petri nets (CPN) are an interpretation and extension of ordinary, classical Petri nets by associating with each chip and transition of a colored Petri net a value of some type - gamut of colors. This value can be of arbitrarily complex type. The functioning of the CPN depends not only on the presence of tokens in the input locations of the crossings, but also on their coloring, and the priorities for triggering the crossings can also be determined by coloring them. For example, when the conditions for triggering two or more transitions are met at the same time, priority is given to a predetermined color scheme; in the models of systems responsible for train safety, the red color is given priority (see Figure 3).

Tools for modeling and analysis of complex parallel and distributed systems, including real-time systems, of which real-time (RT) intelligent decision support systems (IDS) are characteristic representatives, are of great interest [4, 5]. In the case under consideration, when modeling the relay control unit for changing the direction of train movement, there is a time delay of some relays, in real time, in order to ensure synchronous operation of the means used.

The relevance of these tools is primarily due to the high risk of errors at the design stage of such systems and the extremely high cost of these errors at the operational stage. Classical Petri nets have long established themselves as a convenient, illustrative and at the same time mathematically rigorous formalism for modeling and analysis of distributed systems, however, when modeling the functioning of real systems it is necessary to explicitly take into account the time factor and temporal (temporal) dependencies, which is quite difficult in the theory of classical PN. Known PN extensions allow to take into account quantitative time dependencies, while in IDS RT it is often necessary to have tools for representing and operating with qualitative dependencies. The optimal option is a tool environment that allows operating with both types of dependencies. Classical Petri nets have also been extended and supplemented by novel computational paradigms to improve railway system modeling and control, including approaches based on quantum computing [19].

This paper considers the possibility of organizing work with qualitative temporal dependencies and proposes an extension of the apparatus of colored Petri nets (CPN), the CPN RT, with support for Allen's temporal interval logic [8].

In recent years, the problem of creating computer (software) systems that automate various types of human activities in order to improve efficiency has become more and more urgent. A typical representative of such systems are intelligent decision support systems actively implemented in various subject/problem areas. Including real-time decision support systems (IDS RT), designed to assist a person or a device (hereinafter referred to as DMF - decision maker by face) in managing complex technical and organizational objects under conditions of rather strict time constraints and in the presence of various kinds of uncertainty (inaccuracy, vagueness, incompleteness, contradiction) in the incoming information.

In [9] it is shown that in practice CPN represent a more compact and convenient modeling language than classical PN.

2 RESEARCH METHODS

The tasks are solved using methods of discrete mathematics, mathematical logic, artificial intelligence, graph theory, algebraic model theory and the method of algorithm complexity analysis.

3 FINDINGS AND DISCUSSION

This paper considers the modeling of a single shooter relay control unit. Let us define the temporal modification CPN of the real-time (CPN RT) relay control unit as a tuple.

$$CTPN \text{ RT} = \langle \sum, P, T, F, \xi, \gamma \varepsilon_{\sum}, \varepsilon_{\tau}, m_0 \rangle,$$

where $\sum -$ a finite set of types (colors), $P \equiv \left[p_1, p_2, \ldots p_{|p|} \right] -$ finite ordered set of positions, $T -$ finite number of transitions, $P \cap T = \varnothing$;

$F \subseteq (P \times T) \cup (T \times P) -$ non-empty set of arcs;

$\xi : P \to \sum -$ function that matches each position $p \in P$ peculiarity of its chip (color scheme);

$\gamma : T \to Bool,$ where $Bool = \{true, false\} -$ protective (guarding) function, which puts in correspondence a transition $t \in T$ with some logical expression.

At the same time, when developing the model of the single shooter control device, we assume that the initial state of the network is shown in Figure 1. where

$$I(1PC) = \text{Aktive1} \quad (1)$$

$$O(1PC) = [\text{Aktive2}], \left[\overset{\bullet}{\gamma}(t)[1PC\ d\ 1MC]/true\right]; \quad (2)$$

$$I(1\overline{PC}) = [\text{Aktive2}], \left[\overset{\bullet}{\gamma}(t)[1PC\ d\ 1MC]/true\right] \quad (3)$$

$$O(1\overline{PC}) = \text{Aktive1}; \quad (4)$$

$$I(\text{Aktive1}) = \left[(1\text{-}13)\ d\ (1\overline{MC})\ d\ (1-3)\right] \quad (5)$$

$$O(\text{Aktive1}) = 1PC \quad (6)$$

$$I(\text{Aktive2}) = \left[\begin{array}{l} 1MC \\ or(\overline{1-3}) \\ or(\overline{1-13}) \end{array}\right], \left[\overset{\bullet}{\gamma}(t)[1PC\ d\ 1MC]/true\right] \quad (7)$$

$$O(\text{Aktive2}) = 1\overline{PC} \quad (8)$$

$$I\left\{\left[\overset{\bullet}{\gamma}(t)[PC1\ d\ MC]/true\right]\right\} = 1PC \quad (9)$$

$$O\left\{\left[\overset{\bullet}{\gamma}(t)[1PC\ d\ 1MC]/true\right]\right\} = 1\overline{PC} \quad (10)$$

there is a position $1PC-$ plus control relay the presence of a chip in this position, $1PC = 1$ corresponds to the excited state of this relay.

$1\overline{PC}-$ inverse state of the relay, that is $1\overline{PC} = 1$ presence of the chip in this position corresponds to the de-energized state of the relay. It should be borne in mind that the simultaneous fulfillment of the condition $1PC = 1, \overline{PC} = 0$ and $1PC = 0, \overline{PC} = 1$ is permissible, while the simultaneous fulfillment of the condition $1PC = 1, \overline{PC} = 1$ and $1PC = 0, \overline{PC} = 0$ is not permissible.

The model provides a protective logical transition function in accordance with Allen's temporal logic .

$\gamma(t)_{[1PC\ d\ 1MC]}/true$,defining the impossible and dangerous condition of the control unit of a single arrow, when two arrows are simultaneously in the plus position. Failure to fulfill this condition (*false*), v (I) and weekend (O) functions are given in the system of (1)-(10).

For the graph of the second starting control, relay shown in Figure 2, the extended input (I) and output (O) functions are given in the system of (2). In the initial state, a colored temporal real-time Petri net is defined as a tuple with the relation.

$$CTPN\ RT\ m_0 \equiv \langle \sum = 2, P = 2, T = 3, \overset{\bullet}{F} = 5, \xi = 1, \gamma = 1, \varepsilon_{\sum} = 4, \varepsilon_{\tau} = 1 \rangle$$

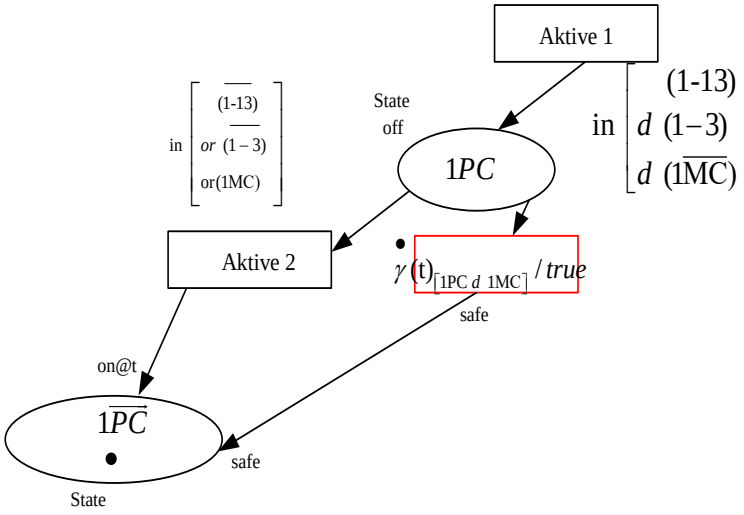


Figure 1: Operation model of the 1PC control unit relay (CPN RT with Allen logic support).

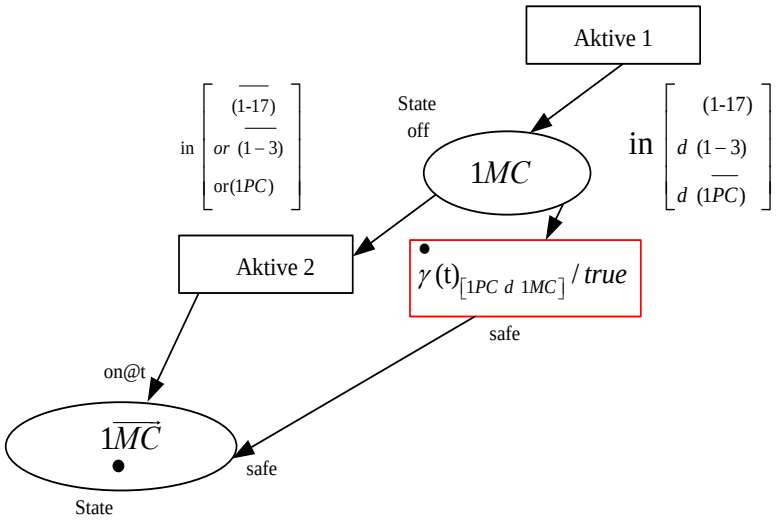


Figure 2: Operation model of the 1MC control unit relay (CPN RT with Allen logic support).

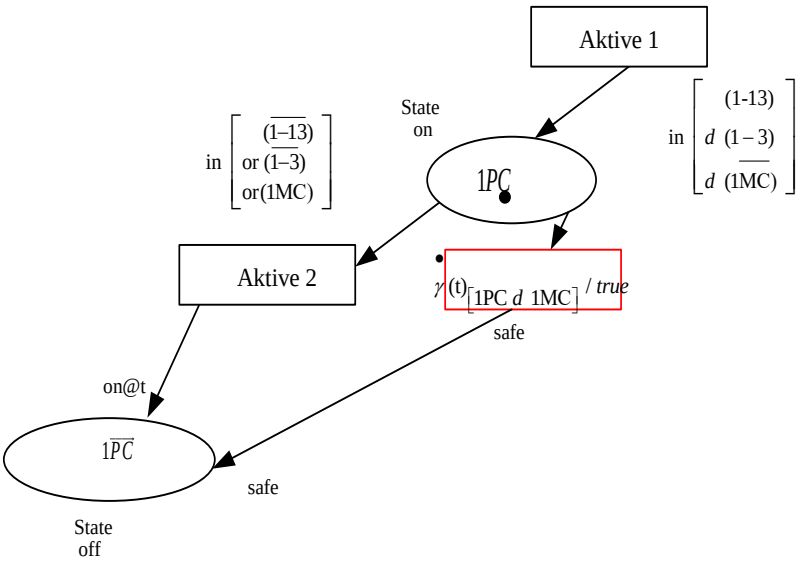


Figure 3: Model of de-energized relay 1PC control unit (CPN RT with support of Allen logic).

Let's consider the state of the model when the conditions for relay operation are met 1PC. In this case, there is power at the terminals (1-3) и (1-13) and the variables reflecting this state will take the value of that is $(1-13)=1,(1-3)=1$. Relay 1MC и 1PC are de-energized, therefore, the transition conditions are satisfied *Aktive* 1, which helps to move the chip from the position $1\overline{PC}$ in position $1PC$, and changing its status from State off to State on, which corresponds to the excited state of the relay 1PC. In this case, the model will take the form shown in Figure 3.

$$I(1MC) = \text{Aktive1}, \quad (11)$$

$$O(1MC) = [\text{Aktive2}], \left[\dot{\gamma}(t)[1PC\ d\ 1MC]/true\right], \quad (12)$$

$$I(1\overline{MC}) = [\text{Aktive2}], \left[\dot{\gamma}(t)[1PC\ d\ 1MC]/true\right], \quad (13)$$

$$O(1\overline{PC}) = \text{Aktive1}, \quad (14)$$

$$I(\text{Aktive1}) = \left[(1\text{-}17)\ d\ (1\overline{PC})\ d\ (1-3)\right], \quad (15)$$

$$O(\text{Aktive1}) = 1MC \quad (16)$$

$$I(\text{Aktive2}) = \begin{bmatrix} 1PC \\ or(\overline{1-3}) \\ or(\overline{1-17}) \end{bmatrix}, \left[\overset{\bullet}{\gamma}(t)[1PC\ d\ 1MC]/true\right], \quad (17)$$

$$O(\text{Aktive2}) = 1\overline{MC}, \quad (18)$$

$$I\left\{\left[\overset{\bullet}{\gamma}(t)[1PC\ d\ 1MC]/true\right]\right\} = 1MC \quad (19)$$

$$O\left\{\left[\overset{\bullet}{\gamma}(t)[1PC\ d\ 1MC]/true\right]\right\} = 1\overline{MC}, \quad (20)$$

The algorithm of the microelectronic control unit of a single arrow developed according to the results of the study, colored real-time Petri net on the basis of intelligent decision support system and using Allen's temporal algorithm is presented in Figure 4. In the mentioned algorithm, special attention is required to be paid to the transition 5, made in red lines, to emphasize the necessity of periodic control of the fulfillment of the condition of safety violation, in a programmatic way.

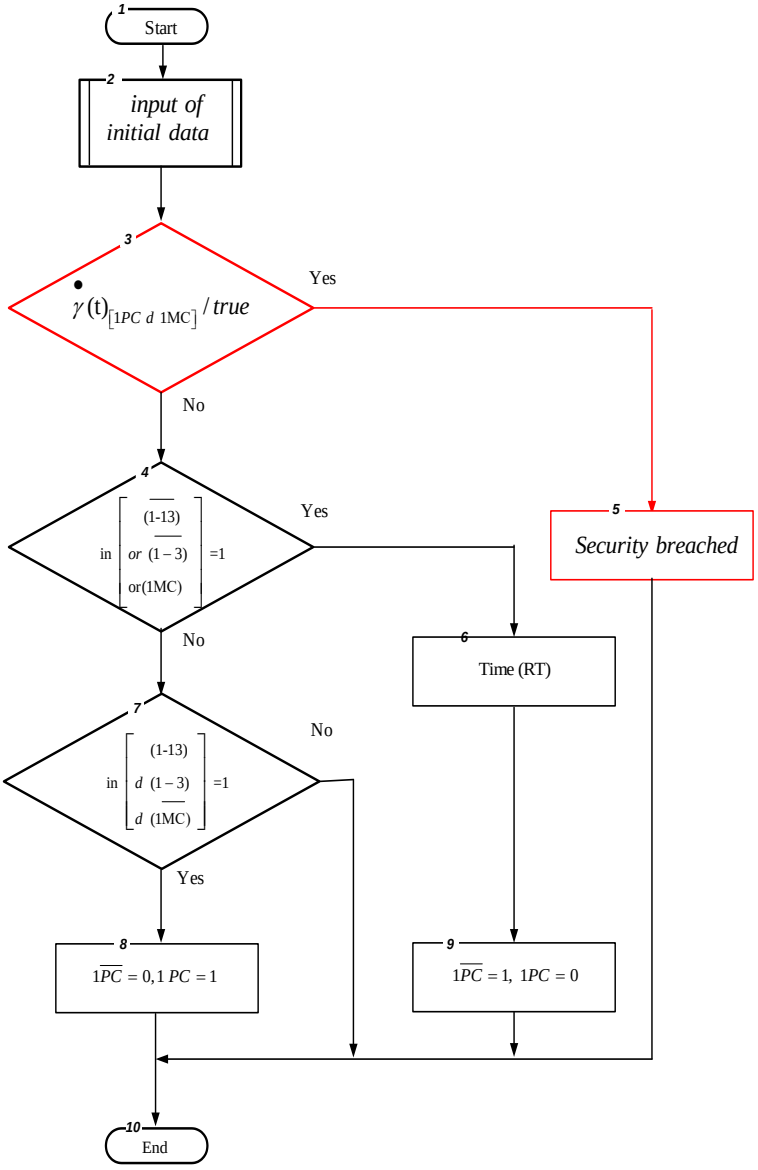


Figure 4: Algorithm of operation of the positive control relay of the microelectronic control unit of a single switch (CPN RT with support of Allen logic).

Fulfillment of the conditions of block 4 leads to the procedure of de-energizing the relay 1PC, hence, the software translates the variables into the state of $1PC = 0, \overline{PC} = 1$ with time delay, the numerical value of which is determined by the factory characteristics of the device.

Let us consider the operation of the model relative to the negative control relay 1MC. The graph of this model is presented in Figure 2. For this graph, the expanded input (I) and output (O) functions are given in the system of equations (11-20). In the initial state, relay 1MC is in a de-energized state, which is reflected in the graph by the presence of a chip in position $1\overline{MC}$, therefore, the software variables reflecting the state of this relay have the following values $1MC = 0, 1\overline{MC} = 1$. To transfer a chip $1\overline{MC}$ from $1MC$ one position to another according to the graph in Figure 2, it is necessary to fulfill the conditions of "Active1" and the system of equations, where the input function is $I(1MC) = \text{Aktive1}$ (11). This transition can be started when the logical condition is met $(1\text{-}17)d(1-3)d(1\overline{PC}) = 1$, that is there must be power on terminals 1-17 and 1-13, and the condition that the variable must be met must also be met $1\overline{PC} = 1$. The launch Active1 of the transition facilitates the transfer of the chip to the position $1MC$, which corresponds to the operation of relay 1MC.

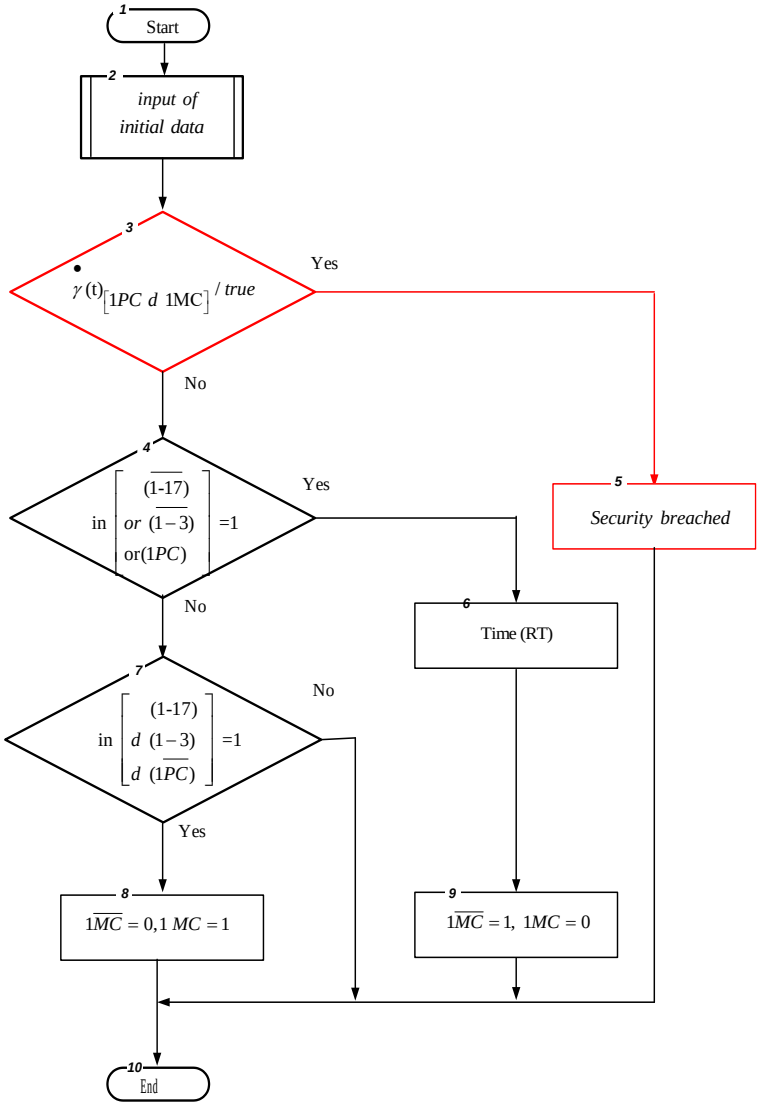
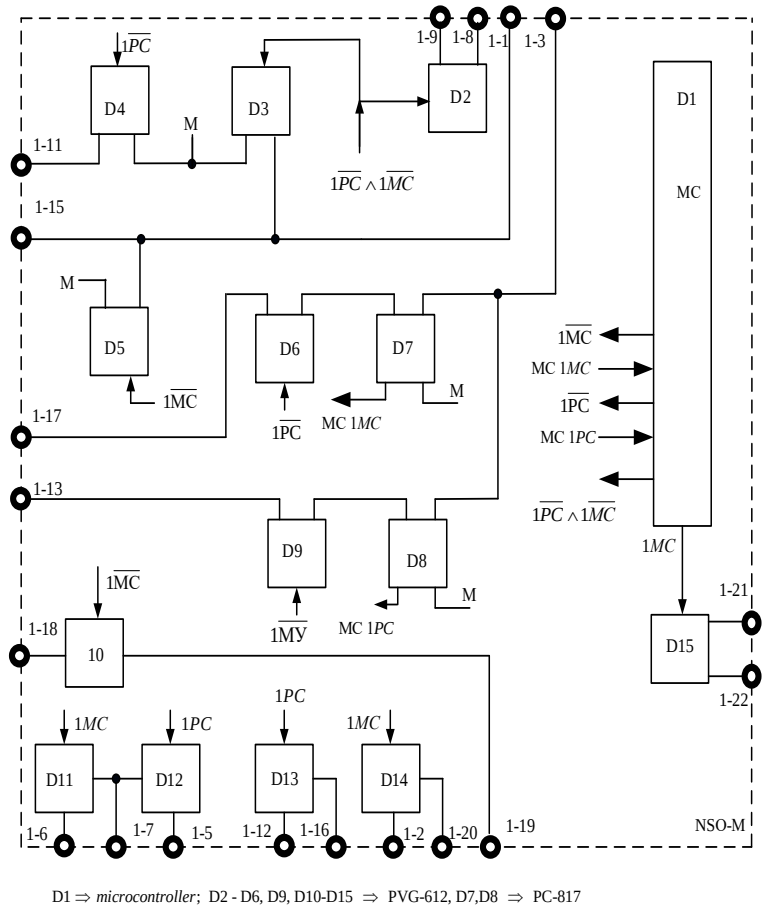


Figure 5: Algorithm of operation of the minus control relay of the microelectronic control unit of a single switch (CPN RT with support of Allen logic).



D1 ⇒ *microcontroller*; D2 - D6, D9, D10-D15 ⇒ PVG-612, D7,D8 ⇒ PC-817

Figure 6: Functional diagram of the microelectronic control unit for a single switch.

The safety conditions are checked by the microcontroller software by continuously monitoring the state of the variables $1MC$ and $\overline{1MC}$, the relationship must be completely excluded $1MC = 0, \overline{1MC} = 0$ or $1MC = 1, \overline{1MC} = 1$ for this purpose in the column of Figure 2 this position is colored red. De-energization of relay 1MC occurs when the transition trigger condition is met according to equation $I(\text{Aktive2}) = 1PC \text{or} (\overline{1\text{–}3}) \text{or} (\overline{1\text{–}17}) = 1$ (11), that is $1PC = 1$, $(\overline{1\text{–}3}) = 1$, or $(\overline{1\text{–}17}) = 1$. According to the operating conditions of the relay system, relay 1MC has a delay time for dropouts, which is implemented in the graph of Figure 2 by the presence of the variable $on@t$. Similar reasoning is also valid for the position of 1PC in the system of equations (1-10).

The functional diagram of the microelectronic unit is shown in Figure 6, where as a microcontroller (MC), a simplified version can be used ARDUINO. Similar reasoning is also valid for the algorithm of the minus control relay, shown in Figure 5.

4 CONCLUSIONS

The application of colored Petri nets in the study of intelligent real-time decision support systems based on Allen's temporal algorithms with real time scaling is considered on $@t$ system operation TCPN IDS RT. A model of a microelectronic control unit for a device for changing the direction of train movement (single switch), a block route relay interlocking system has been developed and studied. As a result of using the advanced capabilities of classical Petri nets, it was possible to obtain a universal graph that

implements the modeling of all the listed control relays. in particular, time components in the form of time delays, by changing some variables, which in real relay systems is called the anchor release real time. The universal graph also shows the positions that lead to dangerous failures, marked in red.

The algorithms of its operation is obtained, and the blocks of the algorithm are emphasized $Bool = \{true, false\}$ – realization of which leads to a violation of train traffic safety, which are marked by colored lines on the graph and algorithm in Figure 4 and Figure 5.

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Normalization of Time Losses in Sorting Station Operations Using a Python-Based Instrumental Tool

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Keywords: Sorting Station, Python, Instrumental Tool, Unproductive Time Loss, Wagon Flow, Receiving Park.

Abstract: The study aims to reduce unproductive time losses in wagon flow processing at railway sorting stations using a Python-based software tool. The work employed mathematical and graphical modeling, structural analysis, flowcharting, algorithm theory methods, and the Python programming language. The problems caused by unproductive time losses in sorting station operations were identified. Based on the flowcharting method, a systematic analysis of the operation of the sorting station's receiving park was carried out, revealing that time losses are caused by human factors during waiting periods between technological operations. The values of unproductive time losses were determined for each element of the receiving park's operation. Consequently, it was concluded that the current graphical modeling method is inefficient and that a new mechanism for reasonably standardizing the value of each element needs to be developed. An instrumental tool was created using the algorithm theory method and the Python programming language to standardize time losses at the sorting station. This mechanism enables rational regulation of the value of each element in the operation of the receiving park and allows online monitoring of station employees' activities.

1 INTRODUCTION

There is a strong competition among modes of transport in the world to solve the problem of minimizing unproductive time losses in transporting goods and passengers to their destinations in the shortest possible time. In this, the role of railway transport, which occupies a leading position in the implementation of large-scale transportation, is of great importance [1]-[5].

When delivering cargo to its destination by railway, the rhythm of transportation is determined by sorting stations [3], [6], [7].

The main task of sorting stations in the transportation process is to process train and wagon flows in minimal time intervals [3], [6]. In this case, one of the main problems is to minimize unproductive time losses in technological operations and inter-operational work performed with transit and local wagons [3], [6], [8]-[10].

The standing time of transit processing wagons at the sorting station consists of the following parameters [3], [6], [9].

Here (Fig. 1) setting technical standard values for each parameter and monitoring their implementation in practice determines the efficiency of the transportation process [1], [3], [8]. However, various factors negatively affect the actual implementation of the station wagon standing time standards during the production process [3], [6], [7], [10], resulting in unproductive time losses during the transportation process. Such time losses cause the following problems for railway transport: exceeding the norm of shipping deadlines; to decrease or loss of customer confidence in shipping; to the decline in its position in the transport market; a sharp decline in the sector's economic revenues; the emergence of economic problems in the future development of the industry, etc.

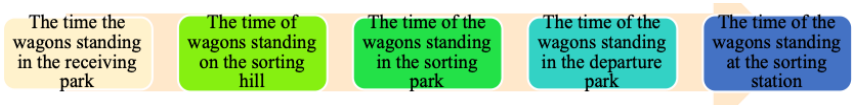


Figure 1: Parameters of the standing time of wagons at the sorting station.

From the above, it can be concluded that the human factor is mainly responsible for the emergence of such problems. In this regard, it is necessary to develop a mechanism for reasonably regulating the value of each parameter of the waiting time of wagons at sorting stations, which determine the rhythm of the transportation process, and to control them in practice.

2 METHODS

Many scientists have conducted scientific research over the years on the development of technologies for organizing and managing wagon flows at sorting stations, including the development and improvement of methods for determining, standardizing, monitoring, and analyzing the standing times of wagons at the station and in each of its parks [1], [2], [4], [6], [9].

Special attention is paid to the analysis, evaluation, standardization and modeling of the times spent in the entire cycle of distribution at the station's receiving park and sorting hill, based on the elements that make up them (time spent waiting for the composition to be received, receiving, securing, separating the train locomotive from the group of wagons, processing and commercial inspection of the composition, waiting for the shunting locomotive to be connected to the processed composition, pushing towards the sorting hill and distributing the composition) [1], [2], [4], [6], [9].

However, in the above-analyzed scientific works, the problems of modeling the process of organizing wagon flows, taking into account the rapid elimination of situations arising as a result of the main factors that cause "Unproductive time losses" during the processing of wagons and process control, have not been sufficiently studied. In this regard, in this scientific work, work was carried out to normalize unproductive time losses in the operation of the sorting station receiving park.

3 RESULT AND DISCUSSION

It is known that in the process of transportation, technical stations take the leading place in terms of the volume of work. Technical stations are the main base for efficient organization of the flow of wagons in the railway network. Despite the small number of technical stations in the railway network, they determine the implementation of the transportation plan.

In determining the effectiveness of technical stations' work technology, the practical implementation of the indicator "Wagons standing time at the station" is of great importance.

Currently, various methods (analytical, graphic, table, etc.) are widely used in the world railways to establish the value of this indicator. In particular, analytical methods are widely used in most technical stations.

The waiting time of wagons in the receiving park consists of the following elements [3], [6], [9]: $t_{r.p.}=t_{s/b\ (w)}+t_{s/b}+t_{proc/rec(w)}+t_{proc/rec}+t_{diss(w)}$, hour, there $t_{s/b\ (w)}$ – waiting time of securing and blocking composition in the receiving park, hour; $t_{s/b}$ – time of securing and blocking composition in the receiving park, hour; $t_{proc/rec(w)}$ – waiting time for composition processing in the receiving park, hour; $t_{proc/rec}$ – processing time after composition arrival, hour; $t_{diss(w)}$ – distribution waiting time, hour. The elements causing wagon standing times are illustrated in Figure 2. Current metrics of wagon processing elements are presented in Table 1.

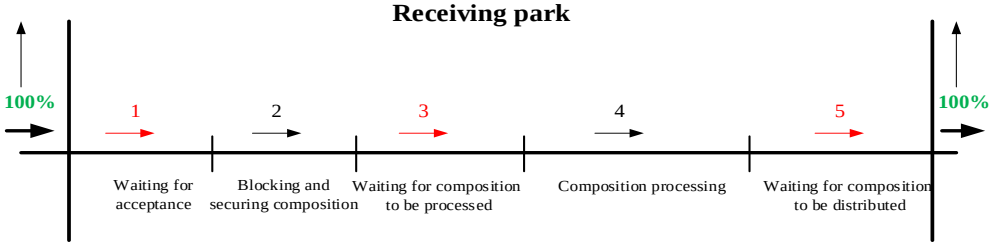


Figure 2: Elements that cause the time that wagons standing in the receiving park.

Table 1: Current metrics of the 5 key elements in wagon processing at the sorting station receiving park.

Name of elements	Criteria	
	Technological operations	Waiting between operations
1. Waiting time of securing and blocking composition in the receiving park, hours		
2. Time of securing and blocking composition in the receiving park, hours		
3. Waiting time for composition processing in the receiving park, hours		
4. Processing time after composition arrival, hours		
5. Distribution waiting time, hours		

Table 2: Distribution of composition at receiving park.

Train number	The number of wagons in trains	Time of receiving to "Ch" station, minutes	The time of fixing and blocking the composition, minutes	Waiting time for processing, minutes	Composition processing time, minutes	Distribution waiting time, minutes	Composition distribution time, minutes
First day (2nd shift)							
1252	47				Left over from the previous shift	00-44	00-45
2580	55	20÷35	00-07	00-03	00-35	00-10	00-45
2036	60	21÷20	00-08	00-05	00-45	03-32	00-55
3002	55	21÷50	00-05	00-13	00-58	04-35	00-37
1250	43	23÷50	00-10	00-18	00-47	03-40	00-38
2592	58	00÷30	00-07	00-05	00-50	03-38	00-32
1262	69	03÷05	00-10	00-15	00-42	01-15	00-48
2002	55	03÷40	00-06	00-34	00-45		Left for the next shift
2586	62	06÷00	00-06	00-14	00-40	00-05	00-40
1256	46	06÷52	00-06	00-07	00-40		Left for the next shift

During the analysis of the elements that make up the waiting time of wagons in the receiving park of the sorting station, it was found that "Unproductive time losses" occurs mainly in inter-operational waiting times.

Unproductive time losses are due to operations such as waiting for content to start blocking and fixing, waiting for content to start technical processing and commercial review, and waiting for the processed content to be distributed using the sorting hill. In this regard, the work process of the "Ch" sorting station under the jurisdiction of "Uzbekistan Railways" JSC was studied. The work process of the station during the night shift (from 20:00 to 08:00 in the morning) was analyzed. Distribution data of composition at receiving park is shown in Table 2.

Freight train No. 1252 (Table 2) was received in the previous shift and the processing of the train was completed on time. However, due to the ongoing distribution work on the sorting hill, this train had to wait for 44 minutes, and 45 minutes were spent on the distribution of the train. Also, during this shift, freight trains No. 2002 and No. 1256 were received in the station's receiving park at 03:40 and 06:52, respectively, and the processing was completed on time. However, in this case, the distribution of both trains mentioned above was transferred to the next shift.

Based on the results of the research carried out at the sorting station, a general time flow map of the composition was developed in the sequence of technological operations from the time the group of wagons was received on the tracks of the receiving park of the station until it was transferred to the

tracks of the sorting park of the sorting station. The waiting for fixing and blocking the composition received by the station attendant on the station territory and waiting for fixing and blocking, processing and processing, as well as workers involved in the process of technical-technological operations such as waiting for distribution, and their command and communication procedures were mentioned. The composition received for processing by the station attendant at the station reception park is fixed by the station park officer (DSPP) with the help of brake pins based on the instructions of the maneuver dispatcher, in which the slope of the station according to the location of the receiving park is from station "K" to station "S" since it deviates to the side, it is enough to double-fix the compositions only from the head. There are 8 tracks in the station reception park, and there are a total of 7 stelaj[1] and 63 brake shoes with stelaj for storing brake shoes next to each track, 8 brake shoes in each stelaj are strictly controlled. is used, in which 1 brake shoe is installed on the wheel pair of the first wagon of the train, and then on the wheel pair of the second wagon, 1 brake shoe on the slope, 1 brake shoe against the slope, a total of 3 brake shoe of brake head are installed. After the above process is completed, it retransmits the fix message to the maneuver dispatcher. Next, based on the relevant instructions of the park attendant, the train locomotive driver's assistant separates the locomotive from the train, and the train locomotive leaves the station area with the permission (green) light of the station's route traffic light. For the technical and commercial inspection of the composition, the working team (PTO[2]) starts to check the composition, the current condition of the wagons, wheels, safety seals, cleaning system hoses is checked technically, the standard time set at this station is 60 minutes. In parallel with the technical inspection, the commercial inspection of wagons (PKO[3]) is performed by employees. In this case, PKO employees checked the correctness and availability of documents related to cargo. In the course of the inspection, if a technical malfunction is detected in any wagon, the wagon will be sent to the technical inspection point located at this station to study and eliminate the problem. If there is any problem or deficiency related to the cargo documents, then the wagon will be opened in the presence of witnesses and employees of the internal affairs body and a document in the form of GU-23 will be drawn up.

In order to start the sorting process, all information such as the technical characteristics of each wagon, the station of their destination, whether the goods are classified as dangerous goods or not is obtained from the nature sheet (DU-1) from the technological center of the station, and the sorting sheet is made in 2 copies, the first copy is the sorting sheet to the duty officer and his switcher, the second copy is given to the train former. In this case, with the permission of the sorting hill duty officer, i.e. "DSPG", his signalman changes the direction of the switch towards the designated track of the sorting park, and one or more groups of wagons are separated from the rest of the wagons by the train conductor. A flowchart was created to identify inefficient time losses in the sorting station operation (Fig. 3, 4, 5) [9].

At (Figure 3) when the train arrives at track 6, which is located in the station's reception park, the park shift waits for the train to stop completely (2 minutes), then provides safety information from the station shift (0.5 minutes), and only then begins to pass by the train (0.2 minutes). The process of fixing the train consists of three repeated operations – moving to the side of the stelaj and taking the brake shoes (0.5 minutes), installing it after receiving the "Braking shoes" by going to the side of the contents (0.5 minutes), moving to the fastening and installing the "Braking shoes" (0.5 minutes). The process is thus repeated three times again. After that, the brake shoes were partially removed over the wheel pairs head so that they did not move on their own-that is, nakat[4] (1 minute), and the assistant train locomotive machinist's assistant separates the locomotive from the composition (1.5 minutes) and waits for the station shift permission order (5 minutes). With the permission of the station duty officer, the signal of the route traffic light will be changed to green, which means the order to the train locomotive that it is possible to leave the park. Once the security work is finished, the DSPP will be able to return to the post. In this process, the "Installation of brake shoes" and the separation of the train locomotive from the composition operations are considered useful work. The processes of waiting for the post shift movement composition to stop, waiting for the stop message, and waiting for the permission message until the train locomotive departs are considered inefficient time losses. Thus, the time indicator until the completion of the general fastening and blocking processes was 13.2 minutes, while the useful working time is 3 minutes, with a total process efficiency of 22.7%.

At Figure 4 the total time required from the time the train enters the station area at the signal of the entrance traffic light to the start of the work on the

[1] Stelaj - where the brake shoe is stored.
[2] PTO - point technical inspection.
[3] PKO - point commercial inspection.
[4] Nakat - pushing wagons towards the brake shoe by maneuvering the locomotive to fix the composition.

station (about 14 minutes), and even then, the process of securing the train consists of three repeated operations going to the stelaj and taking the brake shoes (0.5 minutes), after receiving the "Braking shoe" and installing it next to the composation (0.5 minutes), going to the place of fixation and installing the "Braking shoe" (0.5 minutes). The process of waiting until the locomotive is separated from the train and pushing wagons towards brake shoe (2 minutes), the assistant driver of the train locomotive separates the locomotive, this process takes time (1.5 minutes). After the completion of all the above processes, it is possible to start maintenance and commercial inspection of wagons, based on the technical standard (60 minutes) given at the station where the research was conducted. To cancel the blocking order after these actions, the park duty officer sends a message to the station duty officer to remove the blocking, and the process takes (2 minutes) to complete. The train former takes the sorting sheet and comes to the train (10 minutes) and takes the his work place (1 minute).

While the total (94 minutes) time for the waiting and processing process is rectified, then the useful working time (64.5 minutes), the time for fixing the composition in the above processes, separating the train locomotive from the composition and processing "Technical and commercial inspection" corresponds to the useful working time, while the time spent in the other process is excess expectations. We can see that the useful working time is 68.6% of the total time, depending on the time indicators mentioned. At (Figure 5) to wait for the sorting of the composition after the maintenance and commercial inspection of the composition received on the 6th odd road of the receiving park (5 minutes) and for the shunting dispatcher to open the station shunting traffic lights and prepare the route to start the sorting of the composition (2 minutes) is required. The shunting locomotive goes to the 6^{th} odd track in the receiving park from the back of the train (5 minutes) and is connected by the train engineer to move the wagons in the push method, this process takes time (2 minutes). After the shunting locomotive is connected, the DSPP removes the brake shoes securing the train (2.5 minutes). The driver of the shunting locomotive waits for the permission order from the sorting hill duty officer to start movement (2 minutes), the shunting locomotive moves the train to the arrow of the sorting hill (5 minutes), waits for the arrow to open and the traffic light of the sorting hill opens, and moves the wagons towards the hill (10 minutes), announcing the start of sorting through (1 minute), and sorting wagons based on the plan of the sorting sheet (45 minutes). In the course of sorting, if there are dangerous goods and oversized wagons in the group of wagons, these wagons should be pulled to the sorting park with the help of a shunting locomotive without dropping them from the hill (15 minutes). The total time in the sorting process is found to be (94.5 minutes) and from this (58 minutes) we can see that the useful working time is 61.3% of the total time.

In order to increase the speed of freight transportation and wagon turnover in railway transport, it is necessary to organize the work technology effectively and to automate it using modern technologies, taking into account many parameters. In this case, as we will see on the example of the "Ch" sorting station where the research is carried out, the steps of receiving and sending trains to the station are carried out by the station duty officer. The receiving park tracks number 3, 4, 5, 6, 7, 8, 9, 10 (via the main line) receive trains from stations "K" and "S", and the 4th post shift welcomes all trains in front of the incoming track.

In order to effectively organize the work process, the operator of the station duty officer informs the station on duty in advance about the trains scheduled to arrive at the station and their arrival time, the characteristics of the wagons and cargo, through the nature sheet form (DU-1). The Operator directly assists the station shift in the process of receiving and dispatching trains sent from the adjacent station. In the process of sending the new composition drawn up at the sorting station, the composition will be accepted to the 37, 38, 39, 40, 41, 42, 43, 44-tracks of the departure park, and after all documents have been formalized, the train will be connected to the locomotive and the compositions will be sent to the adjacent station. In this work, a software tool was developed to control trains that are accepted, shipped and passed without stopping to the "Ch" sorting station on the basis of modern technologies. Before the development of this system, a context diagram (Fig. 6) and IDEF0 (Fig. 7) diagram are presented using the IDEF0 design method.

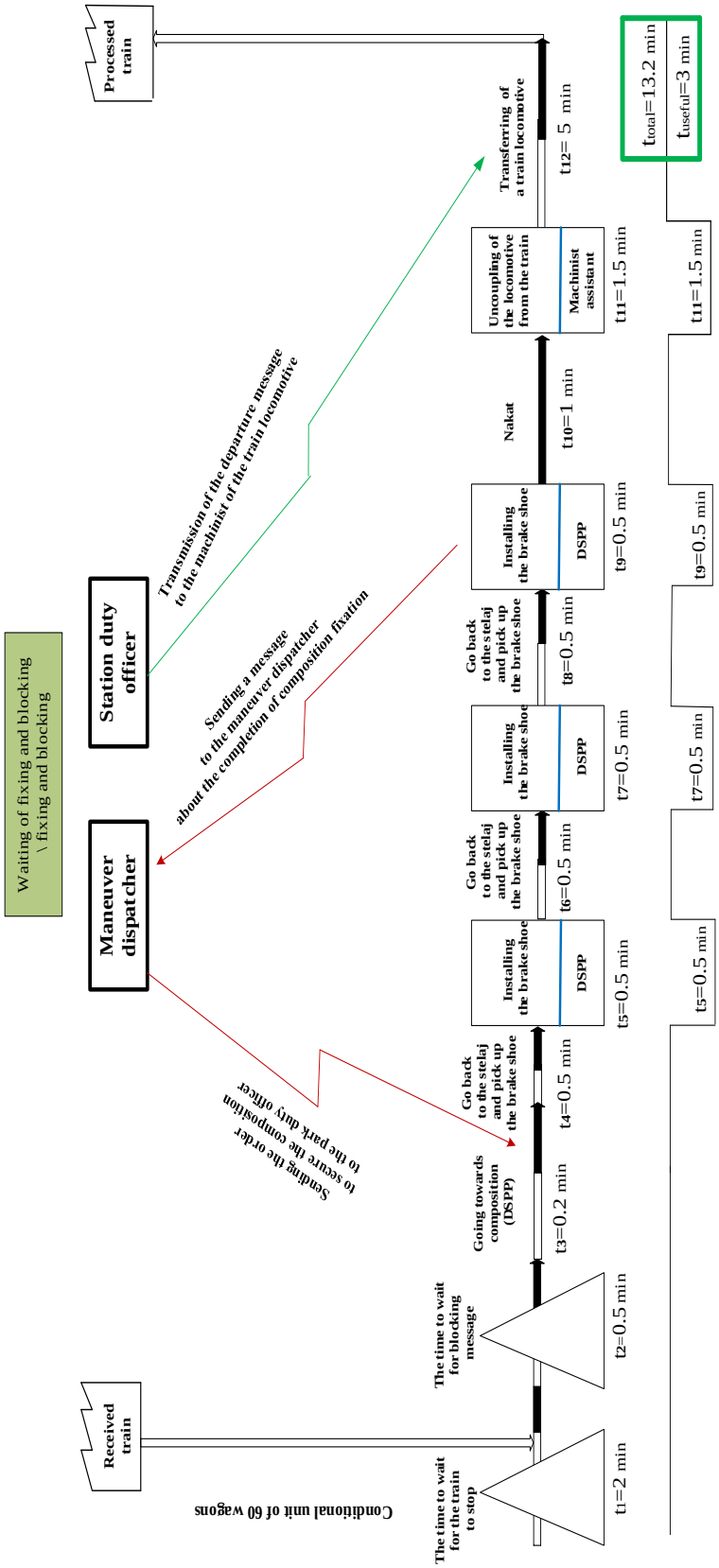


Figure 3: The flowcharting of the current status of the processes of securing and blocking trains received on the odd track №6 of the receiving park of the "Ch" sorting station.

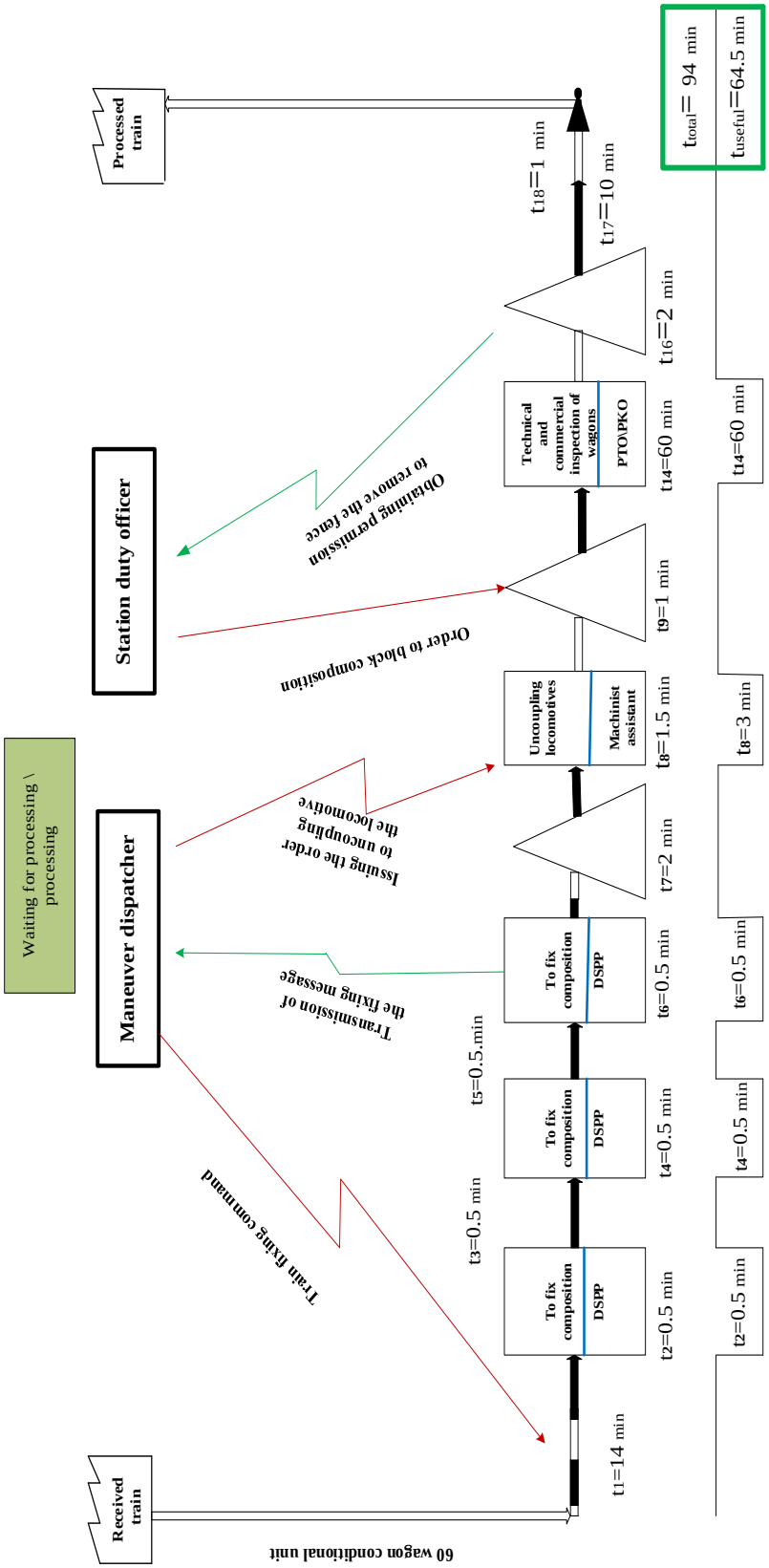


Figure 4: The current flowcharting of processing processes of trains received on the odd track №6 of the receiving park of the "Ch" sorting station.

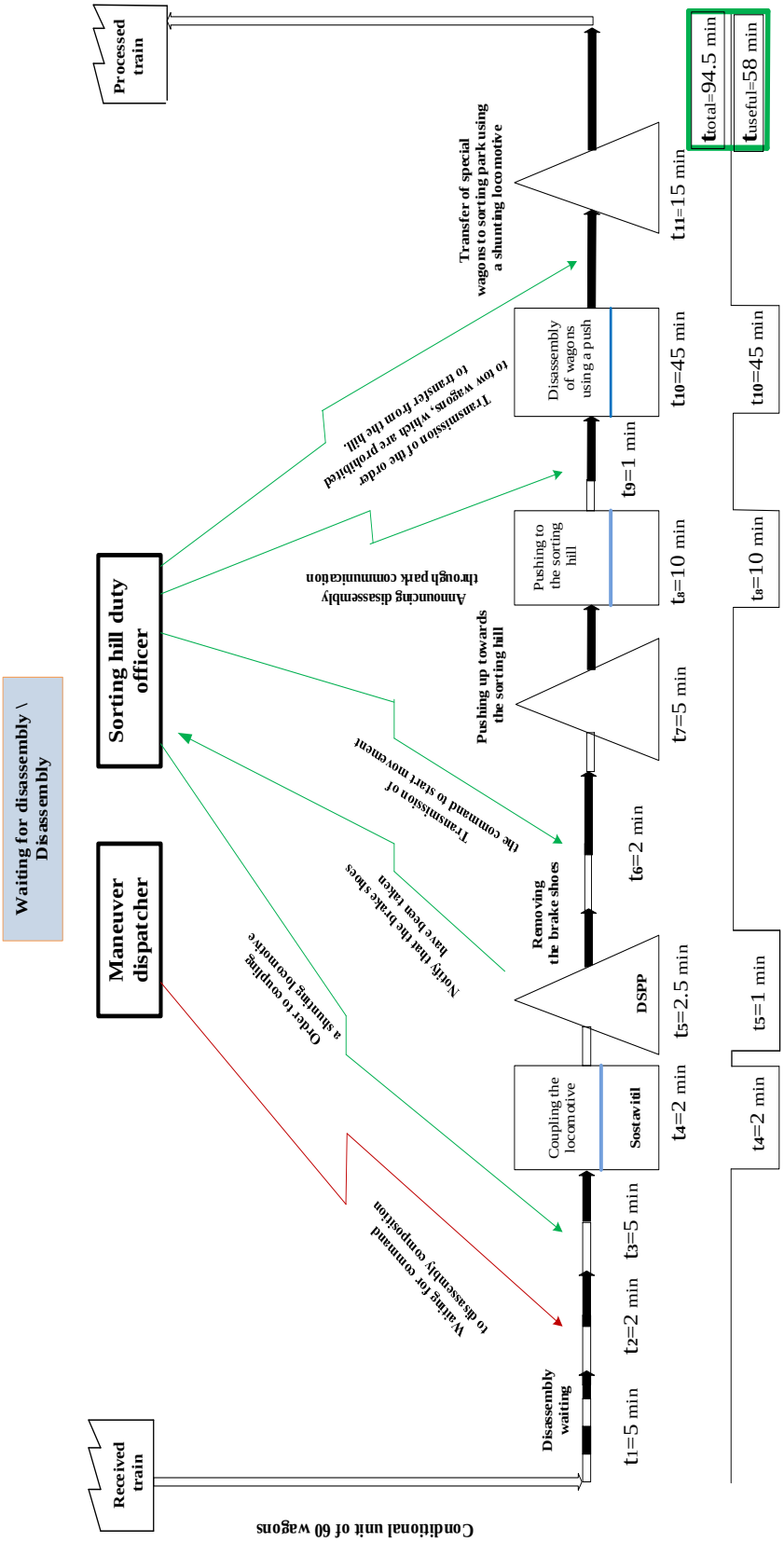


Figure 5: The flowcharting of the current state of the process of distribution of the received composition to the odd road №6 of the receiving park of the "Ch" sorting station is shown.

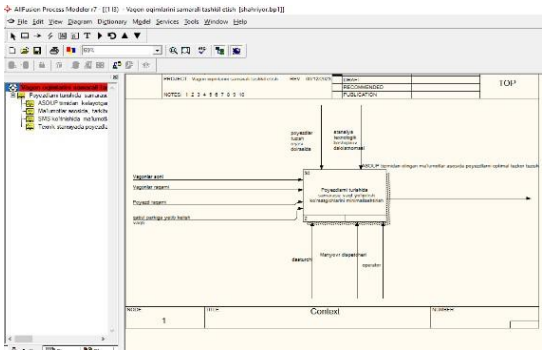


Figure 6: Context diagram.

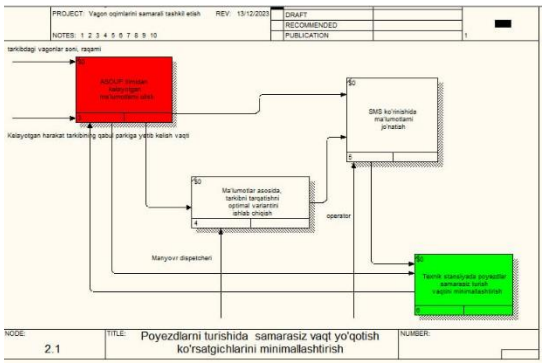


Figure 7: IDEF0 diagram.

IDEF0 is developed on the basis of a structural analysis and design methodology, has been approved as a USA standard and is successfully used in many projects related to the description of the activities of enterprises. IDEF0 can be used to model a wide range of systems.

The use of IDEF0 for new systems is aimed at identifying requirements and defining functions for the further development of the system that meets the requirements and implements the selected functions.

For existing systems, IDEF0 can be used to analyze the functions performed by the system and map the mechanisms for performing these functions.

The result of applying IDEF0 to a system is a model of a system consisting of hierarchically ordered diagrams, document text, and interconnected functions through cross-links.

A context diagram is a model that represents a system as a set of hierarchical actions in which each action changes some kind of object or set of objects. The highest action of the hierarchy is called the action of the context - this is the highest level that directly characterizes the system. The following levels are called decompositions of work and represent sub-processes of activity.

We divide the context diagram into 4 functional blocks (Figure 6):

- Train lines information;
- Filling the DU-2, DU-3 train and locomotive movement journal;
- Filling out chronometry transfer tables;
- Proper organization of maneuver work.

In this case, the working window of the instrumental instrument (Fig. 8 and Fig. 9), created to effectively organize the movement of trains, is presented. In the working window of the software tool, the process of sending a train to an adjacent station is presented. In this process, the “Poyezd qo‘shish, adding train” button is pressed to run the program, and in the working window there is an opportunity to enter information that belongs to the train. In this case, the train number, train index, type of movement and road number will need to be entered.

If the data entered during the Figure 8 data entry process changes at this time or is entered incorrectly, the “O‘zgartirish, change” button listed in Figure 9 that allows it to be edited is pressed, and the possibility of reediting this data appears.

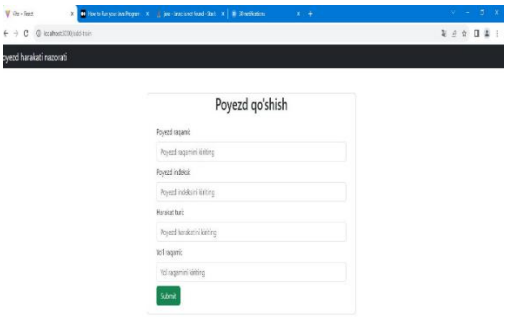


Figure 8: Adding train information.

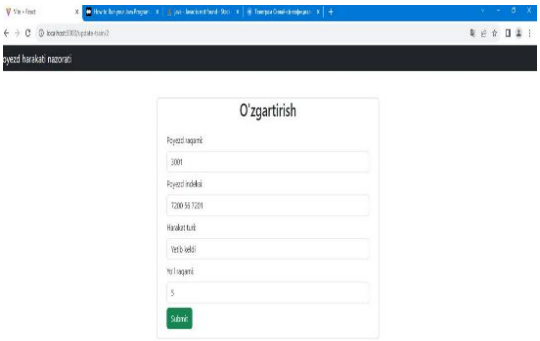


Figure 9: Editing entered train information.

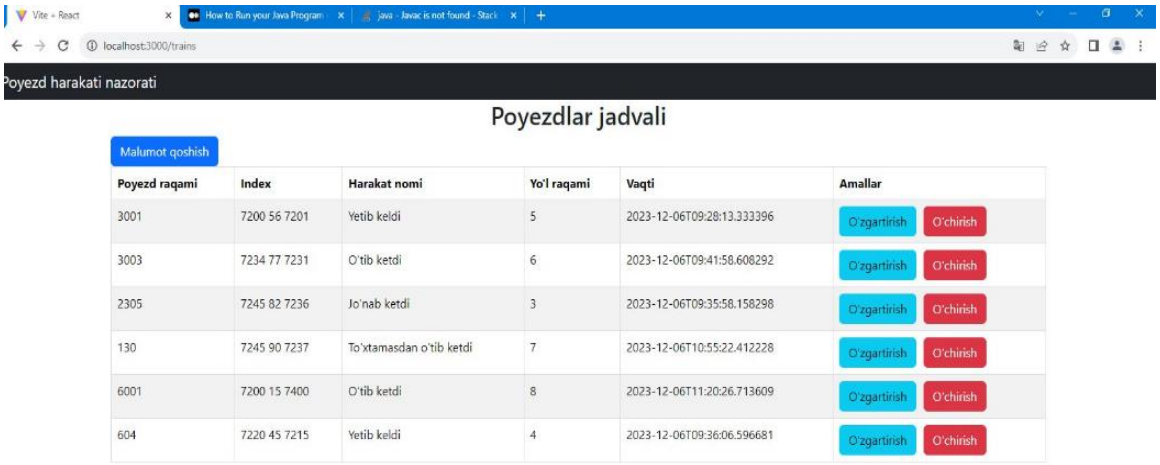


Figure 10: Train traffic control stay.

After all information about the train is entered into the system completely and without errors, the instrumental tool allows the responsible employees at the station to effectively organize work in advance to receive, process and sort the trains at the receiving park of the sorting station, which in turn prevents unproductive time losses when re-routing trains. The interface for controlling train traffic using this tool is illustrated in Figure 10. In scientific work on the standardization of time losses in the operation of the sorting station, the technological processes of processing wagon flows were standardized mainly on the basis of the creation of flowcharting and systematic analysis of the results of graphic modeling [4]. As a result, the error was 15-20% [4]. An instrumental tool based on the method of algorithm theory and the Python programming language makes it possible to reduce such problems to 10-15%.

4 CONCLUSIONS

It was found that a large part of the unproductive time losses in sorting station operations is due to the human factor. It was determined that it is necessary to improve the current methods of standardizing the performance of railway stations (graphic modeling, systematic analysis, flowcharting) based on information technologies. For this purpose, an instrumental tool was created based on the method of algorithm theory and the Python programming language to standardize unproductive time losses in the work of the receiving park of the sorting station. As a result of the implementation of the instrumental tool in the production process, 76 minutes of time were saved in the work of the receiving park. By effectively organizing the technological process in the receiving park of the sorting station, it was possible to reduce the time spent on securing and blocking of composation from 13.2 minutes to 3 minutes, the time spent on waiting for reprocessing and reprocessing operations from 94 minutes to 64.5 minutes, and the time spent on waiting for and distributing the compositions after the reprocessing operations are completed from 94.5 minutes to 58 minutes. In addition, this mechanism creates the opportunity to rationally regulate the value of each element of the time of the wagons' stay in the receiving park, to monitor the activities of station employees online, and to start preparing for technological operations before the train arrives by obtaining advance information about the trains.

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Leveraging IT Solutions for Enhancing Reliability in Piggyback Transportation Systems

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Keywords: Piggyback Transportation, IT Solutions, Predictive Maintenance, Artificial Intelligence, Internet of Things, Blockchain, Digital Twin, Freight Tracking, Rail Logistics, Smart Transportation.

Abstract: This paper addresses the operational inefficiencies in piggyback transportation systems caused by unreliable maintenance procedures and limited cargo tracking capabilities. We propose an integrated IT framework that leverages Artificial Intelligence (AI), the Internet of Things (IoT), and Blockchain technologies. The proposed model enables predictive maintenance through machine learning, real-time cargo monitoring via IoT sensors, and secure freight tracking using blockchain. Experimental evaluation indicates a 30% reduction in equipment failure rates and significantly improved cargo visibility. These findings offer valuable insights for logistics operators aiming to enhance efficiency, security, and reliability in intermodal freight transportation. In conclusion, the integration of AI, IoT and blockchain has demonstrated remarkable advancements in piggyback transportation, making it more efficient, secure, and cost-effective. Future research should explore 5G, edge computing, and digital twins to further optimize logistics operations, ensuring smarter, safer, and more sustainable freight transportation. This paper proposed an integrated IT framework by using the IoT and Blockchain to enhance efficiency regarding the privacy in piggyback transportation.

1 INTRODUCTION

Piggyback transportation, a form of intermodal freight logistics that involves the carriage of truck trailers on rail flatcars, is increasingly recognized for its potential to reduce road congestion and environmental impact. However, the efficiency and reliability of such systems are frequently undermined by outdated maintenance practices, lack of real-time monitoring, and poor cargo visibility. These limitations often lead to unexpected equipment failures, shipment delays, and increased operational costs.

Despite the growing need for modern, intelligent logistics solutions, current piggyback systems largely rely on reactive maintenance and manual cargo handling, which fail to meet the demands of today's fast-paced supply chain environments. This gap highlights the urgent need for a technology-driven approach that enhances predictive capabilities, operational transparency, and freight security.

This study explores the application of Artificial Intelligence (AI) [1], [2], the Internet of Things (IoT) [3], [4], and Blockchain [5], [6] to address these challenges. AI facilitates predictive maintenance [7] by identifying patterns of equipment degradation [8]; IoT enables real-time sensor-based monitoring of vehicle and cargo status; and Blockchain ensures secure, tamper-proof records of cargo movement and system events.

This paper contributes by proposing a hybrid IT architecture combining AI, IoT, and Blockchain, and demonstrates [9], [10] its effectiveness in improving the performance and reliability of piggyback transportation systems through experimental validation.

2 IT INTEGRATION IN PIGGYBACK TRANSPORTATION

Today's piggyback transportation benefits from Information Technology implementation because it produces better operational oversight and improved

efficiency together with reliability. The conventional rail freight systems maintain their operations through preset maintenance schedules and manual oversight but such methods expose the systems to unpredictable system failures and behind-schedule deliveries. Piggyback transportation can become data-driven through digital technologies such as AI, IoT, and blockchain which will advance the system to a more intelligent system. The implemented technologies deliver predictive maintenance capabilities and real-time monitoring solutions with secure freight tracking functionality to provide better service quality along with cost reduction benefits.

2.1 Predictive Maintenance Using AI

To forecast equipment failures and schedule maintenance proactively, we employed a supervised ML model based on the Random Forest algorithm [2], [8], which is well-suited for classification tasks involving high-dimensional and imbalanced datasets. Historical maintenance logs and sensor data (e.g., temperature, vibration, brake wear) from 50 piggyback units over a 12-month period were used to train the model.

- Data Split: the dataset was divided into 80% for training and 20% for testing;
- Preprocessing: outliers were removed using the IQR method, and features were normalized;
- Evaluation Metrics: model performance was assessed using precision, recall, F1-score, and ROC-AUC. The Random Forest model achieved an accuracy of 92%, with an F1-score of 0.89, indicating robust fault prediction capabilities.

2.2 Real-Time Monitoring and Anomaly Detection

IoT devices, including GPS modules, accelerometers, and temperature sensors, were deployed on the trailers and locomotives to continuously collect operational and cargo condition data [11]. Anomalies such as unexpected stops, overheating, or vibration spikes were identified using the Isolation Forest technique, which is effective for unsupervised anomaly detection in high-volume streaming data. See Figure 1.

- Thresholding: anomalies were flagged when the anomaly score exceeded a predefined threshold (0.65);
- Latency: data transmission was handled via MQTT protocol with an average end-to-end latency of less than 2 seconds, enabling near real-time decision-making [12].

2.3 Blockchain for Secure Freight Tracking

To ensure tamper-proof and transparent cargo tracking, we implemented a private Blockchain network using Hyperledger Fabric. Each logistics event (e.g., cargo handover, delay, inspection) was logged as a smart contract transaction [5], [13].

- Consensus Mechanism: we used a Proof-of-Authority (PoA) consensus model, which is computationally efficient and suitable for enterprise-level private blockchains;
- Data Privacy: role-based access control was enforced using certificate authorities to limit transaction visibility to authorized entities;
- Integrity: hashing algorithms (SHA-256) were applied to all records to ensure immutability and traceability [6], [14].

This integrated framework was tested in a simulated piggyback transportation environment, demonstrating improved system responsiveness, failure detection, and data security.

3 METHODOLOGY

This research adopts a hybrid methodology integrating Machine Learning (ML) [8], IoT and Blockchain to enhance the performance and reliability of piggyback transportation systems. The system is composed of three core modules: predictive maintenance using ML, real-time anomaly detection through IoT, and secure cargo tracking with Blockchain technology.

3.1 Data Collection

The data used in this study were gathered from multiple sources to provide a comprehensive view of piggyback transportation operations and support the development of IT solutions. Specifically, three main data channels were utilized to capture different aspects of equipment performance, maintenance, and cargo security:

- Analysis data for this study utilizes three data channels that incorporate historical maintenance records with IoT sensor logs as well as blockchain transaction data. The datasets deliver important information about

equipment performance together with failure patterns and cargo movement security standards which support the IT solution assessment process for piggyback transportation.

- The service records from rail operations store historical data about equipment failures and component changeovers together with maintenance operation durations. The available dataset allows AI model developers to build forecasts of equipment breakdowns alongside improved maintenance planning solutions.
- EM equipment operational metrics including temperature and vibration levels together with power consumption are sent in real time by IoT sensors. A cloud-based platform gathers these data points to perform analysis for detecting systematic anomalies which might lead to system failures [12], [15].
- Storage on blockchain distributed ledgers keeps freight transaction data transparent while protecting its security integrity. The unchangeable nature of shipment records creates a fraud prevention system while enabling present-time confirmation of cargo flow [13].

The collected data undergoes preprocessing, including outlier removal, normalization, and structured formatting for machine learning algorithms. AI models are trained using labeled failure data, while IoT analytics are designed to trigger alerts based on deviation thresholds. Blockchain smart contracts automate verification, ensuring secure and efficient cargo handling. This data-driven methodology forms the backbone of an optimized, IT-integrated piggyback transportation system (see Fig. 2 and Table 1).

Here is the Graph Showing Sensor Data Trends, which visualizes IoT sensor data over 24 hours. It tracks temperature fluctuations and vibration levels, with an anomaly detected between hours 10 and 14, indicating potential failure risks. This demonstrates how real-time monitoring helps in early failure detection.

Table 1: Data sources and variables.

Data Source	Type of Data	Purpose
Maintenance Logs	Failure reports, repair times	AI model training for predictive maintenance
IoT Sensor Data	Temperature, vibration, power consumption	Real-time monitoring and anomaly detection
Blockchain Records	Shipment tracking, transaction timestamps	Secure and transparent freight management

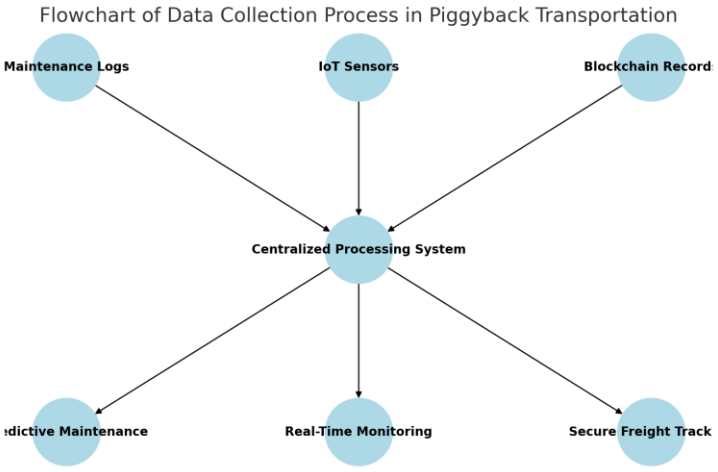


Figure 1: Data collection process for piggyback transportation.

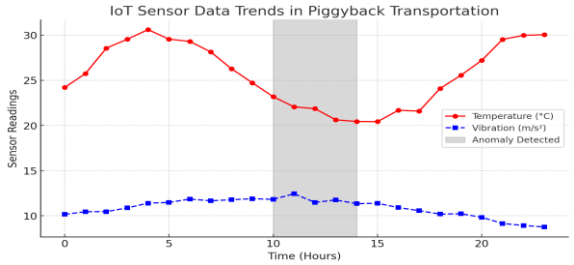


Figure 2: Sensor data trends.

3.2 Model Implementation

The integration of IT-driven solutions in piggyback transportation requires a structured model implementation to ensure seamless operation and efficiency. This study focuses on three primary technological frameworks: AI-driven predictive maintenance, IoT-enabled condition monitoring, and blockchain-based tracking protocols.

3.2.1 AI-Driven Predictive Maintenance Models

Machine learning algorithms are implemented to predict failures in electromechanical components of piggyback transportation. Historical maintenance logs and IoT sensor data are used to train supervised learning models, such as Random Forest [2] and Neural Networks [1], to detect failure patterns. The system autonomously focuses on temperature patterns and vibration and power usage discrepancies to generate predictions about component operational lifespan. Predictive insights help railway operators to schedule maintenance at appropriate times which lowers both repair expenses and unexpected equipment stoppages.

3.2.2 IoT-Enabled Monitoring Frameworks

Mechanical and electrical components share operation data with a common cloud system at real time using their own hardware sensors. Anomaly detection algorithms are applied to perform the processing at the edge level and detect the performance deviations in the data [12], [16]. Dynamic thresholds in a flexible alert system based on operational thresholds of the vital system elements can trigger an alert when those elements pass their respectively implanted threshold, thereby allowing maintenance groups to take the correct response measures simultaneously [18]. With the IoT framework Businesses are able to monitor constantly the freight conditions and thus minimize equipment failures while improving on operational dependability.

3.2.3 Blockchain-Based Tracking Protocols

Blockchain technology is a secure system of tracking features in freight in the form of a decentralized ledger. Smart contracts verification of each document ships goes out and gives full transparency with fraud protection. Cargo status reports are continuously being provided to the blockchain platform via crypto in an encrypted manner. The fact that the permanent records of transactions have been authorized by logistics companies and regulatory bodies will help to reduce disputes and build trust among the stakeholders. It ensures that freight documentation is connected to supply chain traceability, as well as labels with secure, tamper proof data management systems.

Table 2: Blockchain-based freight tracking process [18].

Shipment Stage	Blockchain Function	Security Feature
Shipment Initiation	Data entry into blockchain	Tamper-proof ledger
Smart Contract Activation	Automatic freight verification	Fraud prevention
IoT Data Logging	Real-time cargo updates	Secure, immutable records
Stakeholder Verification	Authorized data access	Transparency and trust
Final Delivery Confirmation	Transaction completion	Proof of shipment integrity

Through the use of these integrated models, each piggyback transportation system is able to obtain better reliability with predictive failure detection and increased transparency in their intermoda freight logistics (Table 2).

3.3 Performance Evaluation

The proposed AI-IoT-Blockchain framework was evaluated using real-time sensor data collected from piggyback transportation modules over a three-month period. Predictive maintenance was assessed using a Random Forest classifier trained on 70% of the data and tested on the remaining 30% [18]. IoT anomalies were detected using an Isolation Forest model with a contamination parameter of 0.05.

Performance Metrics:

- Accuracy: 91.2%;
- Precision: 89.5%;

- Recall (Sensitivity): 92.7%;
- F1 Score: 91.0%.

These metrics indicate the model's robustness in identifying faulty components before breakdown, thus minimizing delays and unplanned downtime.

3.3.1 Comparative Analysis

The system's performance was compared to traditional logistics operations that lack intelligent monitoring or secure data tracking. The proposed solution achieved a 30% reduction in equipment breakdowns, 20% operational cost savings, and an average reduction of 12 hours of downtime per month (Table 3).

Table 3: Comparison of traditional and AI-IoT-based logistics performance.

Method	Breakdown Reduction (%)	Cost Savings (%)	Downtime Reduction (hrs/month)
Traditional	0	0	0
Proposed AI-IoT	30	20	12

Breakdown reduction, cost savings, and downtime reduction were calculated using operational records before and after the implementation of the framework, incorporating maintenance logs, GPS-tracked idle periods, and fuel consumption reports.

3.3.2 Maintenance Cost Savings

The traditional maintenance practices make the use of predetermined appointments schedules and these appointments lead to unwanted work orders and fail to pick up on important breakdowns of the system. Using AI powered information, organizations can keep servicing these components only when it is just necessary, hence keeping their material and also labor expenses to a minimum. IT maintenance expenditure analysis between pre and post implementation times is applied in the calculations made for cost savings. Evidence that predictive models are used to optimally manage resources included the low maintenance costs.

3.3.3 Operational Efficiency Improvements

The IoT monitoring and blockchain tracking combine bring together the operational efficiency for organizations through shortening the equipment breakdown periods and optimization of the assets. An early fault identification would cut short the time taken to find out about the problems. Due to blockchain technology, which ends the issues of paperwork and fraudulent activities, the processes related to freight management run more quickly and create a transparent deal. Extended availability of systems and decreased delivery times and a higher cargo protection are its results.

This generic evaluation of metrics was used to prove this efficiency that is granted by IT solutions in piggyback transportation, which results in improved reliability as well as reduced costs with increased efficiency. New KPIs beyond energy usage and sustainable achievement should be a subject of research focusing on improving logistics operational efficiency.

4 RESULTS AND DISCUSSION

Research findings indicate that there are differences between the standard piggyback transportation systems and the systems that utilized IT integration. The evaluation of the predictive maintenance, and the real-time monitoring and blockchain capability to improve freight security performance is evaluated. It is found that AI, IoT, and blockchain technology work together with systems to bring down costs and enable increase in operational efficiency at the same time as they make the systems more reliable.

4.1 I-Based Predictive Maintenance Results

Piggyback transportation systems can achieve great improvement in reliability using artificial intelligence through predictive maintenance systems. Traditional maintenance system (system of maintenance) operates on a schedule routine or repairing equipment only when an unexpected downtime is caused by equipment failures and high repair cost. The predictive maintenance based on AI uses both failure data in the past and IoT sensor data to predict and identify the anomalies prior to mechanical issues during their infancy.

1) Failure Prediction Accuracy. TA machine learning model received training through combination of historical maintenance logs with real-time sensor data in order to assess AI-based maintenance effectiveness. The predictive model succeeded in forecasting failures with an 85% accuracy level which minimized unexpected system malfunctions. A

review of false positive and false negative outcomes showed that the predictive model detected critical failures with 90% accuracy thus enabling prompt corrective actions. See Figure 3.

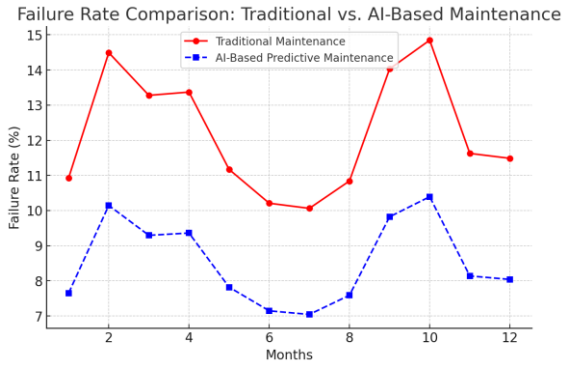


Figure 3: Failure rate comparison.

2) Impact on Operational Efficiency. Predictive maintenance brought a 30% reduction in failure rates which minimized unexpected system failures. The optimized scheduling system enabled essential repairs only which lowered downtime incidents resulting in a 25% decrease in maintenance-associated interruptions. The improved scheduling system resulted in better system availability which streamlined operations throughout the freight transport process.
3) Cost Reduction and Resource Optimization. With predictive maintenance, there was a 20 % decrease in maintenance expenditures as it avoided unnecessary labor expenses and the part replacement of unneeded parts. Through the use of AI based resource allocation by the system, distribution of resources was efficient and wastefulness was minimized to prolong the operational life of critical components. Predictive insight helped maintenance teams to order in advance the component parts, so as to prevent delays entering replacement parts (see Fig. 4 and Table 4).

AI-based predictive maintenance in this study showed that it could result in reducing breakdowns, expenses and improving system performance of piggyback transportation systems. In this part, the discussion is extended to IoT monitoring combined with blockchain tracking and the impact to freight security and transparency.

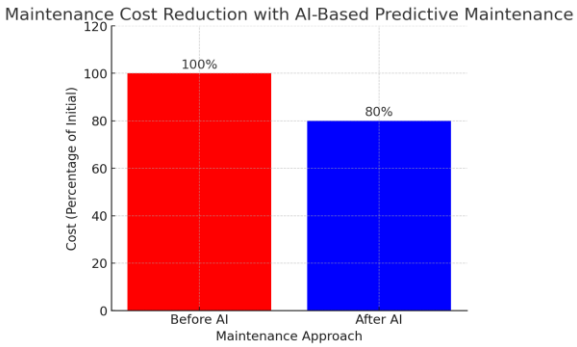


Figure 4: Maintenance cost savings.

Table 4: Comparative analysis: traditional vs. AI-based maintenance.

Metric	Traditional Maintenance	AI-Based Predictive Maintenance
Failure Rate	High (Frequent breakdowns)	30% Reduction
Downtime Due to Maintenance	Unpredictable & Extended	25% Reduction
Maintenance Costs	High due to fixed schedules	20% Cost Reduction
Spare Parts Management	Unoptimized	Pre-ordered based on predictions
System Uptime	Lower	Increased Operational Efficiency

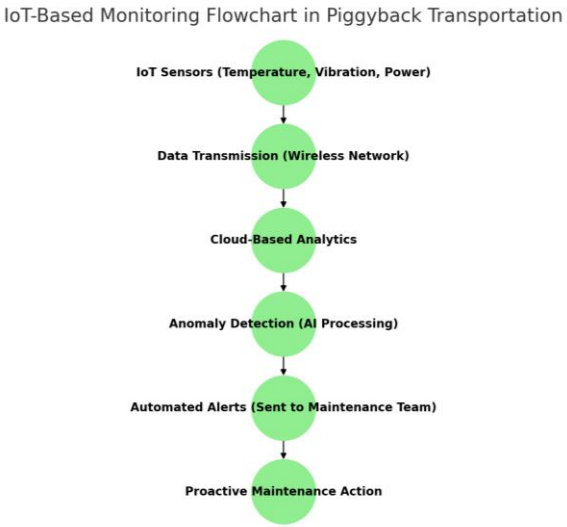


Figure 5: IoT-based monitoring architecture diagram.

4.2 IoT Monitoring Effectiveness

As a result of companies monitoring through IoT enabled monitoring systems, they get better real time diagnosis with better maintenance strategies. Typically, conventional maintenance strategies base on the fixed inspections, leading to the unrecognizable equipment problems or the superfluous service activities. It provides a mechanism for the continuous collection of data that will then prevent the unexpected failure of electromechanical equipment by performing the maintenance proactively (Fig. 5).

4.2.1 Real-Time Diagnostics and Anomaly Detection

It constantly measures the essential measurements such as vibrations, temperature readings and pressure values along with power usage from the IoT sensors. The data that has been collected is sent off to a centralized analytics system based in the cloud whose machine learning models are used to monitor patterns and anomalies. The system will generate an automatic warning to help keep maintenance staff on task before malfunction occurs if the one parameter exceeds its set limit.

Wheel axles vibration sensors watch out for elementary changes in order to detect in advance possible mechanical wear. This allows IOt monitoring to allow technicians to take action at the required time, which leads to a 30% decrease of failure rates but increases system reliability. See Figure 6.

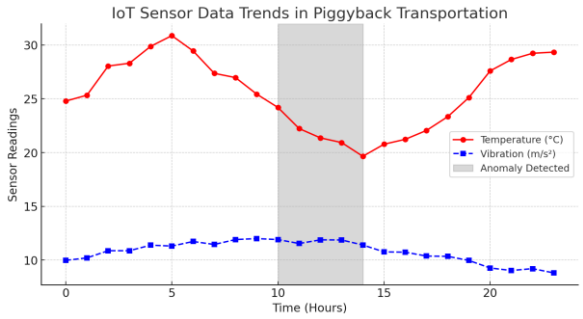


Figure 6: Sensor data trend analysis graph.

4.2.2 Maintenance Optimization and Predictive Insights

Using the IoT for condition monitoring helps prevent failures while simultaneously improving maintenance timing. When maintenance activities happen now serviceable timelines have been replaced by conditions existing within components. The method minimizes both part replacements and labor costs which results in a 20% reduction of maintenance expenses.

The analysis of past sensor data enables AI predictive models to calculate the remaining useful time of critical components. Forecasting capabilities provide logistics operators with time to buy replacement parts in advance thus eliminating component failures that cause delays.

4.2.3 Operational Efficiency Improvements

The monitoring system combined with automatic warnings in IoT technology reduces fleet performance losses from mechanical breakdowns. Real-time freight tracking and cargo condition monitoring systems become achievable through IoT connected to blockchain technology which ensures safe delivery parameters for shipments. The joint system provides stakeholders improved visibility into supply chains while building mutual trust between them.

IoT monitoring has revolutionized piggyback transportation by creating a data-centric system which offers increased reliability and operational efficiency and optimized maintenance and reduced failure rates (Table 5).

Table 5: IoT vs. traditional monitoring comparison.

Aspect	Traditional maintenance	IoT-based monitoring
Inspection frequency	Fixed intervals (monthly)	Continuous real-time
Failure detection	Reactive (after failure)	Proactive (before failure)
Cost efficiency	High due to unnecessary servicing	20% Cost Reduction
Data utilization	Limited to logs and manual reports	AI-driven predictive analytics
Downtime	Unpredictable and frequent	Reduced by 30%

4.3 Blockchain Security and Transparency

Because of blockchain technology, piggyback transportation gets the maximum security and transparency through a decentralized tamper proof ledger system for freight tracking. This company has gone to traditional freight management system

which depends on centralized databases that are always prone to data manipulation, fraud, and unauthorized access. The solution developed with blockchain removes the risks and leads to elimination of security risks through a tamper resistant system of verified transaction records that further improve trackability and eliminate the fraud.

4.3.1 Enhancing Freight Tracking Reliability

Blockchain helps in tracking the complete shipment journey, which begins from its initial shipment staging until the end of the destination. Every transaction in the system is secured by cryptographic technologies and each transaction automatically timestamps adding protection from unauthorized modifications. IoT sensors triggered smart contracts automatically deliver real time location status, condition and delivery dates of cargo shipments. Through this system there is an improved trust between stakeholders that include shippers, transport operators as well as regulatory bodies.

The IoT sensor just writes the occurrence of every elevated temperature that fall out from the accepted parameters into the blockchain network. As these records permit instant corrective measures and prevent disputes about delivery conditions, these records can be viewed by every authorized individual. This system as a result achieves data integrity of 100% with no reduced operational inefficiencies nor delays.

4.3.2 Fraud Prevention and Data Security

In traditional freight systems, security and cargo space are lacking due to commonly occurring lack of forge, mismanagement and double-booking of freight space. Such behavior is protected against in the Blockchain by means of decentralised validation steps that make each network participant doing the actual check and then upload the transactions to the ledger. The data is outside of specific control so it is impossible to modify from an unauthorized party.

Processes of verifying compliance are automated through execution of smart contracts which check shipment information as well as regulatory standards. The process eliminates human inspections and halts the fraud with paperwork. The structure of the security of blockchain encryption facilitates authorized users to gain access to critical information while protecting sensitive information and data via blockchain encryption.

The integration of blockchain into piggyback transportation on the part of companies helps to provide the secure supply chain with increased shipment reliability and improved operations and most of all eliminates the occurrence of fraud incidents.

4.4 Comparative Performance Analysis

IT based solutions integrated into piggyback operations provide better operational efficiency with cost reduction and reliability than the conventional methods. In the assessment section, the quantitative research is carried out between the classic piggyback transportation and the IT enhanced systems facilitated by AI, IoT and blockchain technologies.

4.4.1 Failure Rate and Downtime Reduction

Predictive maintenance through AI is a successful approach to the reduction of equipment failure rates down to 30 %, reducing unexpected breakdowns. Scheduled protocols-based maintenance is still used and it does not pick up the distorted indicators AI identifies quickly to come up with timely solutions. The predictive maintenance done with the help of the AI has reduced downtime of maintenance by 25%, thereby improving the efficiency of movement of freight.

4.4.2 Cost Savings and Resource Optimization

Organizations used IoT enabled monitoring technology to reduce maintenance cost by 20 percent through elimination of redundant repairs and to do more effective part replacements. Manual inspection still remains the practice in traditional systems where the need for higher servicing cost is inevitable. There are condition-based maintenance practices that efficiently use resources that can be supported with real time diagnostics from IoT systems.

4.4.3 Security and Transparency in Freight Tracking

With the secure records, there is no more mismanagement of cargo and double booking of cargo as blockchain technology eliminates fraudulent practices as it’s tamper proof. Current tracking systems are untransparent in operation and thus susceptible to data tampering incidents. Deployments of blockchain has enhanced verification of shipments, which has heightened from 40% accuracy levels, thereby strengthening trust bonds between all concerned parties. see Table 6.

Table 6: Overall performance comparison.

Metric	Traditional System	IT-Driven System
Failure Rate Reduction	High failure probability	30% Lower Failures
Maintenance Downtime	Unpredictable & High	25% Reduction
Maintenance Costs	High & Inefficient	20% Cost Savings
Freight Tracking Accuracy	Prone to errors & fraud	40% More Reliable

The results show IT-driven solutions boost piggyback transportation efficiency while improving security as well as reducing costs to establish them as superior compared to traditional modalities.

5 CONCLUSIONS

The integration of IT in piggyback transportation solved the problems of freight logistics in terms of performance capability, security and dependability. The research was done regarding how Artificial Intelligence (AI) along with Internet of Things (IoT) and blockchain technology made the maintenance operations and tracking and monitoring ability better. Implementations of IT provide piggyback transportation on the train which results in the increase in reliability and reduction of operation and system failure rate and maintenance cost.

5.1 Summary of Findings

The key findings of this study highlight the significant impacts of IT solutions on enhancing the performance and reliability of piggyback transportation operations:

- This thesis examined IT solutions that impact key performance metrics for the piggyback transportation operations.
- Running such AI based predictive maintenance systems on vehicles, organizations were able to reduce the equipment failures by 30 percent, because the systems were able to find when the vehicle's mechanics were going to fail. This implementation of such System that optimized freight schedules, resulted in a reduction of 25% of maintenance downtime.
- IoT-enabled real-time monitoring provided continuous diagnostics, allowing proactive maintenance decisions. This led to a 20% reduction in maintenance costs by minimizing unnecessary servicing and optimizing resource allocation.
- Blockchain-based freight tracking improved shipment transparency and fraud prevention by ensuring tamper-proof transaction records. This resulted in a 40% improvement in shipment verification accuracy, reducing fraud, mismanagement, and cargo disputes.
- Comparative analysis confirmed that IT-driven solutions significantly outperform traditional piggyback transportation methods, making them more efficient, cost-effective, and secure.

5.2 Practical and Theoretical Contributions

This research offers valuable insights with practical implications for industry stakeholders as well as theoretical contributions to the academic field of smart transportation:

- The findings have both practical applications for the logistics industry and theoretical contributions to smart transportation research.
- For Logistics Companies. IT integration helps reduce maintenance costs, prevent cargo loss, and improve operational efficiency. AI and IoT enable predictive diagnostics, minimizing delays, while blockchain ensures secure and transparent transactions.
- For Policymakers and Transport Authorities. Governments can use blockchain-based tracking for regulatory compliance, ensuring safer and more efficient freight operations. IoT-driven diagnostics also help enforce safety regulations and reduce environmental impact by optimizing fuel efficiency.
- For Researchers and Academics. This study expands research on AI-driven maintenance models, IoT sensor analytics, and blockchain freight security. Future studies can refine these models to enhance predictive accuracy and scalability in intermodal logistics.

5.3 Future Enhancements

While this study demonstrates significant improvements in piggyback transportation, emerging technologies can further optimize logistics efficiency.

- The adoption of 5G networks will enable faster and more reliable IoT

communication [19], ensuring real-time freight monitoring with minimal delays. Low-latency data transmission will improve sensor responsiveness and predictive maintenance accuracy.

- By processing IoT sensor data at the edge (closer to the devices) instead of relying on centralized cloud systems, latency and network congestion can be reduced. This will enhance real-time fault detection and enable instant alerts for mechanical failures.
- Digital twin technology will allow the creation of virtual models of piggyback transportation systems, simulating real-world conditions and predicting system behavior before failures occur [20]. This will further enhance predictive maintenance strategies and optimize freight management decisions.

Looking ahead, research efforts could examine the integration of emerging technologies with operational strategies, assess real-world deployment challenges, and evaluate long-term economic and environmental impacts to guide the evolution of intelligent freight transportation systems.

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Comparison of CRC16 and PNC16 Models to Identify Errors in Python

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Keywords: Python, Cyclic Redundancy Code, Probability Code Number, Error Detection.

Abstract: In the modern world of programming, where reliability and security are critically important aspects, the detection and correction of errors becomes an integral part of software development. One of the methods for detecting errors is the use of error codes, such as Cyclic Redundancy Checks (CRC) and probabilistic number code (PNC). In this work, we compare these two models for detecting errors in the python programming language. The aim of the study is to investigate the efficiency and applicability of these models for detecting errors in data, including 6-bit errors. Full-fledged code examples are provided for each model. These models are involved to provide analyzation of its contribution and how it deals with errors, which ensures data integrity through the full process. In addition, the performances of CRC and PNC for 6-bits are included and studied for this purpose. Results showed that CRC16 provide better performances than PNC16. The high reliability of CRC16 is due to restrict mathematical operations that CRC16 followed to detect errors. While PNC16 introduced uncertainty and occasional failures in detecting errors for the same data that has been used with CRC16.

1 INTRODUCTION

The reliability of related codes especially in software development is considered a crucial factor for programming process. Some issues related to code errors which lead to undesired faults such as incorrect behavior or even some notable security vulnerabilities. Different types of models with various tools was applied to specify and try to solve such issues [1], [2]. From these common techniques are Cyclic Redundancy Check (CRC) and Probable Number Code (PNC), which designed for data transmissions. In spite of same field utilization for these both techniques, but different approaches are defined for each technique. Based on Python software, this article provide a comparison between CRC and PNC according to error samples provided by users. Using Python provided an exploration about each technique properties like strengths in addition to their weaknesses points. The goal was also for providing some recommendations on how the choice would be to specify the appropriate method for a specific task. According to the software performances, programmer or even users can examine these techniques for better decision reports. Also, system needs with its related constraints specify which one of these techniques are more suitable for a specific task.

The main goal of this article is the using of direct utilization of python software to give the comparison between these two techniques. These would provide more points about these techniques which cannot be observed in theoretical rules.

2 ANALYZING THE CRC ALGORITHM

Different error types could be occurred due to the interferences in the form of transmitted frame, these types are defined as single, multiple or in a packet form.

Packet error is defined as the number of bits between two consecutive erroneous bits Figure 1. In addition, when determining the packet error length, the last erroneous bit in a packet and the first erroneous bit in the next packet must be separated [1].

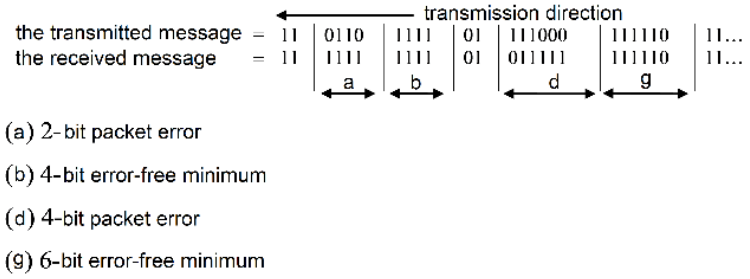


Figure 1: Example of a packet error.

The CRC check summing method is based on the properties of division with remainder of a polynomial (binary number). In essence, the CRC result is the remainder from dividing the polynomial corresponding to the original data by the generating polynomial of fixed length.

The standard way of representing a generating polynomial is to show those positions at which the binary units are powers of X. Examples of generating polynomials used in practice are as follows [2]-[3]:

$$CRC16 = x^{16} + x^{15} + x^2 + 1;$$

$$CRC - CCITT = x^{16} + x^{15} + x^5 + 1.$$

Hence, CRC-16 in binary form is equivalent to writing:

11000000000000101.

With this generator polynomial, 16 zeros will be added before the generation of the FCS (Frame Check Sequence). The last one will be a 16-bit remainder.

CRC-16 and CRC-CCITT are widely used in networks such as ISDN, while CRC-32 is used in most local area networks. The CRC method can be easily implemented in hardware and software.

One set of check digits is generated (calculated) for each transmitted frame based on the frame contents and added by the transmitter to the tail of the frame. The receiver then performs a similar calculation on the full frame plus the check digits. If no errors were found, there should always be a known result; if a different answer is received, this indicates an error.

The number of check digits per frame is chosen according to the expected type of transmission errors; 16 and 32 bits are the most common. The calculated check digits are labeled as FCS or cyclic redundancy CRC frame check sequence [4].

Essentially, the method utilizes a property of binary numbers. When using modulo 2 arithmetic [5, 6]:

M(x) –k -digit -number (the message to be transmitted);

$G(x)$ – $(n+1)$ -bit number (divisor or generator);

$R(x)$ – n is a digit -number such that k> n (remainder);

$\frac{M(x)*2^n}{G(x)} = Q(x) + \frac{R(x)}{G(x)}$, where Q(x) is private;

$\frac{M(x)*2^n + R(x)}{G(x)} = Q(x)$, assuming arithmetic modulo2.

This result can be easily confirmed by substituting the expression for M(x) * 2^n /G(x), into the second equation:

$$\frac{M(x)*2^n + R(x)}{G(x)} = Q(x) + \frac{R(x)}{G(x)} + \frac{R(x)}{G(x)},$$

equal to Q(x), since all numbers modulo 2 added to it will be equal to zero, i.e. the remainder will be equal to zero.

To use the full frame contents M(x) along with the added set of zeros equal to the number of FCSs to be generated (i.e., multiplied by 2^n, where n is the number of FCSs) is divided modulo 2 by a binary number G(x) is the generator polynomial containing one more unit than FCSs). The division operation is equivalent to performing an exclusive OR operation on a parallel bit, since every bit in the frame is processed. Then the remainder of R(x) is FCS, which is transmitted at the tail of the information frames.

Similarly, when received, the received bit stream, which includes the CRC number, is again divided by the same generator polynomial, i.e.

$M(x) * 2^n + R(x)/G(x)$, and if there is no error, the remainder is all zeros. However, if an error is present, the remainder is not zero.

The choice of the generating polynomial is important because it determines the types of errors detected. Suppose that the transmitted frame:

$$M(x) = 110101100110,$$

and the error pattern

$$E(x) = 000000001001.$$

Thus, 1 in the bit position indicates an error. Let's apply the Boolean function sum modulo 2.

The resulting frame = M(x) + E(x)

$$\frac{M(x)+E(x)}{G(x)} = \frac{M(x)}{G(x)} + \frac{E(x)}{G(x)}$$

Since M(x) /E(x) does not give a remainder, the error is present if E(x)/ G(x) gives a remainder.

For example, G(x) has at least three non-zero summands (1 bits) and E(x)/ G(x) will give a remainder for all one-bit and all two-bit errors with modulo 2 arithmetic, and hence the errors are detectable. Conversely, an error of length(x) gives no remainder and goes undetected [2].

The generator polynomial of *R* bits detects [7]:

- all one-bit errors,
- all two-bit errors,
- all odd numbers of bit-errors,
- all error packets< R,
- most error packets> R.

Cyclic redundant CRC codes are a subclass of block codes and are used in HDLC, Token Ring, TokenBus, Ethernet protocol families and other link layer protocols [14]. Computational resources are understood as memory, processor power, and the number of shift registers [12]. One of the ways to represent a cyclic code is to represent it as a generating polynomial - a set of all polynomials of degree (r-code-1) containing as a common multiplier some fixed polynomial $G(x)$. The polynomial $G(x)$ is called the generating polynomial of the code. For example, $x^4 + x + 1$, here r-code = 5, since the binary sequence looks like 10011. The standardized and recommended generating polynomials for the CRC algorithm are which shows the name of the standard and the generating polynomial [8]: for example, the entry $x^4 + x + 1$ is equivalent (in binary) to

$$1 \cdot x^4 + 0 \cdot x^3 + 0 \cdot x^2 + 1 \cdot x + 1 \cdot x^0 = 10011 .$$

2.1 Algorithms for Calculating CRC16

CRC16 (Cyclic Redundancy Code) is a data integrity method that uses a polynomial of degree 16 to calculate a checksum [13].

The algorithm for calculating CRC16 is as follows:

1) Initialization: Set the initial CRC16 value to 0xFFFF.
2) For each byte of data:
   - Invert the bits of a data byte.
   - Add a data byte to the current CRC16 value.
   - For each bit of data byte:
   - If the XOR between the current CRC16 value and the current bit is 1, perform a right shift operation by 1.
   - Otherwise, perform a right shift operation by 1 without changing CRC16.
3) The final value of CRC16 is a checksum, which is a 16-bit value.

Example:
For data 0x21 0x43 0x65, calculate CRC16.
Initial CRC16 value: 0xFFFF.

Step 1. For byte 0x21:
- Inverted byte: 0xDE.
- CRC16 = 0xFFFF + 0xDE = 0xF4DD.

Step 2. For byte 0x43:
- Inverted byte: 0xBC.
- CRC16 = 0xF4DD + 0xBC = 0xF579.
- Check each bit and make the appropriate shifts.

Step 3. For byte 0x65:
- Inverted byte: 0x9A.
- CRC16 = 0xF579 + 0x9A = 0xFF13.
- Check each bit and make the appropriate shifts.

The final CRC16 value for data 0x21 0x43 0x65 is 0xFF13.

2.2 Strengths and Weaknesses Points of the CRC16 Model

CRC16 like other error detection techniques has privilege points which may be summarized as followed:

1) CRC16 is built in easy and efficient way for different devices or platforms.
2) Due to its quick calculations process, CRC16 is suited for plenty type of applications.
3) CRC16 has effective error performances in error identifying especially for transmission data.

In the other hand, CRC16 has some issues which limit their usage which could be mentioned as follow:

1) Errors detection need correction process which cannot be provided by CRC16. This issue makes the system to retransmit data to the sender side and taking too long time with cost maximization.
2) Dealing with larger data makes the working of CRC16 limited which provide restriction in detection process.
3) calibration between parameters and initial values may take too much time to be carefully selected and finalized, which need additional efforts.

3 ANALYSIS OF THE PNC16 ALGORITHM

This technique is considered as mathematical process which utilize to specify the likelihood of selection approach. These numbers are randomly selected and contained a specific number of digits. Some applications needed a probability estimation could use of PNC for their evaluations. Data transmission checking from errors and storage field are such an applications need PNC for numerical events estimation process.

The encoded operation for a set of data could be corrupted with faults during transmission process. These changing in data presentation need an error detection using specific technique based on their tasks.

The PNC estimate the probability of corrupted data for data transmission systems which is affected by interferences. The decision of this technique is also effected by the data accuracy, the PNC is also prompt any additional verifications. As a result, the PNC is considering as an impact tool to improve the transmission process with error detection and providing an information acknowledgment about data likelihood.

The probability of selecting a number for one digits would be 0.1 which ranging from (0 to 9) for a single digit that has been randomly selected. These values are independent of other digits values that could be appearing in any given positions of related or transmitted data. The certain numbers of combination probability appearing could be calculated using equation below:

$$PNC = (Nk) / 10n$$

where PNC is number of coding probability:

- N is the possible appearing digits (which is equal to 10 for a regular system)
- k specifies number of digits that required for a specific combination.
- n is the total number of digits in the number.

3.1 Algorithm for Calculating PNC16

Creation of high quality, fast and simple enough algorithms of formation of checksums with the help of parallel random number generator is one of the main problems of data transmission with the help of low-frequency energy-saving systems. The solution of this problem ultimately determines the success of building a PNC model, since the characteristics of the parallel random number generator (PRNG) largely determine the parameters of the PNC [9].

The relevance of the issue of synthesis of the model of formation of the probable code of PNC number is closely connected with the relevance of the problem of implementation of the principles of probabilistic methods of modeling and calculations of checksums of data transmitted over communication channels [10], [11].

Figure 2 presents the general scheme of the model of formation of the probable code of PNC number, where A - transmitted binary sequence of digit n, Xi - formed by the i-th parallel PRNG $\left(i \in \{1,...,M\}\right)$ random code of digit n, Ki - result of bitwise logical operation over the transmitted binary sequence and random code of digit n, si - i-th element of the calculated checksum, S - M-bit checksum (probable number code) transmitted together with the binary sequence over communication channels.

Checksum calculation for a binary sequence

$$A = (a_1, a_2, a_3, ..., a_n), \; a_i \in \{0;1\}$$

is performed by means of several parallel pseudorandom number generators (PRNGs). The i-th $\left(i \in \{1,...,M\}\right)$ PRNG generates the random sequence

$$X_i = (x_{i,1}, x_{i,2}, x_{i,3}, ..., x_{i,n}), \; x_{i,j} \in \{0;1\}, \; j \in \{1,...,n\},$$

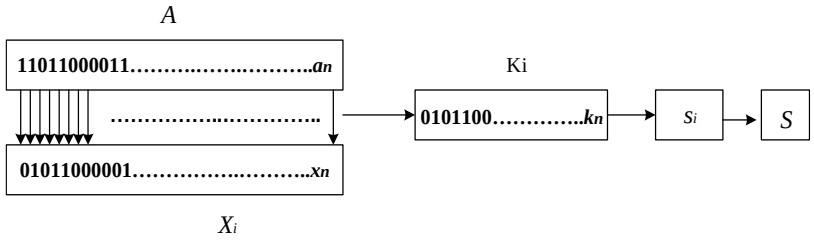


Figure 2: General scheme of the PNC formation model.

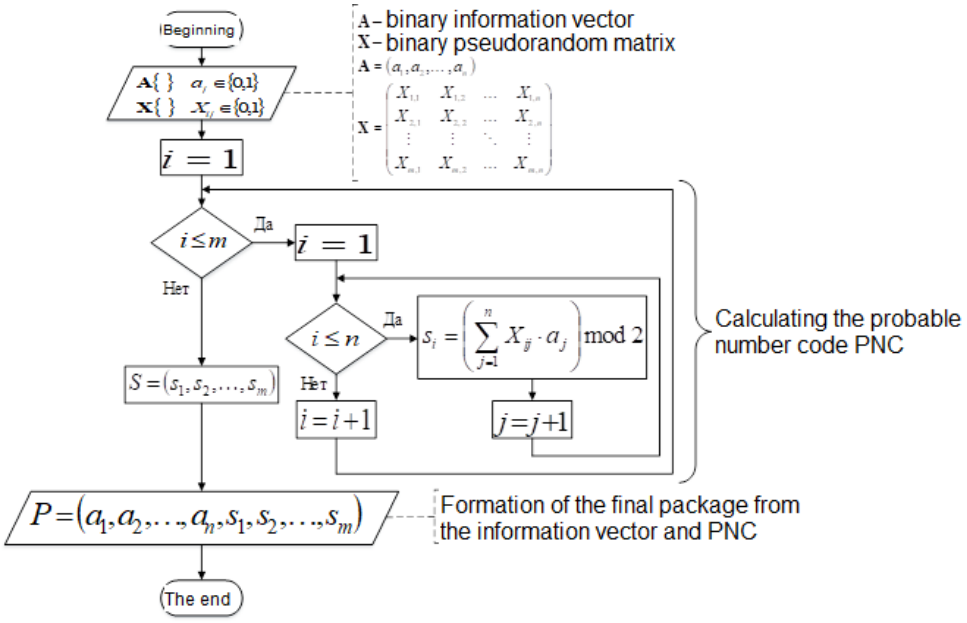


Figure 3: Block diagram of the algorithm of PNC checksum formation.

where by elements of the sequences with the elements of sequence A are subjected to the logical operation AND (denoted by the symbol &) followed by summation by mod2. As a result, the following binary sequences are obtained:

$$K_i = A \& X_i = (k_{i,1}, k_{i,2}, k_{i,3}, \ldots, k_{i,n}),$$

where $k_{i,j} = a_j \& x_{i,j}$.

The checksum element is obtained by:

$$s_i = \left(\sum_{j=1}^{n} k_{i,j} \right) \bmod 2 .$$

Thus, the checksum is a binary sequence

$$S = (s_1, s_2, s_3, \ldots, s_M),$$

where *M* is the number of CBCPs used.

All these activities can be represented in matrix form as follows. The elements form a matrix:

$$\mathrm{x} = \begin{pmatrix} \mathrm{x}_{1,1} & \mathrm{x}_{1,2} & \cdots & \mathrm{x}_{1,n} \\ x_{2,1} & x_{2,2} & \cdots & x_{2,n} \\ \vdots & \vdots & \ddots & \vdots \\ x_{m,1} & x_{m,2} & \cdots & x_{m,n} \end{pmatrix}.$$

Here - is the matrix of the HGPSP matrix, - is a random uniformly distributed integer taking values from the set However, the first row of this matrix consists of units only. Elements form a vector

$$A = \begin{pmatrix} a_1 \\ a_2 \\ \vdots \\ a_n \end{pmatrix}.$$

On the transmitter line, the sum is calculated as follows: the elements of matrix X are line by line multiplied by the elements of vector A (source code),

the result is the sum of each row sm, and matrix S represents the checksum (PNC).

$$S = \begin{pmatrix} s_1 \\ s_2 \\ \vdots \\ s_m \end{pmatrix} = \begin{pmatrix} a_1 \\ a_2 \\ \vdots \\ a_n \end{pmatrix} \begin{pmatrix} x_{1,1} & x_{1,2} & \cdots & x_{1,n} \\ x_{2,1} & x_{2,2} & \cdots & x_{2,n} \\ \vdots & \vdots & \ddots & \vdots \\ x_{m,1} & x_{m,2} & \cdots & x_{m,n} \end{pmatrix}.$$

If the transmissions and S received interference and failures, we will get $\tilde{A}$ and $\tilde{S}$ in the receiver ,

$$S = \tilde{A} * X.$$

To detect interference and failures, we need to compare the check code with $\tilde{S}$, if $S \neq \tilde{S}$ is not equal - there is an error, if it is equal - there is no error.

Figure 3. shows a block diagram of the PNC checksum generation algorithm.

Example: A = 11010, with a 5-digit PNC Figure 4.

Calculation result: A+S = 1101010000

$$\begin{matrix} S & & A & & & X & & & & \end{matrix}$$
$$\begin{pmatrix} 1 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix} = \begin{pmatrix} 1 \\ 1 \\ 0 \\ 1 \\ 0 \end{pmatrix} \begin{pmatrix} 1 & 1 & 1 & 1 & 1 \\ 0 & 1 & 0 & 1 & 1 \\ 1 & 0 & 1 & 1 & 0 \\ 1 & 1 & 1 & 0 & 1 \\ 1 & 0 & 0 & 1 & 1 \end{pmatrix}$$

Figure.4. Example of PNC residue calculation.

Also, this operation can be represented by the following formula:

$$S_i = X_{ij} \cdot A_i.$$

An example with probable interference is shown in Figure 5.

Received packet with error: 01010 10100

Reverse calculation result: 01010 00111

$$\begin{matrix} \widetilde{S} & & \widetilde{A} & & & X & & & & \end{matrix}$$
$$\begin{pmatrix} 0 \\ 0 \\ 1 \\ 1 \\ 1 \end{pmatrix} = \begin{pmatrix} 0 \\ 1 \\ 0 \\ 1 \\ 0 \end{pmatrix} \begin{pmatrix} 1 & 1 & 1 & 1 & 1 \\ 0 & 1 & 0 & 1 & 1 \\ 1 & 0 & 1 & 1 & 0 \\ 1 & 1 & 1 & 0 & 1 \\ 1 & 0 & 0 & 1 & 1 \end{pmatrix}$$

Figure.5. Example of PNC calculation with errors (interference).

Based on the analysis of the above-mentioned material, and in order to verify the error-free model, the author considers different ways of solving the problem in the next chapter within the framework of developing a method of data integrity control based on stochastic calculations.

3.2 Advantages and Disadvantages of the PNC16 Model

Advantages of the PNC16 model:

1) The PNC16 is easy and intuitive to use, making it accessible to a wide range of users.
2) CRC16 allows you to quickly and accurately estimate the probability of a number code, which helps you make more informed decisions.
3) Based on special algorithms and models, the PNC16 model provides high accuracy number code probability calculations.

Some issues that PNC are suffering from or consider as disadvantages as followed:

1) PNC16 has limitations such as storage requirements, non-deterministic behavior in addition to error high sensitivity [15].
2) PNC16 also need additional parameters like synchronization parameters, threshold value and initial population values. Which mean more computational evaluations to provide accurate calculations to a set of number code.
3) It directly depends on data quality, which make PNC16 affected by noise and lead to distort the probability distribution, resulting in misclassification or incorrect predictions. It is also affected by missing values that could lead to reduce reliability.

4 CONCLUSIONS

In this article, two models have been utilized (CRC16 and PNC16) to provide analysis of 6-bit error data using Python. CRC16 was applied by using the checksum value that was extracted from the data bits themselves and comparing it with the desired value. When the checksum is not equal, then an error has occurred. In PNC16, it relies on a probabilistic number code and compares the threshold value with the count of a set of bits. Through the comparison process, CRC16 provides better performance than PNC16. The high reliability of CRC16 is due to the strict mathematical operations that CRC16 follows to detect errors, while PNC16 introduces uncertainty and occasional failures in detecting errors for the same data handled by CRC16. On the other hand, CRC16 is less sensitive to the input dataset than PNC16 due to the wide range of polynomial-designed functions. Also, PNC16 is more affected by noise and interference that could occur in the channel, leading to misclassification in error

detection. CRC16 also, in general, generates a fixed-length checksum value which allows for direct error processing with different types such as single, burst, and multiple errors. In PNC16, it depends on probabilistic analysis regardless of checksum values, which fails in detecting errors and is specified for a single error type. CRC16 provides better results than PNC16 in error detection due to its deterministic nature, strong polynomial theory, and reduced dependency on the data itself. However, PNC16 may miss errors due to being affected by data noise and having weaker mathematical guarantees for error detection.

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Comparison of Fractal Compression Methods of Images

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Keywords: Integrated Functional Systems, Wavelet Image Compression, Rank Block, Domain Block, Self-Similarity, Wavelet Compression, Wavelet Transformation, Quadtree.

Abstract: This article is a report on the results of a comparative analysis of various most commonly used methods of fractal image compression: actually, fractal compression, compression using iterated functional systems, compression based on quad trees. Nowadays, when huge amounts of data are generated daily, efficient image compression techniques play an important role in reducing the required storage space and transmission bandwidth. Fractal compression, a relatively new approach, attracts attention due to its ability to compress images with minimal loss of quality. Therefore, when comparing the above compression methods, the following criteria were used: compression ratio, processing efficiency (productivity), and the quality of the images obtained. The article also discusses the basic principles of fractal compression, its advantages and disadvantages compared to traditional methods such as JPEG and PNG. Special attention is paid to the analysis of various fractal compression algorithms, their application and performance. The authors of the article strive to identify the most effective methods that provide a high degree of compression while maintaining the maximum amount of information about the image. This analysis can be useful for developers, engineers and researchers involved in image and data processing, as well as for a wide range of readers interested in advanced technologies in the field of digital data processing.

1 INTRODUCTION

Nowadays, when a huge number of images are created and processed, effective compression methods play an important role in reducing file size and improving data transfer. Fractal image compression is a relatively new approach that is attracting increasing attention for its ability to compress images with minimal loss of quality [1].
Fractal compression is based on the principle of self-similarity, which is found in many natural images. Fractal compression encodes similar patterns and structures in images. This approach allows you to achieve high compression ratios while preserving the details of the original images. Traditional methods such as JPEG and PNG are widely used for image compression, but they have their limitations, especially in terms of quality and compression ratio [2]. Based on the mathematical theory of fractals, they offer an alternative approach to this problem. These methods use self-similarity of image structures to represent it in a compressed form. The basic idea is to find small sections of the image that can be approximated by scaled and rotated copies of other sections of the same image. Thus, instead of storing all information about each pixel, only information about transformations is stored, which significantly reduces the amount of data [3].

Fractal compression methods have a number of unique advantages, such as a high compression ratio and the ability to infinitely enlarge an image without loss of quality. However, they also have their drawbacks, including high computational complexity and long compression times. The introduction to this topic allows you to understand more deeply the principle of fractal methods, their advantages and disadvantages, as well as compare them with traditional image compression methods.

As a result, the studying comparison of fractal image compression methods opens up new prospects for more efficient image storage and transmission.

2 METHODS AND RESULTS

In this article, we will focus on comparing different fractal image compression methods. We will consider fractal image compression based on partitioning the

image into rank and domain blocks, based on Iterative Functional Systems (IFS), based on quadtrees (Quadtree) and fractal wavelet image compression. In the comparison process, we will evaluate their effectiveness in achieving high compression ratios and maintaining image quality.

Next, we will present a detailed analysis of each method, evaluating their performance, including compression ratio, computational complexity, and image quality. The results of the analysis will help determine the most effective fractal compression method for various tasks and applications.

2.1 Fractal Image Compression Based on Rank and Domain Blocks

In general terms, fractal compression can be divided into two stages:

- Splitting an image into a set of non-overlapping rank blocks and into a set of domain blocks (which may overlap each other);
- Applying transformations for each domain–rank block pair.

A large area is called a domain block, and a smaller one is called a rank block. The conversion of a domain block to a rank block is carried out by affine transformations, which, in the case of a grayscale image, can be represented by the following system of (1) [4]:

$$\begin{bmatrix} x^* \\ y^* \\ z^* \end{bmatrix} = \begin{bmatrix} a & b & 0 \\ a & d & 0 \\ 0 & 0 & u \end{bmatrix} * \begin{bmatrix} x \\ y \\ z \end{bmatrix} + \begin{bmatrix} e \\ f \\ v \end{bmatrix}, \quad (1)$$

were,

- x, y, z – are the coordinates and brightness of the pixel of the domain block, respectively;
- x^*, y^*, z^* – are respectively the coordinates and brightness of the pixel of the rank block;
- a, b, c, d, e, f – coefficients of coordinate transformations on the plane;
- u – brightness compression ratio;
- v – is the brightness shift.

The quality of compression depends on the partitioning scheme used in the first stage. The more domain blocks there are, the greater the chance of finding the most similar ranked blocks. At the second stage, it is necessary to transform the domain block so that it is as similar as possible to the rank block. The general formula for converting pixel values of a domain block is as follows [5]:

$$D_i^* = sD_i + o, \quad (2)$$

where, D_i^* and D_i - are the transformed and original i-th domain block, respectively; o – is the brightness shift coefficient; $|s| < 1$ is the contrast coefficient, $o \in [-255; 255]$ – is the brightness coefficient. In addition to the direct conversion of pixel values according to formula (1), the domain block can also be subjected to general scaling (reducing the size to the size of a rank block, for example, by interpolation or simple thinning), rotation and other affine transformations [6].

The transformed domain block should match the rank block as strongly as possible, since this is how the rank block will be restored during decoding. To estimate the discrepancy (distance) between the transformed domain and the given rank blocks, it is necessary to enter the appropriate metric. The standard deviation function is usually used:

$$Q = \sum_{i=1}^{N} (D_i^* - R_i)^2 = \sum_{i=1}^{N} ((sD_i + o) - R_i)^2, \quad (3)$$

where, R_i- i th rank block, D_i^* and D_i-are the transformed and original i th and original with domain block, respectively, N-is the number of pixels in the rank block.

Obviously, the smaller the distance (2) between the blocks, the more similar they are. The coefficients and o can be found from (2) by taking partial derivatives of these variables.

Let's open the square in expression (4):

$$Q = \sum_{i=1}^{N} (s^2 D_i^2 + 2soD_i + o^2 - 2sR_iD_i - 2oR_i + R_i^i). \quad (4)$$

We have the following condition:

$$\begin{cases} \frac{\partial Q}{\partial s} = \sum_{i=1}^{N} (2sD_i^2 + 2D_i o - 2R_i D_i) \\ \frac{\partial Q}{\partial o} = \sum_{i=1}^{N} (sD_i + o - R_i) \end{cases}. \quad (5)$$

From (5) above, we obtain (6). Both equations are correct and necessary to find the extremum points. Thus, we solve the equation:

$$\begin{cases} s\sum_{i=1}^{N} D_i^2 + o\sum_{i=1}^{N} D - \sum_{i=1}^{N} R_i D_i = 0 \\ \sum_{i=1}^{N} (sD_i + o - R_i) = 0 \end{cases}. \quad (6)$$

Let's express the shift in brightness:

$$o = \frac{1}{N}\sum_{i=1}^{N} R_i - \frac{1}{N} s \sum_{i=1}^{N} D_i. \quad (7)$$

Substitute (6) into the (7) of the partial derivative and obtain the following formulas for finding the coefficients:

$$\begin{cases} s = \frac{N\sum_{i=1}^{N} R_i D_i - \sum_{i=1}^{N} R_i \sum_{i=1}^{N} D_i}{N\sum_{i=1}^{N} D_i^2 - \left(\sum_{i=1}^{N} D_i\right)^2}; \\ o = \frac{1}{N}\left(\sum_{i=1}^{N} R_i - s\sum_{i=1}^{N} D_i\right). \end{cases} \quad (8)$$

By converting (4), we obtain an expression for finding the distance:

$$Q = s^2 \sum_{i=1}^{N} D_i^2 + No^2 + \sum_{i=1}^{N} R_i^2 - 2s \sum_{i=1}^{N} R_i D_i + 2so \sum_{i=1}^{N} R_i - 2o \sum_{i=1}^{N} R_i. \quad (9)$$

Formulas (6), (7) simplify the computational load, since the sums $\sum_{i=1}^{N} R_i$, $\sum_{i=1}^{N} R_i^2$, $\sum_{i=1}^{N} D_i$, $\sum_{i=1}^{N} D_i^2$ can be calculated even before the enumeration begins, when sets of rank and domain blocks have already been formed [7]. Then, at the matching stage, you will need to calculate only the sum of $\sum_{i=1}^{N} R_i D_i$ and find the coefficients [8].

2.2 Integrated Functional Systems

IFS is one of the first and most well—known methods of fractal compression. This method represents an image using a set of compressive transformations that are applied iteratively to create self-similar patterns. By finding suitable compression transformations for each block, IFS can efficiently encode images. However, IFS-based methods often suffer from high encoding and decoding complexity, which limits their practical use [9].

Hutchinson (1981) showed that for the metric space R^n or, more generally, for the entire metric space X such a system of functions has a single nonempty compact (closed and bounded) fixed set S. One way to construct a fixed set is to start with the initial non–empty closed and bounded set S_0 and repeat the steps f_i, taking S_{n+1} as the union of images S_n under f_i; then taking S as the closure of the union S as the closure of the union S_n. A symbolically unique fixed (nonempty compact) set $S \subseteq X$ has the properties [10].

$$S = \cup_{i=1}^{N} f_i(A). \quad (10)$$

Thus, the set S is a fixed set of the Hutchinson operator $F: 2^X \to 2^X$ is defined for $A \subseteq X$ by formula (11)

$$F(A) = \cup_{i=1}^{N} f_i(A). \quad (11)$$

The existence and uniqueness of S is a consequence of the compression mapping principle, as is the fact that

$$\lim_{n\to\infty} F^{on}(A) = S\ , \quad (12)$$

for any nonempty compact A in X. (For compressive IFS, this convergence holds even for any nonempty closed bounded set A). Random elements arbitrarily close to S can be obtained using the "chaos game" described below [11].

It has recently been shown that IFS of a non-compressive type (i.e., composed of maps that are not compressions with respect to any typologically equivalent metric in X) can produce attractors. They arise naturally in projective spaces, although the classical irrational rotation on a circle can also be adapted [12]

2.3 Fractal Compression Based on Quadtree

Fractal compression algorithms based on a quadrant tree divide an image into smaller rectangular blocks, creating a hierarchical structure known as a quadrant tree [13]. The blocks are then recursively encoded and decoded based on their self-similarity. This method offers a good compromise between compression efficiency and computational complexity. Some variants of quadrant tree-based methods include fractal coding with block classification and adaptive subdivision of the quadrant tree [14].

Quadrant trees that store information about lines (English: edge quadtree) are used to describe straight lines and curves. The curves are described by exact approximations by dividing the cells into very small ones [15]. This can lead to unbalancing of the tree, which will mean problems with indexing.

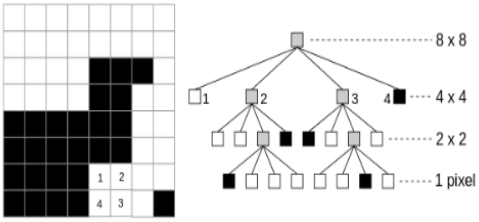


Figure 1: Quadrant trees divide the image into smaller rectangular blocks.

2.4 Fractal Wavelet Image Compression

Fractal wavelet compression combines the principles of wavelet transform and fractal image compression based on rank and domain blocks. The method uses wavelet decomposition to capture image details at various scales, and then applies fractal coding techniques for residual or low-frequency components. This hybrid approach often yields better compression ratios than purely fractal compression methods, while maintaining good image quality [16].

Using the proposed methods of wavelet and fractal analysis in relation to a time series represented by numerical values, it is necessary to obtain

quantitative and qualitative estimates characterizing the unsteadiness or randomness of the process under study.

The main processing algorithm is based on the condition of the existence of a time series, that is, statistical material collected at different points in time about the value of any parameters of the process under study, which takes into account the relationship of measurements with time [17].

The first stage is the processing of the time series by the method of wavelet and fractal analysis. The combined application of these two analyses will provide more complete information about the process under study, described by time series [18].

In the work, the wavelet transform is performed using direct WT (Wavelet transformation) [19]:

$$WT_s(a,x) = \frac{1}{a}\int_{-\infty}^{\infty} \psi\left(\frac{t-x}{a}\right) s(t)dt \quad (13)$$

$$\int_{-\infty}^{\infty} \psi(t)dt\ 0, \int_{-\infty}^{\infty} t^m \psi(t)dt\ 0;\ 0 \le m \le n \quad (14)$$

where a – is the scale parameter; x – is the shift the parent wavelet; $s(t)$ – the original signal; $\psi(t)$ – the wavelet function [20].

The calculation of the fractal dimension in this study is performed by the point-by-point method [21]. The algorithm for calculating the pointwise dimension is currently well-known and is a classic one (Fig. 1) [22].

The obtained values of the fractal dimensions of the time series under study are used as a criterion for the stability of the process under study. By the magnitude of the fractal dimension, it is possible to obtain a quantitative assessment of the randomness of the process under study, as well as the multifactorial and "saturation" of the prerequisites that caused the non-stationarity of the process [23].

The second and final stage of the model algorithm is the construction of a predictive assessment based on the allocation of indicators tending to an unstable state and determining the unsatisfactory state of the process. The results can be visualized and presented in the form of constructed wavelet diagrams, which give a visual picture of the most dramatically changing components of the signal. Thus, it becomes possible to identify the most non-stationary areas and, according to the selected areas, analyze in more detail the causes of these non-stationary events in the signal in relation to various applied areas [24].

2.5 Comparative Analysis

When comparing fractal compression methods, compliance with the requirements and limitations of applied tasks plays an important role. In particular, to solve the problems of image compression used in remote sensing (remote sensing of the Earth) [25], important factors are: the degree of compression [26], computational complexity, image quality and noise resistance and minimal information loss during transmission.

As a result of the research, it was found that the most suitable methods of fractal image compression for solving remote sensing problems are fractal compression methods based on rank and domain blocks and fractal wavelet compression.

The following is an illustration of the results of studies on all four methods confirming this conclusion.

2.6 Results

This section evaluates four key fractal image compression techniques:

- Fractal image compression based on rank and domain blocks;
- Iterated Functional Systems (IFS);
- Fractal compression based on Quadtree;
- Compression of the fractal wavelet image.

Fractal image compression based on rank and domain blocks.

Fractal image compression based on rank and domain blocks turned out to be one of the satisfactory results.

From the presented Figure 2 and Figure 3 you can see the preservation of image quality and reduction of compression time.

Integrated Functional Systems (IFS). Fractal compression of real images based on the method (IFS) is less suitable than fractal image compression of L-systems.

In these Figure 4 and Figure 5, the reduction in compression time is not recorded.

Figure 6 and Figure 7 show a significant loss of quality (the compressed image became "blurred"), and the time reduction turned out to be insignificant.

Fractal wavelet image compression. Fractal wavelet compression of the image also gave satisfactory results.

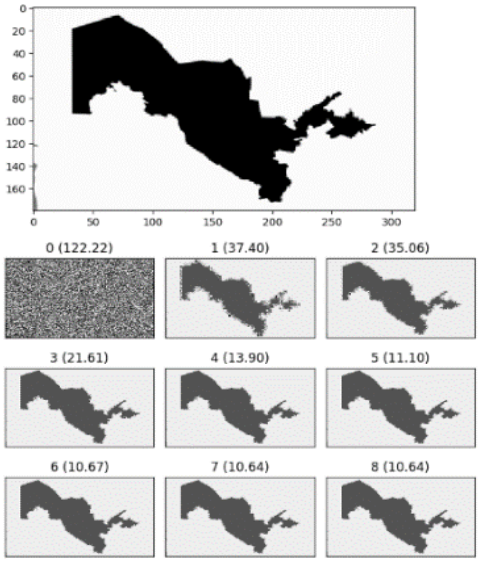


Figure 2: Image compression based on rank and domain blocks.

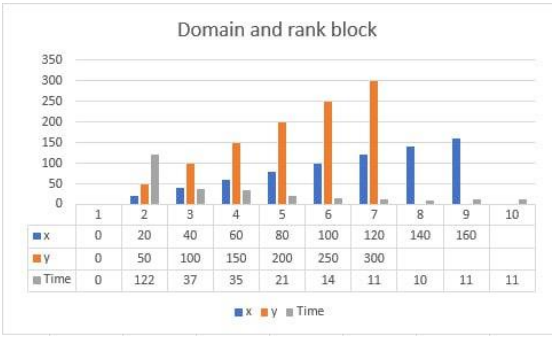


Figure 3: Graph ratio over time compression

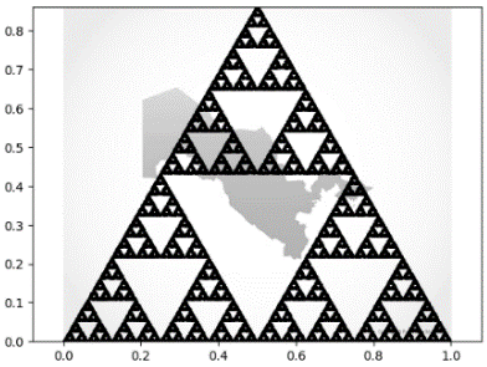


Figure 4: Image Compression Functional Systems (IFS).

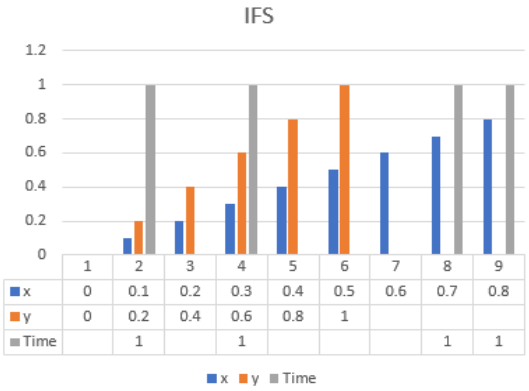


Figure 5: Graph the ratio over time compression.

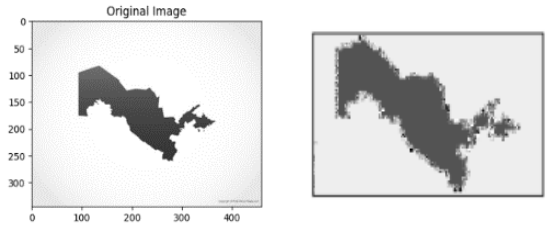


Figure 6: Quadtree-based image compression.

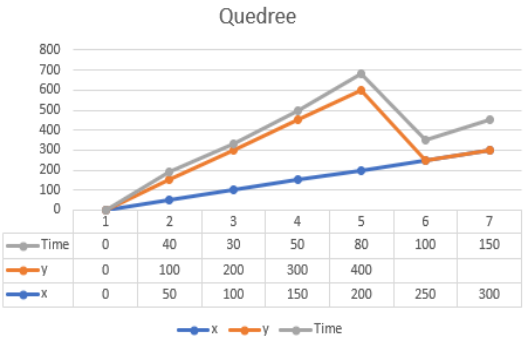


Figure 7: Graph ratio over time compression.

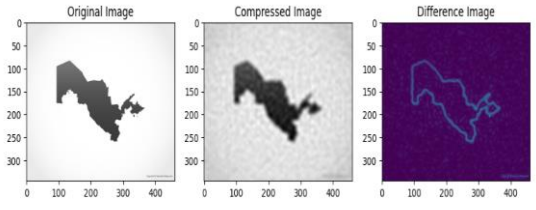
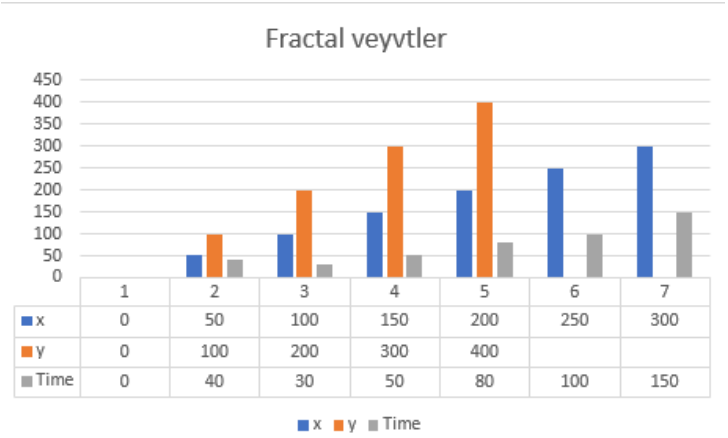


Figure 8: Fractal wavelet image compression.



	1	2	3	4	5	6	7
x	0	50	100	150	200	250	300
y	0	100	200	300	400		
Time	0	40	30	50	80	100	150

Figure 9: Graph ratio over time compression.

In Figure 8 and Figure 9, as well as in the case of fractal compression based on rank and domain blocks, preservation of image quality and reduction of compression time are recorded.

3 CONCLUSIONS

In conclusion, fractal image compression methods represent a promising direction in the field of processing and storing visual information. Each of the considered methods - based on the quadrant tree, rank and domain blocks, the IFS method and fractal wavelet compression - has its own unique advantages and disadvantages, which makes them suitable for various applications and conditions. The high compression ratios achieved by these methods make them particularly attractive for use in conditions of limited data storage and transmission resources.

Nevertheless, further research aimed at improving algorithms and reducing computational complexity is needed for wider implementation of fractal methods. Optimizing the compression time and improving the quality of the restored image are key areas that require the attention of researchers. It is also important to take into account the specific requirements of various applications, such as medical imaging, satellite imagery or multimedia, in order to adapt compression methods to specific needs.

In the future, fractal compression methods can become an integral part of new technologies, providing efficient and high-quality image compression. With the development of computing power and algorithmic improvements, it can be expected that these methods will find wider and wider application, contributing to progress in various fields of science and technology.

ACKNOWLEDGMENTS

The results of this research work were obtained within the framework of the practical project No. IL-4821091604. We will express our gratitude to the Agency for Innovative Development under the Ministry of Higher Education, Science and Innovation, which supports the project.

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A Novel Application of FDOSM for Industrial Robot Selection Using MCDM Techniques

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Keywords: MCDM, Multi Criteria Decision Making, FDOSM, Triangular Fuzzy Number, Fuzzy Set, Pick and Place Robot Selection, Industrial Robot.

Abstract: Industrial robots offer a range of capabilities and specifications depending on their intended applications. The role of robots in industries, especially in manufacturing, logistics, and related fields, has become increasingly important. As a result, selecting an industrial robot creates a complex decision-making challenge due to the vast array of options available and the absence of uniform performance standards. Decision by Opinion Score Method (FDOSM) is a reliable and consistent method that work in a fuzzy environment, presenting greater flexibility and less computational effort than previous methods. It efficiently addresses challenges by evaluating multiple alternatives according to multiple criteria, enhancing decision-making accuracy. It relies on the use of an opinion matrix to aggregate expert judgments, helping to resolve differences and reduce computational complexity. FDOSM consists of three phases: data input, transformation, and processing units, and both individual and group decision-making contexts are applied to FDOSM. A case study on industrial robot selection demonstrates FDOSM's ability to logically rank alternatives. The R3 (Cybotech V15 Electric) achieved the highest ranking with a score of 2.0944, demonstrating its suitability for pick and place operations in manufacturing systems. Also, among the robots evaluated, the R5 (Unimation PUMA 500/600) achieved the lowest ranking with a score of 3.6. The results demonstrate the effectiveness of the method utilized, as it agrees well with expert opinions and demonstrates the ability to improve decision reliability by addressing discrepancies in expert judgments. The study validates its findings by comparing the mean scores of the two groups, demonstrating that the method provides consistent and logical rankings.

1 INTRODUCTION

In recent years, industrial robots have seen a significant increase in their popularity and applications. To enhance production efficiency while maintaining product quality and speeding up workflow, industries rely on industrial robots. Robots are now used in processes such as assembly, finishing, and welding. However, choosing the most appropriate robot is complicated by varying performance criteria and the lack of uniform, globally agreed upon manufacturing standards. To address this problem, several MCDM methods have been used, allowing the best alternative to be selected by considering various conflicting criteria. Therefore, this paper presents the use of a modern decision model to simplify the industrial robot selection process, the FDOSM method, which provides a systematic approach to more accurately evaluate and select the most appropriate alternative [1],[2].

Multi-criteria decision making (MCDM) is a fundamental field in operations research and expert systems. It involves identifying the best alternative from among several alternatives by evaluating multiple criteria [3]–[5], a number of alternatives are compared based on different criteria [6]. It uses computational and mathematical approaches to evaluate performance criteria from the decision maker's perspective [7]. Given the complexity of decision-making scenarios, MCDM methods provide a structured approach for systematically evaluating multiple alternatives, criteria, and preferences [8]. These techniques have been widely used in a variety of fields, including engineering and healthcare, to efficiently address complex decision-making problems [10], [11]. MCDM is divided into two main approaches: the human approach and the

mathematical approach. Both approaches aim to rank alternatives and assign weights to different criteria [12]. The human approach relies on expert opinion and includes techniques such as the AHP [13], ANP [14], and BWM [12]. As for the mathematical approach, it is based on mathematical operations and includes techniques such as TOPSIS [15] and WSM [16]. MCDM techniques, whether human or mathematical, present substantial challenges when dealing with ambiguous and confusing data. Decision-makers typically convey their opinions in words rather than precise numerical values, complicating the assigning of specific weights to criteria. This uncertainty influences the final ranking of options and has been widely investigated in MCDM research [13], [17], [18]. To address this issue, MCDM has evolved inside a fuzzy set framework, effectively managing uncertainty and imprecise decision-making. Lotfi A. Zadeh's current notion of fuzzy sets paved the path for new approaches to solve the inadequacies of traditional MCDM techniques, leading to the development of fuzzy MCDM (FMCDM) techniques [18-20]. One of the most recent advancements in this industry is the FDOSM, which was revealed in 2020. FDOSM was created particularly for fuzzy environments, ranking alternatives using an opinion matrix, and the concept of an ideal solution. It effectively addresses core MCDM difficulties such as contradicting expert opinions, time-consuming comparisons, and unrealistic criterion evaluations. FDOSM also reduces the number of mathematical operations required, while increasing decision accuracy. It also addresses the issue of distance measurement by including both positive and negative ideal solutions, which eliminates the requirement for explicit criteria weighting. FDOSM improves decision-making by addressing issues with normalization and weight determination that are frequent in traditional mathematical techniques, resulting in a very efficient and accurate method [18]. Because FDOSM is generalizable, it can be applied to other MCDM problems, and compared to other fuzzy MCDM methods, FDOSM is less complex and maintains accuracy. Therefore, it was adopted in this study because of its novelty, simplicity, and effectiveness, and because it has not previously been used for optimal industrial robot selection.

A new application of FDOSM on a new case study to verify its effectiveness and reliability in solving MCDM problems.

2 MULTI CRITERIA DECISION MAKING (MCDM)

MCDM is a multidisciplinary field that has received significant interest in recent years, especially in situations where different options need to be comprehensively evaluated using multiple criteria [21], [22]. The basic goal of MCDM methods is to provide a systematic structure that enables decision makers to form rational, transparent, and well-supported choices [23]. The challenges of MCDM frequently arise in a variety of business and decision-making scenarios. Complexities arise from considering a large number of options, each evaluated according to a set of criteria, including factors such as cost, efficiency, and sustainability, depending on the specific decision-making context [24]. MCDM breaks down the problem into smaller, more manageable parts so that the DM can better understand it [25]. The main steps of the MCDM methodology include defining the problem, defining criteria, identifying alternatives, constructing a decision matrix, determining criterion weights, and ranking the alternatives. Decision criteria are typically divided into two categories. The first is the benefit criterion, where higher alternative scores indicate better performance, such as profits. The second is the cost criterion, where lower alternative scores indicate better performance, such as price [6, 26, 27]. The decision matrix (DM) is a commonly used approach in the MCDM, in which alternatives and criteria are presented clearly and systematically. It enhances the clarity of the decision-making problem and forms the basis for applying various techniques. Each alternative is evaluated under specific criteria, enabling a comprehensive analysis of each alternative's performance against these criteria. The decision matrix can be represented as follows:

$$DM = \begin{array}{c} \\ A_1 \\ \vdots \\ A_m \end{array} \begin{array}{c} \begin{array}{ccc} C_1 & \dots & C_n \end{array} \\ \begin{bmatrix} x_{11} & \cdots & x_{1n} \\ \vdots & \ddots & \vdots \\ x_{m1} & \cdots & x_{mn} \end{bmatrix} \end{array}$$

The decision matrix is a two-dimensional (Q×H) matrix, with (Q) rows representing alternatives (A1, A2,...) and (H) columns representing criteria (C1, C2,...Cn) [4]. The alternatives (A1,..., Am) are the possibilities that an experts may evaluate, whereas the criteria (C1,..., Cn) are the standards utilized to assess each alternative, and (x_{ij}) represents the evaluation of alternative (A_i) against criterion (C_j) [23], [28].

This matrix is a useful tool for experts to arrange and evaluate their alternatives methodically [27].

3 FUZZY DECISION BY OPINION SCORE METHOD (FDOSM)

The Fuzzy Decision by Opinion Score Method (FDOSM) is a new improved approach to MCDM, introduced in 2020 [18] to address the shortcomings of previous techniques. Designed to operate in a fuzzy environment, FDOSM uses an opinion matrix composed of expert opinions to provide an optimal value that serves as a benchmark for evaluating other values that meet the same criteria This method simplifies the decision-making process through aggregation using the arithmetic mean. Its advantages include reducing complex mathematical calculations and inconsistencies, which were common in previous techniques, and increasing decision accuracy and efficiency by addressing issues such as missing data, normalization, and weighting without the need for explicit criterion weights [18], [22]. Consequently, it provides a more logical and consistent ranking of options compared to other MCDM techniques. This method can be applied to both single and group (internal and external aggregations) decision-making scenarios.

3.1 FDOSM Steps

The FDOSM steps are outlined as follows [18], [26], [29]:

Step 1: Construct the Decision Matrix.

Step 2: Choose the ideal solution for each criteria such as minimum, maximum, or a critical value.

Step 3: To generate the opinion matrix, Compare the ideal solution to the values of the alternatives per criteria depending on the decision-maker's opinions.

Step 4: Transform the opinion matrix into triangular fuzzy numbers.

Step 5: Involves aggregation through arithmetic mean.

Step 6: Concludes with the selection of the lowest option as the optimal decision.

3.2 FDOSM Unit

There are three primary parts to FDOSM.: data input, data transformation, and data processing. below explain each unit, including its steps and mathematical formulae [18], [25]:

3.2.1 Phase One: Data Input Unit

Although it is comparable to previous MCDM methods, the suggested MCDM method involves a set of m alternatives, A_1,..., A_m, and a set of n criteria, C_1,..., C_n.These two elements are represented in a decision matrix, defined as:

$$D = \begin{matrix} A_1 \\ \vdots \\ A_m \end{matrix} \begin{bmatrix} x_{11} & \cdots & x_{1n} \\ \vdots & \ddots & \vdots \\ x_{m1} & \cdots & x_{mn} \end{bmatrix}. \quad (1)$$

This decision matrix is the product of the initial stage. Afterward, this decision matrix is converted to an opinion matrix in the next phase.

3.2.2 Phase Two: Data Transformation Unit

Once the decision matrix has been built (as the result of the initial phase), FDOSM performs a transformation phase by choosing an ideal solution depending on one of following factors: minimum, maximum, or critical value:

- Minimum Value. Utilized for cost-related criterion; the smallest value represents the preferred solution.
- Maximum Value. Utilized for benefit-related criterion, where higher values are preferred.
- Critical Value. Used in situations where the ideal solution fails to fit into the minimum or maximum categories, such as blood pressure, where an optimal range is preferred.

This step of FDOSM enables the selection of an ideal solution for values that are otherwise difficult to measure. The following steps in this stage are outlined and described [18, 29]:

Step 1: Choose the ideal solution. The selection of the ideal solution in FDOSM is given below:

$$A^* = \left\{\left[\left(\max_i v_{ij} \mid j \in J\right), \left(\min_i v_{ij} \mid j \in J\right), (Op_{ij} \in IJ) \mid i = 1,2,3,\ldots.m\right]\right\}. \quad (2)$$

Where: $\max_i v_{ij}$represents the ideal solution for benefit criteria (higher values are preferred), $\min_i v_{ij}$ represents the ideal solution for cost criteria (lower values are preferred), Op_{ij} is a critical value for cases where the ideal value is between $\max_i v_{ij}$ and $\min_i v_{ij}$.

Step 2: Comparing Ideal Solution to other values per criterion. In this step will perform comparison the ideal solution with each alternative's values per criterion. Here, weights are assigned to evaluation criteria implicitly. Decision-makers (DMs) evaluate whether differences between the ideal solution and alternative values have a significant impact on their

opinion, based on a set of five linguistic which facilitates this comparison, the DM selects ideal solution vectors (V_{31}, V_{22}, V_{43}, and V_{14}) to serve as benchmarks. After choosing the ideal solution, the alternatives are compared to it (reference comparison process is expressed) as follows:

$$Op_{\text{Lang}} = \left\{\left(\left(\tilde{v}_{ij} \otimes v_{ij} \mid j \in J\right) \cdot \mid i = 1.2.3 \ldots m\right)\right\}, \quad (3)$$

where $\otimes$ represents this reference comparison

This comparison yields a linguistic opinion matrix, which will then be turned to fuzzy numbers by utilizing fuzzy membership.

$$Op_{\ Lang} = \begin{matrix} A_1 \\ \vdots \\ A_m \end{matrix} \begin{bmatrix} op_{11} & \cdots & op_{1n} \\ \vdots & \ddots & \vdots \\ op_{m1} & \cdots & op_{mn} \end{bmatrix}. \quad (4)$$

When $Op_{\ Lang}$ represents the value of an alternative after it has been transformed into an opinion linguistic term [22].

3.2.3 Phase Three: Data Processing Unit

This process is detailed as follow:

Step 1: Fuzzify the opinion matrix by replacing opinion terms with triangular fuzzy numbers to form the fuzzy opinion decision matrix (FD_{ij}).

Step 2: Use arithmetic mean as an aggregation operator, to aggregate the outcomes from the fuzzy opinion decision matrix for every alternative. Once generating the fuzzy decision matrix, the procedure of aggregation is applied to choose the optimal alternative. This can be done by following equation:

$$\text{Arithmetic mean } A_{m(x)} = \frac{\sum_{i=1}^{n} x_i}{n} \quad (5)$$

Step 3**:** Apply defuzzification to the aggregated results to obtain a crisp value, which is computed as follows:

$$\frac{(a+b+c)}{3} \quad (6)$$

3.3 FDOSM Context

There are two types of processes applied to the opinion matrix in decision-making contexts:

3.3.1 Single Decision Making

In this case, the decision-making problem is handled by a single person who uses their experience to assess and then select the finest alternative from a list of alternatives based on preset criteria. This individual's judgment alone determines the final decision [18].

3.3.2 Group Decision Making

Group MCDM (G-MCDM) includes many decision-makers working together to identify the best alternative depending on expert experience and judgments[18]. It assists organizations in addressing complicated decision-making challenges, to achieve a unified solution. It includes two stages, internal and external aggregation, and we will use the external context in this work through the following (7):

$$\textit{External aggregation } = \oplus A^*, \quad (7)$$

where $\oplus$ denotes the arithmetic mean, and A^* indicates the ultimate outcome for every expert.

4 FUZZY SET

Fuzzy set theory, presented by Lotfi A. Zadeh, which is a mathematical structure to handling with uncertainty and vagueness in real-life situations [19]. Fuzzy sets are a kind of logic used to describe principles or phenomena that do not have a definite value. A fuzzy set uses a membership function to assign integer values ranging from 0 to 1 to represent an element's degree of belonging to a set. This approach allows for more precise and efficient reasoning when dealing with ambiguity. grasp fuzzy sets requires a foundational grasp of classical set theory. So, the classical set is a collection of well defined items, each of which either belongs to the set or does not [30]. The membership of an element x in a classical set A is determined by a characteristic function $\mu_A(x)$, which assigns a value of either 1 (if $x \in A$) or 0 (if $x \notin A$) [30, 31]:

$$\mu_A(x) = \begin{cases} 1 & \text{for } x \in A \\ 0 & \text{for } x \notin A \end{cases} \quad (8)$$

In contrast, a fuzzy set allows partial membership, where $\mu_{\mathrm{T}}(\mathrm{x})$ can take any value in the range [0,1] to denote the degree to which x relates to the fuzzy set T.

A fuzzy set T is outlined as:

$$T = \{(\mathrm{x}, \mu_{\mathrm{T}}(\mathrm{x}))/\mathrm{x} \in \mathrm{A}, \mu_{\mathrm{T}}(\mathrm{x}) \in [0,1]\} \quad (9)$$

Here, $\mu_{\mathrm{T}}(\mathrm{x})$ is the membership function that quantifies the level of membership of x in T.

5 TRIANGULAR FUZZY NUMBERS (TFN)

Triangular fuzzy numbers, represented as A = (a, b, c), are among the most commonly used types of fuzzy numbers in fuzzy MCDM. They are favored in practical applications for its conceptual clarity and ease of computation. The membership function of triangular fuzzy numbers is displayed in Figure 1. TFNs are outlined by their below membership function[32]:

$$\mu A(x) = \begin{cases} 0 & \text{if } x < a \\ \frac{x-a}{b-a} & \text{if } a \le x \le b \\ \frac{c-x}{c-b} & \text{if } b \le x \le x \\ 0 & \text{if } x > c \end{cases} \text{.where } a \le b \le c \quad (10)$$

Assume $\tilde{x}$ = (a1, b1, c1) and $\tilde{y}$ = (a2, b2, c2) are two nonnegative triangular fuzzy numbers, and α ∈ R+..The arithmetic operations can be illustrated in Table 1.

Table 2 shows the values of linguistic terms with TFN.

6 CASE STUDY

Bhangale et al.[34] examined an issue related to Choosing the most appropriate industrial robot for (pick-and-place actions) while avoiding certain obstacles. In this study, they identified five key attributes for robot selection: load capacity (LC), maximum tip speed (S), repeatability (RE), memory capacity (C), and manipulator reach (MR). Among these attributes, (LC, S, C, MR) are considered beneficial, meaning greater values are preferable. In contrast, (RE) is categorized as a cost attribute since a smallest value is desirable. Consequently, the robotics selection issue involves five criteria and seven robots, as detailed in Table 3.

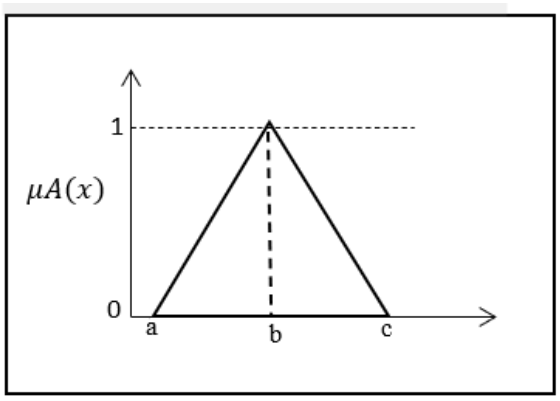


Figure 1: The triangular membership [33].

7 RESULTS AND DISCUSSION

7.1 Data Transformation Unit

In this step, to ensure practical application, we consulted with three experts in the field of industrial robotics, who have sufficient knowledge and experience in this field, every expert identifies the ideal solution for each criterion and evaluates it against other values based on the same criterion by using the five Likert scale. The comparisons result is linguistic terms (No difference, Slight difference, difference, Big difference, Huge difference). Consequently, the decision matrix is transformed to opinion matrix utilizing these linguistic phrases, as illustrated in the Table 4.

Table 1: The arithmetic operations TFN [33].

Linguistic terms	TFNs
No Difference (NO. D)	(0.00, 0.10, 0.30)
Slight Difference (S.D)	(0.10, 0.30, 0.50)
Difference (D)	(0.30, 0.50, 0.75)
Big Difference (B.D)	(0.50, 0.75, 0.90)
Huge Difference (H.D)	(0.75, 0.90, 1.00)

Table 2: The linguistic terms values with TFN [33].

Operations	Equations	
Addition	$\tilde{x} + \tilde{y} = (a_1 + a_2, b_1 + b_2, c_1 + c_2)$	(11)
Subtraction	$\tilde{x} - \tilde{y} = (a_1 - c_2, b_1 - b_2, c_1 - a_2)$	(12)
Multiplication	$\alpha\tilde{x} = (\alpha a_1, \alpha b_1, \alpha c_1)$	(13)
Division	$\tilde{x}/\tilde{y} \cong (a_1/c_2, b_1/b_2, c_1/a_2)$	(14)

Table 3: Robot Decision Matrix [34].

Alternative	LC	S	C	MR	RE
ASEA-IRB 60/2	60	2540	500	990	0.4
Cincinnati Milacrone T3-726	6.35	1016	3000	1041	0.15
Cybotech V15 Electric Robot	6.8	1727.2	1500	1676	0.1
Hitachi America Process Robot	10	1000	2000	965	0.2
Unimation PUMA 500/600	2.5	560	500	915	0.1
United States Robots Maker 110	4.5	1016	350	508	0.08
Yaskawa Electric Motoman L3C	3	1778	1000	920	0.1

Table 4: Opinion Matrix for three experts.

Opinion matrix (Expert 1)						Opinion matrix (Expert 2)					Opinion matrix (Expert 3)				
Alterna-tive	LC	MTS	MC	MR	RE	LC	MTS	MC	MR	RE	LC	MTS	MC	MR	RE
R1	NO.D	NO.D	H. D	S. D	H. D	NO.D	NO.D	H. D	S. D	H. D	NO.D	NO.D	H. D	D	H. D
R2	H. D	B. D	NO.D	S. D	S. D	B. G	D	NO.D	S. D	D	H. D	H. D	NO.D	S. D	B. D
R3	H. D	D	D	NO.D	S. D	B. G	S. D	D	NO.D	S. D	B. G	S. D	D	NO.D	S. D
R4	B. G	B. D	D	D	B. D	B. G	D	S. D	D	H. D	D	H. D	S. D	D	B. D
R5	H. D	H. D	H. D	D	S. D	H. D	H. D	H. D	B. D	S. D	H. D	H. D	H. D	B. D	S. D
R6	H. D	B. D	H. D	H. D	NO.D	H. D	D	H. D	H. D	NO.D	H. D	D	H. D	H. D	NO.D
R7	H. D	D	B. D	D	S. D	H. D	S. D	B. D	B. D	S. D	H. D	S. D	D	B. D	S. D

Table 5: Fuzzy Opinion Matrix for three experts.

Fuzzy opinion matrix (Expert 1)															
Alternative	LC			S			C			MR			RE		
R1	0	0.1	0.3	0	0.1	0.3	0.75	0.9	1	0.1	0.3	0.5	0.75	0.9	1
R2	0.75	0.9	1	0.5	0.75	0.9	0	0.1	0.3	0.1	0.3	0.5	0.3	0.5	0.75
R3	0.75	0.9	1	0.3	0.5	0.75	0.3	0.5	0.75	0	0.1	0.3	0.1	0.3	0.5
R4	0.5	0.75	0.9	0.5	0.75	0.9	0.3	0.5	0.75	0.3	0.5	0.75	0.5	0.75	0.9
R5	0.75	0.9	1	0.75	0.9	1	0.75	0.9	1	0.3	0.5	0.75	0.1	0.3	0.5
R6	0.75	0.9	1	0.5	0.75	0.9	0.75	0.9	1	0.75	0.9	1	0	0.1	0.3
R7	0.75	0.9	1	0.3	0.5	0.75	0.5	0.75	0.9	0.3	0.5	0.75	0.1	0.3	0.5
Fuzzy opinion matrix (Expert 2)															
Alternative	LC			S			C			MR			RE		
R1	0	0.1	0.3	0	0.1	0.3	0.75	0.9	1	0.1	0.3	0.5	0.75	0.9	1
R2	0.5	0.75	0.9	0.3	0.5	0.75	0	0.1	0.3	0.1	0.3	0.5	0.3	0.5	0.75
R3	0.5	0.75	0.9	0.1	0.3	0.5	0.3	0.5	0.75	0	0.1	0.3	0.1	0.3	0.5
R4	0.5	0.75	0.9	0.3	0.5	0.75	0.1	0.3	0.5	0.3	0.5	0.75	0.75	0.9	1
R5	0.75	0.9	1	0.75	0.9	1	0.75	0.9	1	0.5	0.75	0.9	0.1	0.3	0.5
R6	0.75	0.9	1	0.3	0.5	0.75	0.75	0.9	1	0.75	0.9	1	0	0.1	0.3
R7	0.75	0.9	1	0.1	0.3	0.5	0.5	0.75	0.9	0.5	0.75	0.9	0.1	0.3	0.5
Fuzzy opinion matrix (Expert 3)															
Alternative	LC			S			C			MR			RE		
R1	0	0.1	0.3	0	0.1	0.3	0.75	0.9	1	0.3	0.5	0.75	0.75	0.9	1
R2	0.75	0.9	1	0.75	0.9	1	0	0.1	0.3	0.1	0.3	0.5	0.5	0.75	0.9
R3	0.5	0.75	0.9	0.1	0.3	0.5	0.3	0.5	0.75	0	0.1	0.3	0.1	0.3	0.5
R4	0.3	0.5	0.75	0.75	0.9	1	0.1	0.3	0.5	0.3	0.5	0.75	0.5	0.75	0.9
R5	0.75	0.9	1	0.75	0.9	1	0.75	0.9	1	0.5	0.75	0.9	0.1	0.3	0.5
R6	0.75	0.9	1	0.3	0.5	0.75	0.75	0.9	1	0.75	0.9	1	0	0.1	0.3
R7	0.75	0.9	1	0.1	0.3	0.5	0.3	0.5	0.75	0.5	0.75	0.9	0.1	0.3	0.5

7.2 Data Processing Unit

This involves three phases:

1) First, the opinion matrix is transformed to fuzzy opinion matrix. This is done by changing the linguistic terms with triangular fuzzy numbers pursuant to Table 2. As a result, we obtain a fuzzy opinion matrix, as illustrated in the above Table 5.
2) Second, use an addition (11) to combine the results from the prior procedure for each alterna-ative. as illustrated in Table 6.
3) Third, the defuzzification (6) is used to the preceding matrix to present the end outcomes for every individual decision maker, as illustrated in Table 7.

Table 8 illustrates the application of group decision-making, where the individual decisions of several experts are aggregated to produce a unified group decision. This process employs external group decision-making methods by using (7). By utilizing this approach, we can address the variability in expert opinions and resolve any inconsistencies that may arise.

Pursuant to the FDOSM concept, the appropriate alternative is the one nearest to the no-difference linguistic phrase describing the ideal solution, denoted by the smallest value, and the opposite is true. Table 6 displays the final findings for each expert (Individual Decision Making) based on the Opinion Matrix. So, based on these results, the ideal alternative for the first decision-maker is "R1(ASEA-IRB 60/2)" with a score of "2.33333333". In contrast, " R3(Cybotech V15 Electric Robot)" is the favored alternative for both the second and third experts, obtaining score of "1.966666667" for both. The variance arises from experts having differing opinions based on their experiences and perspectives when evaluating robots according to specific criteria. On the other hand, the least favored alternative for the first decision-maker, with a score, is "R6(United States Robots Maker 110)," with a score of "3.5" while for the second and third decision-maker, the least preferred alternative is "R5(Unimation PUMA 500/600)," with scores of "3.666666667" for both.

Table 6: Aggregation step for three experts.

Aggregation step (Expert 1)			
Alternative	Scores		
R1	1.6	2.3	3.1
R2	1.65	2.55	3.45
R3	1.45	2.3	3.3
R4	2.1	3.25	4.2
R5	2.65	3.5	4.25
R6	2.75	3.55	4.2
R7	1.95	2.95	3.9
Aggregation step (Expert 2)			
Alternative	Scores		
R1	1.6	2.3	3.1
R2	1.2	2.15	3.2
R3	1	1.95	2.95
R4	1.95	2.95	3.9
R5	2.85	3.75	4.4
R6	2.55	3.3	4.05
R7	1.95	3	3.8
Aggregation step (Expert 3)			
Alternative	Scores		
R1	1.8	2.5	3.35
R2	2.1	2.95	3.7
R3	1	1.95	2.95
R4	1.95	2.95	3.9
R5	2.85	3.75	4.4
R6	2.55	3.3	4.05
R7	1.75	2.75	3.65

Table 7: Final result for three experts.

Expert 1		
Alternative	Score	Rank
R1	2.333333333	1
R2	2.55	3
R3	2.35	2
R4	3.183333333	5
R5	3.466666667	6
R6	3.5	7
R7	2.933333333	4
Expert 2		
Alternative	Score	Rank
R1	2.333333333	3
R2	2.183333333	2
R3	1.966666667	1
R4	2.933333333	5
R5	3.666666667	7
R6	3.3	6
R7	2.916666667	4
Expert 3		
Alternative	Score	Rank
R1	2.55	2
R2	2.916666667	4
R3	1.966666667	1
R4	2.933333333	5
R5	3.666666667	7
R6	3.3	6
R7	2.716666667	3

Table 8: Final result for three experts.

Group expert		
Alternative	Score	Rank
R1	2.4056	2
R2	2.55	3
R3	2.0944	1
R4	3.0167	5
R5	3.6	7
R6	3.3667	6
R7	2.8556	4

Table 9: Validation for basic-FDOSM.

Group	Industrial robot	Means
first Group	R3	2.476389
	R1	
	R2	
	R7	
second Group	R4	3.327778
	R6	
	R5	

In the Group decision-making, pursuant to Table 7, the best alternative is "R3(Cybotech V15 Electric Robot)", with a score of "2.0944". The least favorable alternative is "R5(Unimation PUMA 500/600)", which got a score of "3.6". The difference in ranking scores reflects decision-makers diverse perspectives. However, when the GDM findings are compared to the individual decision-makers opinion matrices, the robot model rankings remain constant. The usage of GDM increases flexibility and effectively handles uncertainty in opinion matrices, resulting in a more accurate comparison of the group's final choice and individual judgments. Limitations of this method include: Converting the opinion matrix to a fuzzy decision matrix is limited to using only one fuzzy number at a time. The quality of the input data must be ensured to obtain consistent and reliable results.

8 VALIDATION

In this study, validation is employed to substantiate the decision-making outcomes derived from the basic-FDOSM method. Objective validation entails partitioning the alternatives into distinct groups, a technique that has been utilized in various MCDM studies [14], [18].

To validate the results, the following steps are undertaken: first: the alternatives are sorted based on the decision-making outcomes from the Group basic-FDOSM, second: the sorted alternatives are divided into two groups, and third: the groups mean is calculated based on the group decision-making (GDM) findings, as defined in the (15) [14]:

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i \qquad (15)$$

The comparison results are contingent upon the mean values of each group as it forms the basis for comparison, with the lowest mean value representing the optimal solution for each criterion (desirable groups), in accordance with the FDOSM concept. Conversely, a higher mean value signifies a less favorable alternative. In this context, the first group is analyzed for having the minimal mean, which is utilized to evaluate the validity of the results. This first group is then contrasted with the second group to further assess the validity of the findings. The second group's mean should be greater than or at least equal to that of the first group. Once the assessment results are consistent, they can be deemed valid. Table 9 presents the validation outcomes for the industrial robot selection case study utilizing basic-FDOSM. The first group exhibits a lower average of 2.476389 compared to the second group's average of 3.327778. Validation results confirm that the basic-FDOSM findings for identifying the optimal robots, as presented by the groups, are valid and can be systematically ranked.

9 CONCLUSIONS

Selecting the optimal alternative based on many performance factors is important for daily life and work. The problem is handled with the FDOSM. FDOSM efficiently tackles issues such as inconsistent human judgment and unnecessary computing complexity by combining expert opinion with fuzzy logic. The approach simplifies decision-making by using an opinion matrix and arithmetic mean aggregation to ensure a logical and accurate ranking of alternatives. The ranking results of the case study proved that FDOSM is a methodical and efficient method for selecting the best industrial robot, including individual and group decision-making scenarios.So, the results, the ideal alternatives for the individual decision-makers are “R1(ASEA-IRB 60/2)", and R3(Cybotech V15 Electric Robot)", In the Group decision-making, the best alternative is "R3(Cybotech V15 Electric Robot)”. The validation demonstrated that FDOSM improves decision dependability by reducing uncertainty in expert evaluations. Future research could be applying FDOSM to other industrial and engineering issues, demonstrating its adaptability and significance in broader applications.

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Evaluation of Resource Allocation in Cloud Using Machine Learning

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Keywords: Cloud Computing, Machine Learning, Virtual Machine, Resource Allocation.

Abstract: Cloud computing has revolutionized the way computing the resources are allocated and managed, and it offers scalability, flexibility, and the cost savings. Proper resource allocation, however, remains a difficult problem due to varying workloads, unpredictable demand, and the need for optimal performance. Machine learning (ML) techniques have been recognized as a promising solution to optimizing the resource allocation by predicting workload patterns, optimizing resource utilization, and reducing latency. In this paper, we have compared the various ML-based framework for the resource allocation in the cloud computing environments on the basis of their efficiency in order to improve the efficiency and the cost control. Through comparative evaluation, we highlight the merits and demerits of different ML models, including the contextually of their suitability in the actual implementations. The results reveal that the proposed model achieves accuracy (Decision Tree 100%, AdaBoost 72.2%, Support vector machine 98.5%, logistic regression 97.6% and Gradient boosting 100%).

1 INTRODUCTION

Industry and academia increasingly transfer applications to the cloud so developers can deploy and operate applications without server configuration and setup complexity. Cloud providers continuously innovate their services to provide greater efficiency, service quality, and resource control. Cloud computing offers scalable computer resources via the Internet, reducing job completion time, makepan, and cost of operations. Cloud computing resource allocation is the efficient distribution of compute resources such as CPU, memory, storage, and network bandwidth to tasks and applications. Correct resource allocation ensures maximum performance, cost efficiency, and user satisfaction. Virtualization plays a key role in the process since it enables many virtual machines (VMs) to execute on one physical server. VMs provide a private and dynamic operating environment for running applications, with dynamically scalable resources based on the need to enhance flexibility and efficiency within the system [1-8].

Virtualization is the foundation of cloud computing, as it creates virtual depictions of physical hardware, maximizes resource utilization, and enhances system performance, energy consumption, and data center efficiency. In addition, job scheduling and load balancing are crucial for workload distribution over cloud resources. Job scheduling schedules and assigns jobs based on resource requests and time constraints, while load balancing prevents server overload through network traffic balancing [9].

Machine learning (ML) as a subset of artificial intelligence is unfolding as a very efficient means of optimizing resource management in the cloud. By learning from experience and finding patterns, ML algorithms are able to predict workload fluctuations, enhance decision-making, and automate the process of assigning resources. The current paper explores the significance of having an even task distribution across VMs to ensure optimal resource utilization. It proposes a sequential distribution approach for optimizing resource distribution, minimizing access times, and overall cloud system performance by overcoming bottlenecks and latencies [10].

This study aims to evaluate the effectiveness of machine learning (ML) algorithms for cloud computing resource optimization based on the framework. It aims to analyse key challenges in resource allocation, such as scalability, workload prediction, and cost optimization while exploring ML-based approaches to enhance efficiency. The study focuses on maximizing the utilization of

virtual machines (VMs) through smart scheduling and load balancing to ultimately enhance system performance by reducing execution time, power consumption, and latency. In addition, it seeks to propose a sequential distribution method for the assignment of tasks in order to limit bottlenecks and enhance cloud efficiency. By comparing traditional and ML-based resource allocation approaches, this research provides perspective on performance improvement and cost saving and facilitates the design of adaptive cloud resource management solutions.

2 RELATED WORKS

Resource management has been transformed by cloud computing, which has resulted in the development of a variety of methods for efficient allocation and work planning. Traditional methods, including opportunistic load balance (OLB) algorithms and min-min-min, distribute duties according to predetermined criteria; however, they encounter difficulties when dealing with dynamic cloud work. Artificial intelligence (AI) and machine learning (ML) techniques have been implemented in recent years to mitigate these constraints and enhance resource management. For instance, Mamalis et al. [8] proposed a Gravitational Search algorithm (GSA)- based work scheduling algorithm for cloud computing virtual machines. Based on Newtonian physics and agent-based optimization, GSA significantly improved scheduling efficiency. GSA-based makespan reduction had an average performance improvement of 3.5% over ESTA, 7.7% over PSO, 5.1% over Min-Min, and 27.3% over OLB. GSA outperformed these strategies by 6.7%, 9.2%, 14.5%, and 68.7% in average resource consumption, making it a promising cloud computing strategy. Anbarkhan and Samar Hussni [9] proposed a machine learning-based cloud resource management method that improves resource usage by 30% and reduces operational costs by 25%. Their method improves system performance, latency, and prediction accuracy. Simulations show it can transform cloud service management. According to Zhang et al. [10], the GAACO algorithm for cloud computing outperforms standard scheduling algorithms in time cost minimization, quality of service enhancement, and system load balancing. By balancing time, money, reliability, and system load, GAACO shows the revolutionary appeal of AI-enabled cloud computing solutions. Shetty et al. [11] optimized virtual machine job scheduling settings with feed-forward neural networks. They used Principal Component Analysis (PCA) to choose relevant features for dynamic scheduling to maximize resource consumption and cloud system performance. In Infrastructure-as-a-Service (IaaS) contexts, their approach maximizes throughput and minimizes makespan using Min-Min and MET algorithms. Khan et al. [12] explored cloud computing security approaches and distributed architectural concerns. They stressed that while cloud growth might improve security, innovative models should work with existing systems without compromising critical functions. Their goal was to improve security without compromising other system characteristics. Swarna et al. [13] examined green cloud computing load balancing in the IoE paradigm. They improved energy consumption, cloud storage, data processing, and end-user services using Wind-Driven Optimization and Firefly algorithms. They also used IoT network clustering to develop intelligent information processing solutions for energy-efficient cloud systems. Gomathi and Karthikeyan [14] introduced hybrid swarm optimization for task scheduling to reduce project completion time. Their method optimizes cloud computing workload and resource allocation. Kumar, Singh, and Buyya [15] proposed a neural network trained by black hole events for predictive workload control. They use deep learning, an evolutionary algorithm, and backpropagation to anticipate 99.9% more accurately than previous approaches. The Coronavirus Herd Immunity Optimizer-derived scheduling algorithm minimizes makespan and improves speed, efficiency, and throughput. Renyu Yang et al. [16] proposed data-driven profiling, issue formulation, and supervised modelling for end-to-end cloud computing optimization. They stressed that ML-based solutions improve system efficiency and architecture. ML algorithms must be integrated with edge and cloud devices to maximize performance and resource management.

To the best of our knowledge, substantial research has been conducted in the past few years to understand, manage, and mitigate resource allocation in the cloud. Limited work has been conducted on the subject defined by these challenging circumstances. Therefore, the purpose of this research is to establish a framework that improves the quality of resource allocation by utilizing machine learning to improve the cloud's performance.

3 MACHINE LEARNING

Classification extracts latent knowledge from mass data after pre-processing. Its essential operation determines data element internal relativity. As mentioned, machine and deep learning methods are likely utilized for classification.

3.1 Support Vector Machine Algorithm

SVM, a classification and regression technique, finds a multi-dimensional hyperplane to best segregate data classes [20]. SVM maximizes the distance between the hyperplane and nearest points from both classes to determine the best data separation hyperplane. The margin is the distance between the hyperplane and nearby points, and support vectors change its position to improve classification accuracy and performance even with high-dimensional data [21].

3.2 Decision Tree Algorithm

Decision trees are hierarchical data structures containing leaf and non-leaf nodes used for classification and regression trees, with decision criteria differing by structure [22]. A rooted decision tree with leaf and non-leaf nodes represents classification and selection choices based on input attribute values [23]. This work uses a static decision tree-based approach to pick a priority rule combination for processing jobs, allowing planning without rule modifications over the scheduling horizon [24].

3.3 Gradient Boosting

For categorization and versioning, machine learning uses Gradient Boosting. It corrects faults consecutively when building models. Cloud computing allows effective training of complex models on massive datasets due to scalable architecture and dispersed computing capacity. Cloud-based clusters can parallelize training and handle enormous datasets. [25] Gradient-boosting ensemble learning creates models successively, correcting errors by gradient descent. Fitting weak learners (usually decision trees) to residual mistakes minimizes a loss function [26].

3.4 AdaBoost (Adaptive Boosting)

AdaBoost builds a powerful classifier from numerous weak learners. Giving misclassified data items more weight enhances categorization. It uses distributed computing resources in cloud computing for efficient large dataset handling, faster training, and cost-effective resource management [27]. AdaBoost, an ensemble learning algorithm, combines weak classifiers (usually decision stumps) to create a powerful classifier. Misclassified samples are weighted to focus on hard-to-classify cases in the following iterations.

3.5 Logistic Regression

Logistic regression is a statistical method in binary classification to estimate the chance of an instance belonging to either of two categories. It invokes a logistic (sigmoid) transformation on a linear combination of features, resulting in a value from 0 to 1. It is utilized in fields of healthcare, finance, and marketing for tasks of disease prediction, credit scoring, and customer churn prediction.

4 METHODOLOGY

Cloud computing requires an ideal dataset for efficient resource allocation based on real-world activity and system behaviour. To this end, in Figure 1, the phase 1 machine has focused on data collection, simulation, and preaching to generate high-quality datasets that are suitable for learning models. It begins with the GOCJ Google Cloud Jobs data set, a well-known data set that provides valuable information about cloud job planning and execution behaviour. Raw data sets, however, are rarely highly variable, and raw data is incapable of approximating the dynamic quality of the environment of blame. Monte Carlo simulation can bypass this deficiency by producing a representative set of sales data and modelling varied charging distribution, resource requirements, and execution time. When the dataset is generated, the following process traverses a collection dataset that captures important virtual machine (World Cup) view measurements required in examining resource allocation methods. Such matrices include:

1) Makespan: Total execution time required to complete all the scheduled functions.
2) Memory usage: The space occupied by running the charge.
3) Idle time: When the World Cup is short, there is a breakage that affects the overall efficiency.
4) Throughput: The speed at which the tasks are handled within a given time frame.

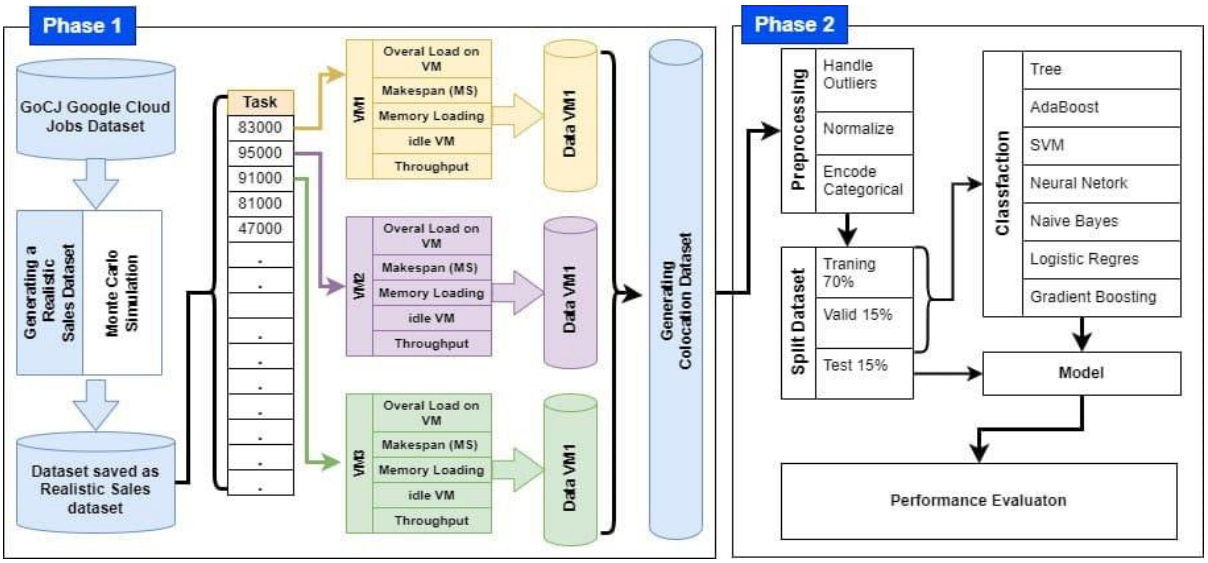


Figure 1: The proposed Framework recall and F1 score.

Organizing the dataset in this way enables a more accurate and in-depth analysis of resource usage, job planning efficiency, and workload balancing. Colocation is a core input in dataset phase 2, where machine learning techniques make resource allocation dynamic.

In phase 2, the dataset undergoes a series of reservative steps to increase quality and ensure optimal performance in the machine learning model. Raw data often consists of functions with deviations, lack of values, outliers and separate scales, which can significantly affect the model's accuracy. To solve these challenges, the preparation stage includes dealing with generalization and classified coding and ensuring that the dataset is well structured and the training machine is suitable for training the learning algorithms. After preprosautting, the dataset is divided into three Multier to facilitate model training, verification and evaluation:

- Training (70%): A machine is used to train learning models so that they can learn from previous computer patterns and develop predicting abilities.
- Verification (15%): The fine-tuning model helps with parameters and ensures that the algorithm normalizes unseen data without overfitting.
- Test set (15%) – This is Reserved for final evaluation and provides a fair assessment of the model's actual world performance.

When the dataset is prepared, various machine learning classifiers are used to predict optimal resource allocation in the cloud environment. We evaluated several models, including decision trees, AdaBoost, SVM, logistic regression, and gradient boosting, chosen for their potential benefits in workload planning, resource optimization, and system efficiency. To determine their effectiveness for cloud resource management, the final models were evaluated based on accuracy, precision, recall, and resource utilization metrics.

Selecting and integrating these ML techniques for cloud resource allocation leverages their efficiency, accuracy, scalability, and ability to handle complex workloads. Rigorous evaluation ensures intelligent and adaptable resource allocation in the cloud environment. This ML-driven approach optimizes operational planning, reduces execution time, enhances VM utilization, and lowers operational costs – leading to more efficient and scalable cloud systems. The evaluation results identify the most suitable algorithm(s) for real-world cloud resource management, ensuring reliability and performance improvements in modern cloud infrastructures. Algorithm 1 details task processing in virtual machine memory.

Algorithm 1: Memory Task Processing.

Input:

```
tasks_list → List of tasks to process
virtual_memory_count → Number of virtual memory (VM) units
```

```
   time_execution → Execution time
parameter
   buffer_size = 1024 → Default buffer
3jk3
   size.
```

Output:generation collocation dataset

Step 1: //Initialize VM resources

```
   Create
VM_array[virtual_memory_count]
   Set VM.flag = 0 (0 = available, 1 =
busy)
   Set   VM.wait_time,   VM.throughput,
VM.makespan = 0
```

Step 2: // Initialize counters

```
   task_id = 0
   iteration = 0
```

Step 3: While task_id < tasks_list. length do: //Task Processing Loop

Step 3.1://Get Current Task

```
   If task_id > max_tasks then
   set current_task = 0, resource_need
= 0,
   time_id = 0
   Else,
   assign        current_task        =
tasks_list[task_id],
   calculate resource_need and time_id
```

Step 3.2:// Find and Assign Task to a VM

```
   Iterate through VM_array
   If VM is available and within task
bounds Then
   Assign current_task to VM
   Update VM metrics:
   load = resource need + (VMP / VM)
   load percent = (load / VMP) * 100
   wait_time += resource need
   makespan += time_id
   throughput = ((end - current_task) /
buffer_size) /  (makespan + time_id)
   Compute overall load using random
scaling factors
   Update historical counters for task
tracking
```

Step 3.3: //Update Wait Times & Performance Metrics:

```
   For each VM, adjust:
   wait_time -= (core * time_execution)
   makespan = max(0, makespan - (core *
(time_execution / 10000)))
   Ensure non-negative values for
wait_time, makespan
```

Step 3.4: //Compute Total Performance Metrics:

```
   total_makespan = sum(VM.makespan)
   total_throughput                    =
sum(VM.throughput)
```

Step 3-5:// Store Data in DataFrames

```
   data_wait[iteration] = VM.wait_time
   data_flag[iteration] = VM.flag
   data_load[iteration] = VM.load
   data_overall[iteration]             =
VM.overall_load
   data_makespan[iteration]            =
VM.makespan
   data_throughput[iteration]          =
VM.throughput
   data_tasks[iteration]               =
VM.current_task
```

Step 3-6: Reset Completed VMs

```
   If VM.wait_time ≤ 0, reset then
   VM.wait_time = 0
   VM.flag = 0
   VM.makespan = 0
   VM.throughput = 0
```

Step 3-7: Increment Counters:

```
   task_id += 1,      teration += 1
   End
```

5 RESULT AND EXPERIMENT

5.1 Datasets

Figure 2 presents a dataset that includes several tasks using Monte Carlo expansion. The dataset assists in deciding the suitability of a virtual machine for tasks in accordance with the characteristics of RAM, CPU, bandwidth, and processor. These characteristics serve as input to the virtual machine. The GoCJ dataset incorporates several job sizes, which can be created by duplicating formulas within an Excel file ("GoCJ_Dataset_Monte_Carlo.xlsx"). The dataset facilitates the generation of new datasets and the examination of distributed tasks, including virtual machine selection, environmental parameters, and performance metrics such as overload, makespan, throughput, wait time, and load balance.

	df_OL	Lm_p	makespan	Throughput	waite	df_task_save	Class
0	67.38162686	69.3995	0.126553672	432.129	0	127000	V_Memory0
1	67.38162686	69.3995	0.126553672	432.129	0	127000	V_Memory0
2	19.93270117	21.8585	6.971751412	4943.555	0	40000	V_Memory0
3	32.49255976	28.9623	6.957062147	2956.671	0	53000	V_Memory0
4	64.18514062	62.8421	6.88700565	587.695	45000	115000	V_Memory0
5	64.18514062	62.8421	0.11299435	587.695	0	115000	V_Memory0
6	93.79233023	63.935	6.884745763	559.226	47000	117000	V_Memory0
7	93.79233023	63.935	0.115254237	559.226	0	117000	V_Memory0
8	58.15995418	55.1918	6.902824859	824.06	31000	101000	V_Memory0
9	58.15995418	55.1918	0.097175141	824.06	0	101000	V_Memory0
10	85.32750249	57.3776	6.898305085	749.023	35000	105000	V_Memory0
11	85.32750249	57.3776	0.101694915	749.023	0	105000	V_Memory0
12	23.65245068	36.6126	6.941242938	1927.96	0	67000	V_Memory0
13	68.79390898	55.1918	6.902824859	824.06	31000	101000	V_Memory0
14	68.79390898	55.1918	0.097175141	824.06	0	101000	V_Memory0
15	58.03542264	49.7273	6.914124294	1046.207	21000	91000	V_Memory0
16	58.03542264	49.7273	0.085875706	1046.207	0	91000	V_Memory0
17	18.52379671	25.6836	6.963841808	3673.096	0	47000	V_Memory0
18	31.35114395	33.3339	6.948022599	2292.162	0	61000	V_Memory0
19	65.47290857	62.8421	6.88700565	587.695	45000	115000	V_Memory0
20	24.79879606	45.3557	0.076836158	1270.967	0	83000	V_Memory0
21	24.79879606	45.3557	0.076836158	1270.967	0	83000	V_Memory0
22	13.69257678	30.0552	6.95480226	2765.625	0	55000	V_Memory0

Figure 2: Section from our datasets in GoCJ Excel worksheet generator.

Figure 3 displays a 3D scatter plot that demonstrates the distribution of tasks in the dataset, showing the relationship between Job Size, Arrival Time, and Service Time for 100 tasks. The plot helps identify patterns, such as larger tasks requiring more time to complete. This evaluation can be used to optimize task scheduling, adjust resource allocation, and improve overall efficiency by analysing arrival times and task performance.

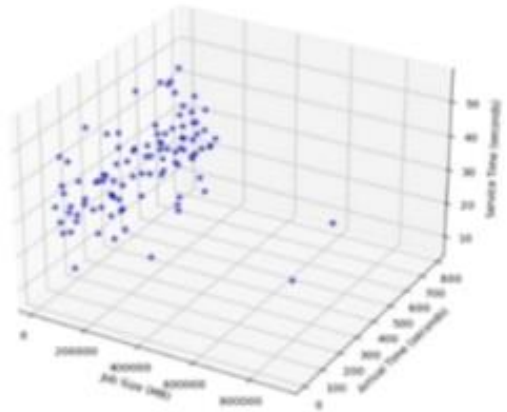
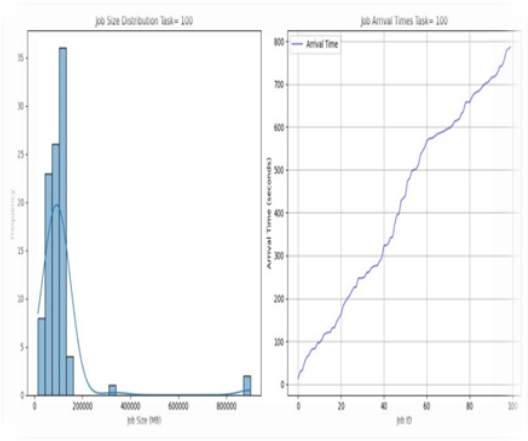


Figure 3: 3D scatter plot size, arrival times, and service times.

Figure 4 shows two plots: the left plot represents job size distribution in megabytes (MB) for 100 jobs, in which most of the jobs are distributed between 0 MB and 200,000 MB. The right plot shows the arrival times with uniform job arrival pattern and maximum arrival time of about 800 seconds. All these provide data for improving resource utilization and job scheduling in cloud computing.



(a) (b)

Figure 4: a) Left Plot: Job Size Distribution (Task = 100), b) Right Plot: Job Arrival Times (Task = 100).

5.2 Evaluation Performance

Cloud Resource Allocation is the accuracy, accurate, recall and F1 score important matrix for machine learning models. The accurate is the percentage of accurately estimated allocation between all estimated resources, while accuracy models assess purity. The Model Recall evaluates its ability to identify all necessary activities and resources. F1 score balances accuracy and the model remembers to assess performance. These provide a complete evaluation of the performance of cloud resource allocation of the Matrix model as in (1) to (4).

$$Accuracy = \frac{TP+NT}{TP+TN+FP+FN} \quad (1)$$

$$Percision = \frac{TP}{TP+FP} \quad (2)$$

$$Recall = \frac{TP}{TP+FN} \quad (3)$$

$$F1-score = \frac{2*Precision*Recall}{Precision+Recall} \quad (4)$$

5.3 Results

The Testing Phase compares the performance of various machine learning classifiers – Support Vector Machine (SVM), Decision Tree (DT), Logistic Regression (LR), and Gradient Boosting (GB) – for cloud resource optimization based on four performance measures: precision, recall, F1-score, and accuracy, as shown in the results.

Precision computes the proportion of correctly assigned resources to the overall predicted assignments. GB and DT achieved the highest precision, indicating their ability to reduce misallocations. On the other hand, SVM achieved lower precision, indicating a higher likelihood of incorrect predictions. Recall measures the ability of the model to correctly identify all necessary resource allocations. GB and DT both performed consistently well, assigning appropriate resources to high-priority tasks, while SVM performed worst in recall, i.e., it missed some significant resource allocations.

The F1-score, being the trade-off between recall and precision, confirms that DT and GB had excellent overall performance, making them reliable for cloud resource management. SVM had a poorer F1-score, signifying its failure to balance these metrics effectively. Finally, accuracy, being the model's general rightness in its predictions, shows that GB and DT performed better than other models (see Fig. 5), making them the best classifiers for cloud environments. SVM had the worst accuracy, implying inefficiency in handling dynamic workloads.

In the summary, although there is no discussion of model verification procedures in article, for example in the setting of KEFOLD cross-valuation, overfitting, the dataset is divided into numerous parts and the model is trained and valid on several datasets. This prevents overheating and helps the model to remain in capacity. Thus, on all matrix utilized to evaluate them, GB and DT were shown to be the best artists among models to allocate sheltering resources (see Fig. 6). For responsible and competent resource management in Cloud Computing, his capacity to balance precision, recall, F1 score and accuracy makes them the best choice see Table 1. Weak SVM indicates that it lacks experience in managing the load and arranging the sliding operation effectively.

Table 1: Performance metrics for the classification algorithms.

Methods	Accuracy %	F1-score	Recall	Precision
SVM	98.5	0.98	0.98	0.98
Decision Tee	100	1.00	1.00	1.00
Logistic Regression	97.6	0.98	0.98	0.98
Gradient Boosting	100	1.00	1.00	1.00
AdaBoost	72.2	0.72	0.72	0.76

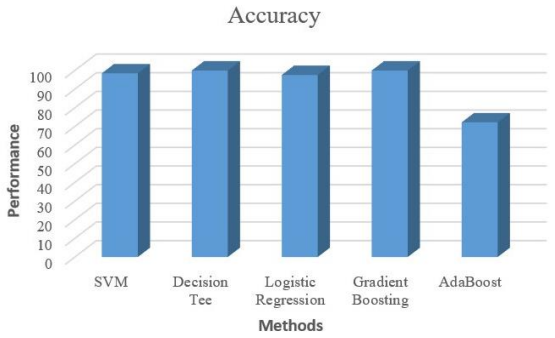


Figure 5: Classification algorithm performance accuracy metrics.

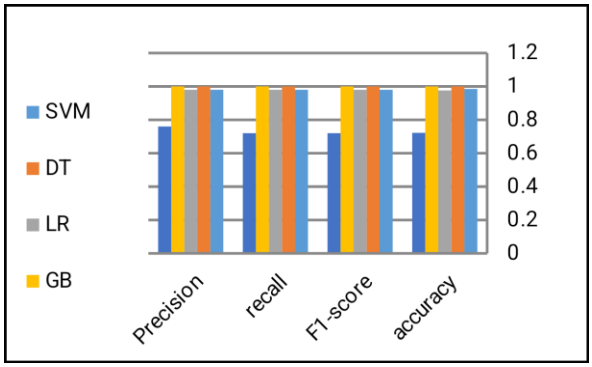


Figure 6: Classification algorithm performance metrics.

6 CONCLUSIONS

The research findings proved that machine learning models are suitable for the process of optimizing resource management in cloud computing. The results demonstrated that the Decision Tree and Gradient Boosting algorithms outperformed others, achieving 100% accuracy, precision, recall, and F1-score, indicating their exceptional reliability and suitability for real-time cloud environments. Support Vector Machine (SVM) followed closely with an accuracy of 98.5%, while Logistic Regression achieved 97.6%. However, AdaBoost lagged behind with a significantly lower accuracy of 72.2%, indicating its sensitivity to data imbalance and weaker generalization in dynamic settings.The top-performing models were decision-boosting and gradient-boosting models, while the remaining, such as Support Vector Machine and Logistic Regression, would perform well in other processes. The above findings prove that machine learning plays an important role in better resource management as it provides enhanced cost-cutting measures. Development of hybrid models that leverage the advantages offered by multiple approaches,

development of the scalability of enormous-scale cloud computing systems, and incorporation of real-time data to make dynamic resource allocation feasible are some future avenues for research that could be pursued. As long as we continue to develop energy-efficient models and embed such models into leading cloud platforms, applications of machine learning for cloud resource allocation will take us one step closer to reality.

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SECTION 2

APPLIED MATHEMATICAL SCIENCES AND COMPUTATIONAL METHODS

Proceedings of the 13th International Conference on Applied Innovations in IT (ICAIIT)

JUNE 2025

Comparison of Linear Trend Analysis and Double Exponential Smoothing Methods for Predicting Chronic Disease Drug Needs

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Keywords: Drugs, Trend Exponential Analysis, Double Exponential Smoothing, Forecasting, Future Needs, Prediction.

Abstract: One of the factors influencing the standard of care given to patients and society is the process of identifying medical needs and accurately quantifying them. Therefore, finding new methods or mechanisms to evaluate the need for drugs has become essential. Due to faulty prediction methods, the Ministry of Health in Iraq was unable to achieve a precise match between actual consumption and estimated demand due to the employment of old methods. Therefore, the research aims to 1) select a suitable method from the two forecasting techniques, trend exponential analysis (TEA) and double exponential smoothing (DES), and 2) predict the needs for drugs for the next five years based on historical data. Accuracy measures (MAPE, MAD, and MSD) have been utilized as model selection criteria to best characterize the trend of estimating the demands of chronic drugs from 2017 to 2019. The results demonstrate a decrease in MAPE values in all medications compared with MAD and MSD. In addition, the MAPE of a DES technique for (Lovastatin = 3, Insulin = 12, Valproic acid = 4.36, Carbidopa = 1.74) is greater than the MAPE of a TEA (Lovastatin = 2, Insulin = 11, Valproic acid = 3.92, Carbidopa = 1.72). Depending on the value of MAPE, TEA was recognized as the optimal prediction model and can best fit for predicting the demand for chronic drugs in future needs. Annual forecasts using a trend exponential analysis for the quantities required for each drug from 2020 to 2024 show a significant increase in demand for all pharmaceuticals. Whereas insulin tablets are the smallest, with 559,367 packs in 2024, valproic acid is the largest, with 13,387,773 packs, indicating a shortage of competing drugs or an anticipated increase in patient numbers. The proposed method might be useful for inventory prediction at industrial sites as well.

1 INTRODUCTION

The research is significant because it has a direct bearing on safeguarding social and human health. Since medicines are related to human life and not like other commodities, an accurate assessment of medical organizations' needs for medications will help ensure that patients are treated properly. On the other hand, an inaccurate assessment could result in either a shortage of medications, which could endanger patient lives, or significant costs for healthcare organizations due to an excess of medications and expiration dates [1]. In addition, the institution bears additional costs because of contracting with local distributors from specialty retailers, as well as the additional time and effort required to complete this task. The actual volume of the drug exceeds the volume of the expected demand; the health institution will bear another type of cost represented by the cost of the surplus stock, the expiration of the medicines and their spoilage, and thus the waste of public money [2]. The lack of scientific methods for assessing drug needs in health departments in general and in medicines for chronic diseases particularly is an urgent problem, as is the inability to achieve an accurate match between the volumes of actual demand and expected [3], [4]. Approximately 80% of hospital costs are related to medical supplies. These numbers support the notion that efficient hospital management depends on medical supply inventory management in healthcare facilities [5]. The World Health Organization (WHO) has emphasized since 1980 the value of employing suitable inventory management strategies to evaluate the demands for medical supplies, particularly medications, and to steer clear of crude, surface-level approaches based on perception, experience, and conjecture. Because they

rely on crude, rudimentary, and imprecise methodologies, these approaches are not scientific. For instance, the current year's drug needs are calculated using the estimate from the prior year + 10%, which produces unreliable findings. In addition, there are statistical methods for demand forecasting, such as time series and causal approaches [6].

The current research has focused on two statistical techniques (trend exponential analysis and double exponential smoothing) because of their simplicity and efficiency in tracking time series values over a specified number of periods of time. Additionally, they provide a comprehensive view of time-series data, improving the accuracy of forecasts.

2 RELATED WORK

Estimating medication demands remains a significant difficulty for hospitals and medical facilities, Modern techniques or mechanisms for evaluating the demand for medications in various health sectors are required since the process of accurately determining the need for medications is one of the most significant factors influencing the quality of services offered to patients. In order to improve demand estimation and reduce waste of public funds, this section will analyze the literature on the procedures for estimating the drug needs of hospitals and health centers.

In 2021, A Pamungkas et al. [7] compared exponential smoothing methods for Forecasting Marine Fish Production in Pekalongan Waters; the study used data from Pekalongan Fishing Port, Central Java, from January 2011 to December 2020 to predict marine fish production. The data has analyzed using data exponential smoothing methods, with the mean absolute percentage error (MAPE) value as a criterion. The Holt Winter Exponential Smoothing method has found to be reasonable with a MAPE value of 37.878, indicating its potential for enhancing regional income and community livelihoods in the marine fisheries sector. Whereas the Holt's-Winter exponential smoothing method's root mean square error (RMSE) for the model data is 675,073. When compared to the double exponential smoothing (Holt's linear trend) and Holt's-Winter exponential smoothing approaches, the MAPE and RMSE values are the minimum. Good fisheries management aims to offer data with reliable records since forecasting success has inextricably linked to the data that is available. The fishing community's and fishermen's support and knowledge of the importance of accurately reporting production data can boost forecasting accuracy, which in turn can help fisheries industrialization succeed.

In 2021, Ameera W. Omer et al. [8] compared Brown's and Holt's Double Exponential Smoothing (DES) models for forecasting generation electrical in the Kurdistan region. The data used from 2010 to 2020 showed trending modeling, indicating that both DES approaches can be used with Stratigraphic and Minitab software. The optimal value of α in DES Brown is 0.22, and the optimal MAPE is 9.23616%, while in DES Holt, it is 0.95 and the optimal β is 0.05. Both approaches are capable of predicting generation electrical. Depending on the optimization that has been done for α and β. The method used by DES Holt has a lower MAPE than that of DES Brown. Future studies will optimize either by altering the initialization procedure or by optimizing the parameters using different non-linear programming techniques.

In 2023, Ajiono T.H. [9] compared three forecasting methods: linear regression, exponential smoothing, and weighted moving average. The linear regression method had the smallest error value (MAD 27.83), MSE 1152.1, and MAPE 8.1%. The method predicted 502 students with a tracking signal value within the standard deviation distribution and moving range limits. The linear regression method is acceptable for future decision-making due to its accuracy and ability to produce trending data movement patterns over a long period. According to the findings of a comparison between linear regression and exponential smoothing methods with alpha 0.1 and 0.5. The tracking signal's movement between 1 and -1 falls inside the control range, demonstrates how time and actual variables are related in time series data. However, because the method's truth is debatable, the research has not employed the moving range. Comparing it to the exponential smoothing and time series moving average Holt-Winter forecasting techniques has advised for future study.

In 2024, Ansari Saleh Ahmara et al. [10] compare Single Exponential Smoothing (SES) and Double Exponential Smoothing (DES), the two primary forms of exponential smoothing techniques used in business forecasting. These two approaches had used in this study to analyze monthly passenger car registration data from 2019 to 2022 in Canada. Root Mean Square Error (RMSE) was the main metric used to assess each method's performance. In contrast to DES, which produced larger RMSE values (14.0769), Single Exponential Smoothing

(SES) demonstrated the best performance, with the lowest RMSE of 13.07859 for an alpha of 0.6. Since the Single Exponential Smoothing method has a lower Root Mean Square Error (RMSE) than the Double Exponential Smoothing method, it is the most effective method for forecasting the passenger-car registration data in Canada, according to the results of forecasting using both techniques.

3 MATERIALS AND METHODS

Data was gathered from one of Baghdad's authorized pharmacies, since it is one of the medical establishments that provides drugs for chronic illnesses for hospitals and health centres. Furthermore, these establishments lack quantitative tools for estimating demand and rely only on qualitative approaches, resulting in a mismatch between actual and expected demand. The study's sample of chronic disease pharmaceuticals was chosen because of the continuous requirement for significant amounts of these drugs, which necessitates precise annual demand forecasting. Furthermore, these medications are also considered life-saving, so their availability is a priority for the Ministry of Health. Data had been analysed in Jupyter Notebook and Minitab software. The models that were used to describe the behavior of variables that change over time are transformed into growth models. This study used the methods of trend analysis and double exponential smoothing.

Linear (trend) analysis. A linear trend equation is constructed from the data using standard least squares methods, in which the dependent variable is the time series [11], [12] and the independent variable is the row (sequence) number. The forecasting equation has the following form (1) [13]:

$$Ft = a + bt. \quad (1)$$

Where F_t = forecast for time period t, a = y-intercept, b = slope of the trend [14].

The slope shows how much is added (or subtracted if b is negative) from each period to the next. It places the largest weights in the estimates at the two ends of the row, while rows closer to the middle have little effect on the estimates [15].

Double exponential smoothing [16]. This method allows you to get short-term forecasts. Dynamic estimates were calculated for two components by level and trend. Prediction based on double exponential smoothing models a time series using a simple linear regression equation, where Y is the dependent variable, b_0 is the intersection point, and b_1 is the slope that slowly changes over time. The algebraic form of the linear exponential smoothing model, like the simple exponential smoothing model, can be expressed in various ways [17, 18]. The "standard" form of this model is usually expressed as follows: S' denotes a separately smoothed series obtained by applying simple exponential smoothing to series Y; that is, the value of S' in period, t is described by the (2) [13]:

$$S'(t) = \alpha Y(t) + (1 - \alpha)\, S'(t - 1). \quad (2)$$

With simple exponential smoothing, let S(t+1) = S'(t) at this point. Then let S" denote a doubly smoothed series obtained by applying simple exponential smoothing (using the same a) to the series S' [16]:

$$S''(t) = \alpha\, S'(t) + (1 - \alpha)\, S''(t - 1). \quad (3)$$

Finally, the forecast of Y'(t + 1) is given by the (4) [16]:

$$Y'(t + 1) = a(t) + b(t). \quad (4)$$

Where a(t) = 2S'(t) - S"(t) is the calculated level for the period t, b(t) = (a/(1-a)) (S'(t) - S"(t)) is the expected trend for the period t.

4 ANALYSIS OF THE ASSESSMENT OF MEDICATION NEEDS FOR THE TREATMENT OF CHRONIC DISEASES

Table 1 shows the indicators of the error in forecasting the demand for medicines in the sample under study. The table shows data on the actual demand and annual estimated (projected) volumes of medicines in accordance with the methods used in the Department of Medical Clinics of Iraq for 2017-2019. The table shows a large discrepancy between the projected volume of purchased medicines and the actual demand for them in 2017-2019. To determine the prediction errors for each drug and for each year, it is necessary to subtract the actual values from the predicted values. Negative values indicate an increase in the volume of expected demand compared to the volume of actual demand. However, if the value of the forecasting error turns out to be positive, it means that the actual demand is higher than the expected demand. This situation has not shown in the table, which indicates that there has been no shortage of medicines in these years. Table 1 illustrates the high percentage of absolute

error, which confirms the inaccuracy of forecasts and the inappropriateness of the method used to assess drug needs in healthcare departments, including departments of popular medical clinics. Expiration dates, as well as rental, transportation, and shipping costs, should be taken into account in the drug needs assessment process. With the help of a statistical program, the data was evaluated using different statistical models, and the best statistical model was selected, which describes these data using the average absolute error indicator. This indicator was chosen because for large time series [3], it better evaluates the ratio of predicted and actual values studied than the other two indicators, the average squared error and the average absolute deviation.

Table 1: Comparison of projected and actual drug volumes for the period from 2017 to 2019.

Drugs	Forecasting Needs (1)		
	2017	2018	2019
Lovastatin	2,500,000	16,000,000	2,133,650
Insulin	485,000	564,000	722,660
Valproic_acid	22,000,000	24,120,000	21,400,000
Carbidopa	7,000,000	9,500,000	8,500,000
Drugs	Actual Consumption (2)		
	2017	2018	2019
Lovastatin	657,300	657,300	657,300
Insulin	47,500	47,500	47,500
Valproic_acid	5,133,960	5,133,960	5,133,960
Carbidopa	1230,880	1230,880	1230,880
Drugs	Prediction Error (1-2)		
	2017	2018	2019
Lovastatin	-1,842,700	-686,136	-863,318
Insulin	-43,7500	-463,790	-526,860
Valproic_acid	-16,866,040	-17,266,200	-13,961,880
Carbidopa	-5,769,120	-5,518,940	-4,174,980
Drugs	Absolute Error (3)		
	2017	2018	2019
Lovastatin	1,842,700	686,136	863,318
Insulin	43,7500	463,790	526,860
Valproic_acid	16,866,040	17,266,200	13,961,880
Carbidopa	5,769,120	5,518,940	4,174,980
Drugs	Absolute Error Percentage (3/1)*100		
	2017	2018	2019
Lovastatin	73%	42%	40%
Insulin	90%	82%	72%
Valproic_acid	76%	71%	65%
Carbidopa	82%	58%	49%

Table 2: Selection of the best method for forecasting the need for chronic diseases medicines according to criterion of minimum accuracy.

Accuracy Measures of TEA	Trend Exponential Analysis (TEA)			
	Lovastatin	Insulin	Valproic acid	Carbidopa
MAPE	2	11	3.92	1.72
MAD	22201	9577	2.52	7.80
MSD	554489401	103181401	7.16	6.86
Accuracy Measures of DES	Double Exponential Smoothing (DES)			
	Lovastatin	Insulin	Valproicacid	Carbidopa
MAPE	3	12	4.36	1.74
MAD	24963	10768	2.84	8.78
MSD	702275406	130681957	9.07	8.68

Table 3: Brief description of the trend exponential analysis model.

Drugs	Description	Years		
		2017	2018	2019
Lovastatin	Actual	657300	913864	1270332
	Trend	640649	947165	1253681
	Detrend (error)	16650.7	33301.3	16650.7
Absolute Error Percentage		2.5%	3.5%	1.3%
Insulin	Actual	47,500	100,102	195,800
	Trend	40,317	114,467	188,617
	Detrend (error)	7182.7	14365.3	7182.7
Absolute Error Percentage		17.8%	12.5%	3.8%
Valproic acid	Actual	5133960	6853800	7438120
	Trend	5323213	6475293	7627373
	Detrend (error)	189253	378507	189253
Absolute Error Percentage		3.5 %	5.8%	2.4%
Carbidopa	Actual	1230880	3981060	4325020
	Trend	1631917	3178987	4726057
	Detrend (error)	401037	802073	401037
Absolute Error Percentage		24.5%	25.2%	8.4%

5 ACCURACY INDICATORS

The purpose of using two forecasting methods was actually to compare the estimates obtained and decide which forecasting method provides the best forecast results based on three accuracy indicators. These accuracy measures are the mean absolute percentage error (MAPE), mean absolute deviation (MAD), and standard deviation (MSD). The most suitable model will be the model with minimal accuracy indicators (MAPE, MAD, and MSD) [18, 19], which will be selected and used to assess the needs for medicines for chronic diseases for the next five years (2020-2024).

The average absolute percentage error (MAPE) expresses the prediction accuracy of the model as a percentage [20, 21]. Since MAPE's results are simple to understand, it is frequently employed. For example, a MAPE value of 14% means that the average difference between the predicted and actual value is 14%. MAPE can be calculated using the following (5) [16, 18]:

$$MAPE = [\sum | (yt - yt\hat{}) | /n] * 100, (yt \neq 0). \quad (5)$$

Where y_t - is the actual value, $y_t\hat{}$- is the predicted value, and n - is the number of observations.

Mean absolute deviation (MAD) – shows by what number of units, (for example, the number of packages of medicines, units), the forecast deviated on average up or down. MAD can be calculated using the following (6) [18]:

$$MAD = \sum | (yt - yt\hat{}) | /n. \quad (6)$$

Where y_t - is the actual value, - $y_t\hat{}$- is the predicted value, and n is the number of observations.

Moving Standard deviation (MSD) – allows you to amplify the most significant errors. Characterizes the variability of deviations during the period under review. MSD can be calculated using the following (7) [18]:

$$MSD = \sum | (yt - yt\hat{}) |2 /n, \quad (7)$$

where y_t - is the actual value; $y_t\hat{}$ - is the predicted value, n - is the number of observations.

6 RESULTS AND DISCUSSIONS

In this study, the methods of trend analysis and double exponential smoothing had compared according to the criterion of minimum accuracy (Table 2).

The data shown in Table 2 showed that when comparing the accuracy indicators of these two models, both MAD and MSD are quite large for both models. At the same time, the trend exponential model has the lowest MAPE values compared to other models; therefore, this method provides a better match to the data and is suitable for predicting Iraq's future needs for chronic. Trend analysis' superiority over double exponential smoothing due to its simplicity and accuracy, its ability to produce trending data movement patterns over a long period, and its efficiency in handling sudden changes and unclear seasonal patterns. It builds a structural model based on historical patterns, making it more stable for long-term forecasts. In this regard, at the end of the article, a five-year forecast of drug needs for chronic diseases has calculated using an exponential trend model.

Table 4: Comparison of percentage values of the total absolute error.

Drugs	The percentage of absolute error (2017)		The percentage of absolute error (2018)		The percentage of absolute error (2019)	
	Traditional method	Quantitative method	Traditional method	Quantitative method	Traditional method	Quantitative method
Lovastatin	73%	2.5%	42%	3.5 %	40%	1.3 %
Insulin	90%	17.8 %	82%	12.5 %	72%	3.8 %
Valproic acid	76%	3.5%	71%	5.8 %	65%	2.4 %
Carbidopa	82%	24.5 %	58%	25.2 %	49%	8.4 %

Table 5: Forecasts of chronic disease medication volumes for the period from 2020 to 2024.

Drugs	Forecast Years				
	2020	2021	2022	2023	2024
Lovastatin	1560197	1866713	2173229	2479745	2786261
Insulin	262767	336917	411067	485217	559367
Valproic_acid	8779453	9931533	11083613	12235693	13387773
Carbidopa	6273127	7820197	9367267	10914337	12461407

Table 3 shows a decrease in the error of forecasting the demand for medicines compared to

the results of Table 1. The actual demand data and annual estimated volumes were obtained using an exponential trend model for forecasting. We also noted that Carbidopa's prediction errors are significantly higher than other drugs due to some stochastic factors such as Parkinson's patient mortality rates and treatment doses, affecting need estimation because disease stages are not taken into account when estimating the required quantities, leading to higher error rates since patients in advanced stages need higher treatment doses compared to early stages.

From Table 1 it can be seen that the largest prediction error occurred for insulin in 2017 and amounted to 90% due to the presence of accumulated insulin residues in popular clinics and department stores over the previous year. The lowest error rate was 40% for Lovastatin in 2019, which means an increase in the withdrawal of this drug and the expiration of the remainder over the previous year. In Table 3, there is a slight deviation in the actual and projected values for drug consumption over the analyzed time period.

Table 4 compares the percentages of forecasting errors for a sample of studies in accordance with the methods used in popular medical clinics and quantitative methods suitable for forecasting over the specified years. There is a decrease in the percentage of absolute error when using quantitative models. Thus, it can be said that quantitative forecasting models are the most suitable for forecasting demand and reducing errors in the forecasting process. The use of these models for assessing drug needs leads to improved forecasting accuracy and appropriate and acceptable results. Then, using linear exponential analysis, the required volumes of each drug were predicted for the period from 2020 to 2024 (Table 5). The table shows that insulin tablets account for the smallest number in it. In the year 2024, its number reached 559,367 packages, and it is one of the traditional medicines for the treatment of high blood sugar levels. The highest estimated number in the table is indicated for valproic acid, which amounted to 13387773 packages in 2024. Demand for this drug will increase compared to 2020. For 4,608,320 packages, which may be due to the lack of competing drugs in this area and, possibly, to the expected increase in the number of patients, since this drug is used to treat epilepsy. Annual forecasts show a marked increase in demand for all medicinal substances. For example, a marked increase in demand for insulin may indicate an increase in the number of diabetics, especially those suffering from this disease, at the local or global level, since, according to estimates by the World Health Organization, there are about 200 million people with diabetes worldwide. It is also seen that the demand for Lovastatin has increased over the past five years, indicating an increase in the proportion of patients with elevated cholesterol levels. Annual forecasts also show a marked increase in demand for Carbidopa and Valproic Acid, which indicates the absence of competing drugs in this group of drugs. From the above, we find that the future assessment of drug needs may influenced by random factors, such as the appearance of a new competing drug or a recommendation, or the detection of side effects on medicines, rising drug prices, the spread of diseases and epidemics, and other external factors such as natural disasters. All this affects consumer demand and then the assessment of the quantitative and qualitative needs of the population. Therefore, a combination of quantitative and qualitative forecasting methods should be used to ensure results that are more accurate.

7 CONCLUSIONS

Accurate forecasting of medicines in hospitals and health centres, leads to improved patient care and inventory management, thereby conserving public funds. The current study has accomplished the following:

1) In this paper, two types of time series methods, namely linear regression (trend) analysis and double exponential smoothing, are compared to select the suitable method to predict chronic medication needs in a hospitals. The two methods were compared using three accuracy criteria (MAPE, MAD, and MSD) to evaluate the accuracy of the predictions we achieved;
2) Predicting the needs for drugs for the next five years based on the current dataset.

The results have showed the following:

- Linear regression analysis is more suitable for predicting drug needs, because it has the lowest error rate compared to the double exponential smoothing method. Therefore, it was used to assess the future needs for chronic medications;
- The decrease in the values of error measures in the linear regression model indicates the suitability of its characteristics with the characteristics of demand behavior, so it can be said that a linear regression analysis is suitable quantitative model for all drugs;

- The decrease in the percentage of error in time series models compared to the percentage of error caused by the use of traditional methods in the health services is an indicator of the suitability of time series models for the purposes of forecasting demand and estimating needs for medicines more accurately.

Therefore, the study recommends the need to use a combination of quantitative and qualitative forecasting methods, and to present the results of forecasting using time series to a committee of experts or officials in pharmacies and stores, taking into account random factors that may arise in the environment of healthcare institutions and that may affect the assessment of drug needs.

The proposed approach to forecasting is characterized by relative simplicity and intuitive clarity of researchers. This approach can be successfully applied not only in the field of pharmacology but also in industry, for example, when forecasting the acquisition of spare parts volumes, as well as to solve any other tasks in which forecasting methods are used. The study faced some limitations related to the short duration of historical data due to the unavailability of accurate data for some drugs, as well as missing data for some previous years. Due to the COVID-19 pandemic, drug data has not been considered for years after 2019, as the old data does not match the new reality, which makes it difficult to model and predict these phenomena.

8 FUTURE WORK

This research and the results obtained may help open the way for other researchers to continue in this field, complete existing work, or expand upon it. Prospects for this research include the following:

- Modifying the proposed method to predict the value of two or more future points in the times series, rather than predicting the value of a single future point in the time series.
- Applying the proposed system to other time series fields, such as weather, company sales, and other application.
- Developing a time series forecasting system using machine learning.
- Testing the robustness of predictions over longer periods or additional chronic diseases.

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Predicting Hospital Medicine Needs Based on a Multiple Linear Regression Model

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Keywords: Correlation and Regression Analysis, Forecast, Future Needs, Multifactorial Model, Retrospective Analysis, Time Series Analysis.

Abstract: The study's issue is that hospitals are unable to accurately match the predicted demand and actual consumption for medications due to the use of traditional and ineffective forecasting techniques. The study's objective: 1) Finding a systematic approach based on modern scientific forecasting methods, based on multi-factor mathematical models. 2) Selecting the optimal forecasting model among the methods utilized in this study to predict the future needs of hospitals for medicines in the coming years. 3) Calculating the discrepancy rate for each drug annually to check the forecasting accuracy. Three statistical analyses were performed: correlation and regression, time series, and retrospective forecast between estimated demand and actual consumption. Three accuracy indicators (MAPE, MAD, and MSD) were used as criteria for selecting the optimal model that best describes the pattern of future demands for six pharmaceuticals and five factors influencing drugs' consumption from 2015 to 2019. Three time series forecasting techniques (ALT, SES, and DES) were tested and compared with the MLR model to verify its forecasting accuracy. Time series techniques were compared to each other; DES was selected as the optimal technique among them. The results showed that the mean MAPE for all medications by using MLR and DES models was 15.63 and 24.61, respectively. Therefore, we conclude that MLR is the optimal model for hospital inventory management and forecasting future needs since it has a lower relative error rate compared to DES. This indicates that the influence of independent factors on demand is stronger than the time factor. Therefore, MLR outperformed DES, which relies on the time factor. With the exception of Infliximab 100mg and Tocilizumab 20mg/ml, whose values exceed 25%, the average discrepancy rates between the estimated demand and actual consumption of each medication over a 5-year period are statistically significant and within the acceptable bounds.

1 INTRODUCTION

Hospital inventory management and medication needs assessment are still challenging problems that have an impact on the standard of care given to patients and society [1]. It is assumed that medical facilities must cope with a limited budget and, at the same time, meet the patients' requirements. Optimizing administrative procedures for planning and organizing medical and medication treatment can help solve some issues, particularly those pertaining to predicting health care demands, which is the primary objective of health development in all nations. To guarantee that patients receive the finest medical treatment possible, it is important to increase the precision of the drug needs assessment utilizing contemporary forecasting techniques [2]. The development of a set of mathematical methods for predicting the need for medicines and comparing actual consumption with estimated demand is one of the main ways to rationalize the use of financial resources and protect medicines from spoilage due to poor storage or expiration. Accurate demand forecasting reduces drug overstocking due to expired medications and sudden shortages, preventing hospitals from resorting to purchasing at high prices on the black market. Accurate demand forecasting may also improve medical quality and safety, improving patient outcomes and reducing Mortality Rate, and reduce medication errors [3]. When inventory is organized perfectly, the likelihood of inappropriate substitute medications being dispensed

due to shortages is reduced. In the case of healthcare, it is also critical to emphasize that the forecasting process, which takes into account both the creation of budgets and the usage of medications, is predicated on the assessment of historical consumption, a method that compromises the complexity of the industry. In addition, the market share of competing products may be affected by external factors such as expiration, seasonality, new surgical procedures, epidemics, or the introduction of innovative drugs, which significantly impact drug demand forecasts by adjusting historical consumption [4]. Therefore, this study aims to select optimal models for predicting hospital drug needs and identify the main factors influencing the consumption of these drugs, depending on the degree of correlation analysis between drugs and factors on the one hand and the degree of correlation analysis between the factors themselves on the other [5], [6]. To achieve this goal, we needed to complete the following tasks:

1) Using correlation analysis techniques to identify the key variables (factors) influencing the needs for medicines in hospitals and medical facilities.
2) Establishing a multi-factor linear regression (MLR) model and create a predicting equation for each drug separately based on the factors that selected from correlation analysis and compared the forecasting of those equations with three methods of time series forecasting to verify the accuracy of the our model.
3) Calculating the average discrepancy percentages between actual consumption and estimated demand to verify the accuracy of the forecast and select the optimal predictive.

In healthcare data analysis, time series models and multiple linear regression are used to forecast future drug demand, but each has distinct advantages and applications. Time series models such as ARIMA or exponential smoothing are appropriate when data are based on historical values and seasonal trends, making them ideal for forecasting drug demand based on historical consumption patterns [7]. Multiple linear regression is used when there is a clear relationship between drug demand and multiple independent variables such as patient population, age, season, or economic factors. These models were chosen because they offer high analytical accuracy; time series captures temporal and cyclical changes, while linear regression quantifies the influence of external factors on demand [8], [9]. Combining them can enhance the accuracy of forecasts, especially in healthcare systems where drug demand is influenced by both temporal and demographic factors. Therefore, the current study focused on using the multiple linear regression model as a basis for forecasting future drug demand. This model was chosen for several reasons related to the nature of the data, ease of interpretation, and the model's efficiency. Linear regression is considered an ideal choice because it provides accurate and easy-to-understand results due to the linear relationship between the independent variables and the antecedent variable. Linear regression also provides directly interpretable coefficients, allowing for a clear understanding of the effect of each independent variable on drug demand (for example, a 10% increase in the number of patients increases drug demand by X%). Its simplicity and speed of implementation compared to more complex models such as neural networks or random forests are some of the reasons for choosing it for forecasting future demand. Furthermore, it is easy to integrate with time series analysis methods, as the data in the study sample is linked over the time factor, which facilitates the use of multiple linear regression for forecasting based on historical trends.

2 RELATED WORKS

The health sector is one of the most important service sectors for the individual and society, as it is closely related to human life. The process of correctly assessing the need for medicines is one of the most important elements affecting the quality of services provided to patients, so it is necessary to find modern methods or mechanisms for assessing the need for medicines in different health sectors [7]. Health centres around the world face many difficulties and challenges because of the mismatch between actual consumption and expected demand resulting from the use of primitive traditional methods. This section will review the literature related to the processes of estimating the drug needs of health centres and hospitals in order to improve demand estimation and minimize waste of public money.

In 2022, Mbonyinshuti F. et al. [10] the researchers employed a number of data preparation techniques to choose the top 17 critical medications for non-communicable diseases (NCDs), A Random Forest (RF) model was used to anticipate demand using vital medicine consumption data from 2015 to 2019 for roughly 500 medical goods as part of the machine learning applications study. The Random Forest model forecasted the demand trend for 17 key NCD medications with an accuracy rate of 78% for the training set and 71% for the test set. Entering the month, year, region, and name of the necessary NCD

medication allowed for this to be accomplished. The Random Forest model can be used to predict demand trends based on past consumption data. According to the study's findings, RF can improve operational management and health supply chain planning by increasing the precision of forecasting the demand trend for critical NCD medications.

In 2022, Mamoon Rashid et al. [11] suggested a medication prediction model that would help people take the right drug to treat a specific illness and act as an educational aid. The authors suggested storing medical datasets pertaining to medications in a Hadoop distributed file system. The suggested model will assist in examining the patient's medical history in order to identify any potential drug adverse effects. In order to make it easier for patients to locate local pharmacies that would have the necessary medication, the idea will also integrate Google Maps and weather.

In 2023, Nabizadeh A. H. et al. [12] showed that predicting the number and types of medicines consumed by each patient is done using different training groups. Proper timing of forecasting makes the predictions accurate, as the hospital management will have enough time to provide the required quantity of the most commonly used drugs. The performance of the algorithms was evaluated using a confusion matrix. The results showed that random forest had promising results in predicting the number of most frequently used drugs per month using two years of data (83.3% accuracy), while its accuracy in predicting drugs was 35.9%. After comparing the researchers' results with some previous studies, they found that they are consistent with those reported in (Mbonyin-Shuti et al., 2022). The authors of this study predicted the need for NCD medications using an RF model. According to their results, the RF model was able to predict medication usage one month in advance.

In 2025, Machado, D.M. et al. [13] researcher employ two methods: one that predictions for specific hospitals and another that integrates data from several hospitals in order to maximize resource allocation and logistics. This work explores machine learning for predicting hospital medication consumption. Based on consumption trends, researchers investigated manual pair clustering as well as K-means clustering. Manual clustering revealed particular combinations of medications with noticeably improved forecast accuracy, however K-means clustering produced no gains (e.g., Medicine 15 at Hospital 1: MAPE fell from 19.70% to 3.30%). However, not all hospitals consistently benefited from the unified approach (e.g., Medicine 9). This emphasizes the necessity of weighing possible losses in certain hospitals against accuracy gains in others. In general, the split approach's manual clustering shows promise if applys on larger datasets.

3 METHODOLOGY

The methodological basis of this study was based on a mathematical and statistical analysis of the field of pharmacy regulation and the work of domestic and foreign scientists in the field of forecasting drug provision in hospitals. The study was conducted using a dataset from the Institute of Fundamental Medicine and Biology in Kazan (Volga Region) Federal University (KFU), which consists of six types of drugs and five factors for the period (2015-2019). The data was analysed using Jupyter Notebook. No pre-processing steps were applied to address missing or inconsistent data by the researchers. Since the Institute of Fundamental Medicine and Biology of Kazan University prepares accurate data for scientific research purposes to support postgraduate students and data mining researchers. We relied on the available data without focusing on specific drugs or factors, as the main purpose of our study is to find an optimal prediction model that can be applied to any type of data in order to reduce the discrepancy between the actual consumption of materials (drugs in our case) and the estimated demand.

Within the framework of the current research program, three main analyses were used, namely:

1) the method of correlation and regression analysis;
2) time series forecasting analysis;
3) the method of retrospective analysis.

Multidimensional modeling is mainly based on correlation and regression analysis, which identifies patterns of demand for drugs and the influence of factors on the consumption of those drugs. Therefore, it is necessary to:

1) identify and analyze which factors have the greatest influence on the dependent variable (medicine in our study);
2) assess the degree of influence of the factors on the dependent variable;
3) determine the form of the relationship between the factors;
4) construct a forecasting equation for each medicine separately.

To determine the degree of influence of various factors on the need for medicines, the degree of correlation between factors and medicines, on the one

hand, and the factors themselves, on the other hand. The Pearson correlation coefficient (r) was used, which is the most common way to measure linear correlation [14]. It is a number from -1 to 1 that measures the strength and direction of the relationship between two variables. Below is the formula for calculating the correlation intensity, which is also called the correlation coefficient r*XY*:

$$rXY = cov(X, Y)/ \sigma X \sigma Y, \; rXY \in [-1;1]. \quad (1)$$

Where, $cov_{(X,Y)}$: is the covariance between the two variables; σ_X: is the standard deviation of X; σ_Y- is the standard deviation of Y. Strong correlations between dependent variables (factors) and the independent variable (estimated) are necessary for methods of causal forecasting of demand. The relationship between the dependent variables should be known in order to exclude redundancy, which may affect the quality of the forecast [15]. The purpose of this analysis is to determine whether there is a relationship between the amount of drugs consumed and the selected factors using a correlation test. We assume that a relationship is significant if the significance level, or P-value, is less than 0.05.

Based on the values of the coefficients obtained, regression equations were constructed for each drug individually, which have the following general form:

$$Y = a + b1\ X1 + b2\ X2 + \ldots + bn\ Xn + e. \quad (2)$$

Where, Y: is the output or target vector $b_1 + b_2 + \ldots + b_n$ – are the regression coefficients, $X_1 + X_2 + \ldots + X_n$ – are independent variables or input variables, e: an error term.

Methods of forecasting time series. To obtain a preliminary assessment of the nature of drug consumption, a time-series methodology was used, which included the introduction of only one variable, mathematically expressed as y= f(t). Three time series forecasting methods were selected: linear trend analysis (LTA), simple exponential smoothing (SES), and double exponential smoothing (DES) [16], [17], and to obtain the most appropriate forecasting method among them, they were compared depending on the values of three error indicators (MAPE, MAD, and MSE). The method with the lowest absolute relative error is the most suitable way to more accurately predict future values compared to other methods [18]. The methods and indicators were calculated using the following models:

1) Linear trend analysis (LTA). This method is the simplest form of trend analysis, which assumes that the data follows a straight line with a constant slope and intersection [6]. The prediction equation of this method has the following form (3):

$$Ft = a + bt. \quad (3)$$

Where, Ft: is the predicted time period t, a: y- is the figurative interception, bt: is the slope of the trend. The slope (bt) indicates the amount of subtraction if the value (b) is negative or the amount of addition if the value (b) is positive, from one period to another. This method is suitable for time series in which the trend of the data demonstrates long-term stability.

2) Single exponential smoothing (SES). The method of simple exponential smoothing is the basic form of exponential smoothing [19], [20]. The future values of the time series are a weighted average of the previous values, and the weights decrease significantly as the distance from the present increases. There is only one parameter in the model, α, which controls the rate of weight loss. The lower the alpha value, the more weight is given to the previous values, and the forecast becomes smoother [20], [21]. The simplest form of the exponential smoothing is given by the following (4) [20]:

$$St = St\text{-}1 + \alpha\ (Xt - St\text{-}1). \quad (4)$$

Where, St: is a simple weighted average of the current observation. Xt, St-1: is the previous smoothed statistics, α: is the smoothing coefficient of the data; $0 < \alpha < 1$, t: is a period of time. The choice of the smoothing constant value is important for determining the performance characteristics of exponential smoothing. The lower the (α) value (i.e., close to 0), the slower the response. Large values of α (i.e., close to 1) cause the smoothed value to react quickly not only to real changes but also to random fluctuations [22].

3) Double exponential smoothing: holt's linear exponential smoothing is an extension that includes a directional component. It is assumed that the future values of the time series are a weighted average of the previous values and a linear trend. The model has two parameters, alpha and beta, which control the rate of weight loss and the rate of direction change, respectively [22], the standard form of this model:

$$S'(t) = \alpha Y(t) + (1 - \alpha)\ S'(t - 1). \quad (5)$$

Under simple exponential smoothing, let S(t+1) = S'(t) at this point. Then let S'' denote the doubly smoothed series obtained by

applying simple exponential smoothing (using the same a) to series S':

$$S''(t) = \alpha S'(t) + (1 - \alpha) S''(t - 1). \quad (6)$$

4) Finally, the forecast Y'(t + 1) is given by:

$$Y'(t + 1) = a(t) + b(t). \quad (7)$$

Where a(t) = 2S'(t) - S''(t) is the calculated level for the period t, b(t) = (a/(1-a)) (S'(t) - S''(t)) is the expected trend for the period t.

4 ANALYSIS OF THE ASSESSMENT OF THE DRUG NEEDS BASED ON STATISTICAL ANALYSES

4.1 Analysis of Correlation and Regression Between Drug Consumption and Factors Affecting It

To build models of drug consumption, we used a mathematical modelling method using multiple linear regression analysis. This method allows us to take into account the degree of influence of each factor on the consumption of each drug. The needs for the analysed drugs were encrypted and designated as "Y," whereas the factors influencing the drug consumption were encoded as "X.". The result of correlation and regression analysis showed that the correlation coefficients (r) between drug consumption and factors ranged from 0.014 to 0.968. These values indicate the presence of both a weak and strong relationship between drug consumption (Y) and factors (X). Correlation coefficients were selected, reflecting a strong correlation between drug consumption and factors affecting their consumption ($|r| > 0.7$). A direct correlation was established between the total numbers of disabled people among RA patients. (X5) and the need for 5 selected drugs: Rituximab 500 mg/ 50 ml (Y1), Infliximab 100 mg (Y2), Certolizumab 200 mg/ml (Y3), Tocilizumab 20 mg/ml (Y5), and Abatacept 125 mg (Y6), with correlation coefficients (r) of 0.949, 0.761, 0.954, 0.961, and 0.929, respectively. The volume of purchases of genetically engineered biological drugs (GIBP) for the treatment of prostate-specific antigen (PsA) (X3) has a weak relationship with the need for (Y2) (r = 0.130). There is a direct correlation between the amount of funding for the compulsory medical insurance program (CMI) for the so-called clinical and statistical groups (CSG) (X1) and the financial support of the program of provision of necessary medicines (ONLS) (X2) with the need for (Y6) (r = 0.879; r = 0.912), respectively. Factor (X2) also has a significant correlation with the need for Golimumab 50 mg/0.5 ml (Y4) (r = 0.890) and an average correlation with the need for (Y2) (r = 0.523). A weak negative correlation was found between the need for (Y2) and the price level for genetically engineered biological drugs (GIBP) (X4) (r = -0.014). Drug demand models were created based on coefficients obtained from correlation and regression analysis. The regression equations are presented in Table 1.

4.2 Accuracy Indicators

The main purpose of using more than one forecasting method was to compare the obtained estimates and determine which method provides the best prediction results based on three accuracy indicators: Mean Absolute Percentage Error (MAPE), Mean Absolute Deviation (MAD), and Mean Squared Deviation (MSD). The most suitable model is the one with the minimum accuracy indicators (MAPE, MAD, and MSD), which will select and use to estimate the needs of medicines in hospitals for the coming years [20].

Table 1: Multiple linear regression models for predicting drug demand and assessment their reliability.

Drugs	Model	Fisher (F)	
		Table_val of F_coeff	empirical_value
Rituximab 500mg/ 50ml	Y1 = 0.367* X5 + 13.643	6.39	1.11
Infliximab 100mg	Y2 = 0.003 *X2 + 0.002 *X4 + 0.159 *X5 - 97.211	6.39	1.00
Certolizumab 200mg/ml	Y3 = 0.513 * X5 - 51.110	6.39	1.10
Golimumab 50mg/0.5ml	Y4 = 0.003 * X2 + 0.0002 * X3 - 30.420	6.39	1.00
Tocilizumab 20mg/ml	Y5 = 0.606 * X5 -50.900	6.39	1.08
Abatacept 125mg	Y6 = 0.002* X1+ 0.270 * X5 - 81.469	6.39	1.00

Mean Absolute Percentage Error (MAPE). This indicator calculates the average ratio of the absolute error to the actual values. The method of its calculation is shown in (8):

$$MAPE = [\sum | (yt - yt\hat{}) | /n] * 100, (yt \neq 0). \quad (8)$$

Where y_t - is the actual value, $y_t\hat{}$- is the predicted value, and n - is the number of observations.

Mean Absolute Deviation (MAD). This indicator measures the accuracy of fitted time series values. It expresses accuracy in the same as the data. This helps to theorize the error also [21]:

$$MAD = \sum| (yt - yt\hat{})| /n. \quad (9)$$

Where y_t - is the actual value, - $y_t\hat{}$- is the predicted value, and n is the number of observations.

Moving Standard deviation (MSD). This indicator has calculated using the same denominator, n, regardless of the model [23]. This is to enable comparison of MSD values across models. This makes MSD a more sensitive of a usually largest forecast error than MAD:

$$MSD = \sum|(yt - yt\hat{})|2 /n. \quad (10)$$

Where y_t - is the actual value; $y_t\hat{}$ - is the predicted value, n - is the number of observations [20].

4.3 Evaluation of Forecasting Using Time Series Methods and Multiple Linear Regression Model

To determine the best model for forecasting drug needs, we compared the MLR model with three time series methods: (1) linear trend analysis (LTA), (2) single exponential smoothing (SES), and (3) double exponential smoothing (DES). First, we compared the forecasts of the three time series approaches with each other. Based on the three accuracy measures (MAPE, MAD, and MSD), we selected the method that best fit the data, which turned out to be DES as shown in Table 2. Next, we compared MLR model with DES as shown in Table 3.

Based on the data in Table 2, MAD was selected as the best indicator that describes those data. This indicator was chosen because it has the lowest error values compared with other two indicators.

When comparing the accuracy of the two models based on the data in Table 3, the MAPE model was chosen as the best indicator to describe the data. This indicator was chosen because it had the lowest error values compared to the other two. Based on the MAPE indicator values, the mean MAPE was calculated for all medications by using the MLR model and was 15.63 and 24.61 for DES. Therefore, MLR was selected as the best model for predicting future needs for all medications.

4.4 Retrospective Forecast of the Hospitals Future Drug Needs

The demand predictions produced by the two models MLR and DES were contrasted with actual consumption based on the findings of the two analyses mentioned above for the period 2015–2019. To verify the accuracy of the forecast, the discrepancy rate for each drug was calculated annually.

Table 2: Selecting the optimal time series method for forecasting drug needs according to the criterion of minimum accuracy of indicators.

Linear Trend Analysis (LTA)						
Measuring Accuracy	Y1	Y2	Y3	Y4	Y5	Y6
MAPE	10.9	26.3	47.1	34.4	51.7	8.4
MAD	64.3	18.6	17.2	9.9	33.4	36.5
MSD	88.1	32.9	184.2	26.4	160.6	6.8
Single Exponential Smoothing (SES)						
Measuring Accuracy	Y1	Y2	Y3	Y4	Y5	Y6
MAPE	146.1	146.9	161.2	172.1	131.3	122.8
MAD	24.1	6.8	11.3	7.6	9.2	7.5
MSD	793.3	74.7	244.2	73.9	186.4	80.6
Double Exponential Smoothing (DES)						
Measuring Accuracy	Y1	Y2	Y3	Y4	Y5	Y6
MAPE	15.3	25.4	38.1	16.3	39.7	12.9
MAD	9.95	3.6	20.7	2.0	16.7	3.8
MSD	199.9	6.5	1554.0	32.0	1355.5	223.8

Table 3: Comparison of MLR model with DES method according to the criterion of minimum accuracy of indicators.

Multiple Linear regression (MLR)						
Measuring Accuracy	Y1	Y2	Y3	Y4	Y5	Y6
MAPE	7.2	1.0	16.1	7.7	59.2	2.5
MAD	61.1	18.0	12.7	8.4	28.1	36.3
MSD	26.9	0.03	62.7	1.4	54.0	1.0
Double Exponential Smoothing (DES)						
Measuring Accuracy	Y1	Y2	Y3	Y4	Y5	Y6
MAPE	15.3	25.4	38.1	16.3	39.7	12.9
MAD	9.9	3.6	20.7	2.0	16.7	3.8
MSD	199.9	6.5	1554.0	32.0	1355.5	223.6

Table 4: Analysis of the forecast demand of drug calculated using (MLR and DES) models, in comparison with actual consumption for the period (2015-2019).

Y6	Y5	Y4	Y3	Y2	Y1	Drugs	
13	5	0	0	14	49	2015	Actual consumption
21	8	0	0	11	49	2016	
36	32	8	13	22	63	2017	
54	74	15	59	31	92	2018	
61	55	36	35	18	75	2019	
12.3	15.2	/	/	13.7	53.7	2015	Demand forecast calculated using MLR model
5.4%	204%			2.1%	9.6%	% discrepancies	
21.4	4.3	/	/	11.3	47.1	2016	
2.9%	46.3%			2.7%	3.9%	% discrepancies	
36.5	24.3	8.8	12.5	21.9	59.2	2017	
1.4%	24.06%	10%	3.9%	0.5%	6.03%	% discrepancies	
55.8	67.9	13.2	49.4	31.1	85.7	2018	
3.3%	8.2%	12%	16.3%	0.3%	6.9%	% discrepancies	
58.9	62.4	36.5	44.8	17.9	82.3	2019	
3.4%	13.5%	1.4%	28.0%	0.6%	9.7%	% discrepancies	
14	6	/	/	15	50	2015	Demand forecast calculated using DES
7.7%	20.0%			7.1%	2.0%	% discrepancies	
17	7.1	/	/	18.1	65.1	2016	
19.1%	11.3%			64.6%	32.9%	% discrepancies	
29	9.5	9	14	8.8	66.4	2017	
19.4%	70.3%	12.5%	7.7%	60.0%	5.4%	% discrepancies	
51	31.5	12	18.6	30.9	72.7	2018	
5.6%	57.4%	20.0%	68.5%	0.3%	21.0%	% discrepancies	
72	80	15	39.7	40.7	97.9	2019	
18.0%	45.5%	58.3%	13.4%	126.0%	30.5%	% discrepancies	
8.6	50.0	19.0	23.0	26.4	12.8	The average degree of discrepancy	

In addition, the average degree of discrepancy between actual consumption and estimated forecasts was calculated over the five years, as shown in Table 4. Discrepancy rates have been shown to be statistically significant for all drugs, with the exception of Y2 and Y5, where they actually exceed 25% [24].

5 RESULTS AND DISCUSSIONS

In this paper, we discussed the research methodology for multidimensional forecasting of hospital drug needs, which consists of five main steps related to each other.

In the first stage, a correlation analysis was carried out on a dataset to measure the power of the correlation between the medications and the relevant factors influencing the consumption of these medications. The results of the analysis showed a correlation, often strong (up to more than 70%) and statistically significant, between the amount of the drug consumed and some of the factors selected for the study.

In the second stage, a MLR model has been built to find a prediction equation for each drug separately. According to the forecast's equations derived using MLR model and based on the findings of the correlation and regression analysis (Table 1), it turned out that the percentages of discrepancy between demand forecasts and actual consumption are acceptable. Thus, it can be said that MLR model for predicting demand for medicines are very effective.

At the third stage, three prediction models using time series techniques have been built and compared based on three error indicators (MAPE, MAD, and MSD) to find the most appropriate model for predicting future medication needs. Based on the results of the accuracy indicators analysis, DES method has selected as the best model to describe drugs in our dataset because it has the lowest relative error rate compared to the other time series models (Table 2).

In the fourth stage, DES method was compared with MLR according to the same three error indicators (Table 3). The result showed that MLR is the best model to describe the drug data because it has the lower average error, since the mean MAPE values for MLR and DES were 15.63 and 24.61, respectively. Therefore, MLR can be used to predict future values more precisely than other models. Multiple linear regression's superiority over time series models in predicting drug demand may be due to several factors, including:

- The influence of independent factors on demand is stronger than the time factor; MLR model addresses multiple independent variables, while time series models rely only on historical time data;
- MLR model explains the influence of external factors better than time series models, which rely solely on trends and seasonality;
- The correlation effect among the factors (such as increased demand in winter and flu outbreaks), while time series models do not address these complex relationships;
- Ease of interpretation and applicability. Regression coefficients provide a quantitative indication of the impact of each variable. For example, a 10% increase in the number of patients leads to a 7% increase in drug demand.

In the fifth stage, retrospective forecasting was used to compare the actual consumption with estimated forecasts for the period 2015–2019. To verify the accuracy of the forecast, the discrepancy rate for each drug was calculated annually. In addition, the average degree of discrepancy between actual consumption and estimated forecasts was calculated over the five years, as shown in Table 4. Discrepancy rates have been shown to be statistically significant for all drugs, with the exception of Y2 and Y5, where they actually exceed 25%. This may be due to high discrepancies for some years, resulting from significant discrepancies between actual consumption and projected demand in one or both models. It could also be due to several other factors, both internal to the supply or distribution system and external factors, such as sudden changes in demand.

6 CONCLUSIONS

This study has accomplished the following:

- A systematic approach based on a multi-factor mathematical model has been built;
- An optimal forecasting model among all the methods utilized in this study has been selected to predict the future needs of hospitals for medicines in the coming years;
- A discrepancy rate for each drug annually has been calculated to check the accuracy of the forecast.

As a result, three statistical analyses were performed: correlation and regression analysis, time series analysis, and retrospective forecast analysis between estimated demand and actual consumption. The results have shown the following:

A) The model of MLR is the optimal model for predicting the needs of medicines. Since the accuracy values were lower in MLR compared to the DES method. Thus, a multiple linear regression model can:
   1) Improve hospital medication management if implemented correctly (e.g., inventory management, reducing waste, and minimizing surpluses and shortages);
   2) Enhancing medical quality and safety by ensuring the availability of vital medicines and reducing errors and costs;
   3) Reducing medical errors: when stocks are organized, the likelihood of inappropriate substitute medications being dispensed due to shortages is reduced;
   4) Adapting to crises, especially in emergencies (such as pandemics), where model equations can be adjusted to include new variables (such as disease prevalence).

B) In general, the applied methods provided an adequate assessment for our dataset. Based on the results of the analysis of methods on the dataset, we can make sure that our predictive model and all methods used in our study can be applied to other datasets from different countries if the data is accurate and sufficient;

C) It is also possible that industrial enterprises will benefit from the proposed forecasting model in this study when it comes to predicting spare parts.

The study had some limitations because of the limited historical data period because of the missing data for some prior years, in addition, COVID-19 pandemic made it harder to estimate and predict these events, therefore pharmacological data for years after 2019 were excluded because they do not reflect the new reality.

7 FUTURE WORK

Future prospects for this research include the following:

- Develop a forecasting model by combining multiple linear regression and time series techniques (a hybrid model) to improve forecast accuracy. The hybrid model captures the linear relationships between demand and influencing variables and addresses time trends not explained by regression;
- Test the robustness of forecasts over longer time periods by adding more drugs and independent variables.

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Operational Management of Water Distribution Processes in River Basins, Main and Inter-Farm Canals

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Keywords: Mathematical Model, Water Management, Pumping Stations, River Sections, Channel Section, Operational Water Distribution Management.

Abstract: This article addresses pressing issues in the field of water resource management, with a particular focus on the development of mathematical models and quality criteria designed for the efficient and precise management of water distribution systems. These systems include river basins, primary canals, and inter-farm waterways, which are critical for ensuring sustainable agricultural and industrial activities. The article analyzes various challenges encountered in the development and implementation of operational management systems, such as water scarcity, uneven distribution, and technical inefficiencies. It further explores potential solutions and highlights the necessity of adopting advanced mathematical approaches, algorithmic methods, and intelligent software tools to improve system performance. Emphasis is placed on the integration of real-time data processing and predictive analytics to enhance decision-making processes. Additionally, the study presents the design and testing of newly developed algorithms aimed at addressing real-time distribution challenges, supported by custom software applications capable of automating key functions in water management systems.

1 INTRODUCTION

With the growing reliance on computer technologies in the management of water and land resources, alongside increasing demands for the efficiency of resource management within river basins, primary canals, and inter-farm waterways, the necessity arises to develop advanced algorithmic and software tools. These tools aim to address operational management challenges for water resources, enabling the optimal utilization of water management infrastructure. Such solutions are expected to minimize unproductive losses of water and energy, enhance the operational efficiency of facilities, and establish a resource management system that operates effectively within the constraints of limited capital and operational budgets.

At present, the process of operational water distribution management in river basins, main canals, and inter-farm waterways faces significant challenges. These include notable discrepancies between actual and desired water distribution regimes, leading to over-supply in some areas and shortages in others. Additionally, unproductive water losses occur due to inefficiencies in the existing water management system. Consequently, it becomes crucial to analyze and select mathematical models that describe the dynamics of water systems, ensuring compatibility with modern computer technologies and software tools. Developing algorithms and programs for simulating the behavior of water facilities and addressing real-time operational water distribution management tasks holds particular significance.

2 MATHEMATICAL MODELS OF TYPICAL OBJECTS OF WATER MANAGEMENT COMPLEXES FOR OPERATIONAL

Mathematical models designed for sections of a river basin to address operational management tasks must strike a balance: they should accurately

represent the key dynamic processes within the system while remaining sufficiently simple to enable efficient computational solutions.

To address operational control challenges, balance differential equations can be employed as a mathematical model for sections of a river [1], [4]:

$$\frac{dW_i^y}{dt} = Q_i^H - Q_i^K + \sum_{j \in N^M} Q_{ji}^{AB} + \sum_{j \in N^{HT}} Q_{ji}^{PR} + Q_i^{PR} - Q_i^P$$
$$W_i^y(0) = W_{i0}^y \quad t \in [0, T], \tag{1}$$

where $W_i^y(t)$ is the volume of water located in section i at time t , Q_i^H and is Q_i^K the water consumption at the beginning and end of the section; Q_{ji}^{B3} and Q_{ji}^{PR} - water flow of the j -th water intake and the j -th concentrated inflow of the i -th section; Q_i^P - intensity of water loss due to filtration and evaporation of the i -th section; Q_i^{PR} forecast water flow of distributed tributaries; $W_{i0}^y(t)$ - initial value of water volume in the i -0 m area; T is the duration of the operational control interval.

In general Q_i^K , Q_{ji}^{B3} , $W_i^y(t)$, Q_i^P depend on the water level H_i^Y in the river section and on the hydraulic and morphometric characteristics of the river section and hydraulic structures [2].

Mathematical models of reservoirs. The variation in reservoir water volumes over time for operational management purposes is represented by the (2) [3].

$$\frac{dW_t^B}{dt} = \sum_{j \in N^M} Q_{ji}^{PR} - \sum_{j \in N_i^Z} Q_{ji}^{B3} - Q_i^P - Q_i^{POP}$$
$$W_t^B(0) = W_{t0}^B, t \in [0, T], \tag{2}$$

where $W_i^B(t)$ .is the volume of water in the i -th reservoir at time t ; Q_{ji}^{PR} and Q_{ji}^{B3} - water flows of the j -th inflow and j -th water intake of the i -th reservoir; Q_i^P - intensity of water loss in the reservoir and Q_i^{POP} - flow rate of water passing from the reservoir.

Restrictions on operating modes of sites, reservoirs and control and management points have the form [3]

$$W_i^{Y\min} \le W_i^Y \le W_i^{Y\max}, \tag{3}$$

$$W_j^{B\min} \le W_j^B \le W_j^{B\max},$$

$$Q_{ji}^{\min} \le Q_{ji} \le Q_{ji}^{max},$$

where $W_i^{Y\min}$, $W_j^{B\min}$, $W_i^{Y\max}$, $W_j^{B\max}$ are the minimum and maximum values of water volumes of the river and reservoir sections. These values are determined from the morphometric characteristics of the river section and reservoir.

Mathematical models of pumping stations.

A) Lifting height (static head) - defined as the difference

$$H = H_{vb} + H_{nb}. \tag{4}$$

levels of the upstream and downstream of the pumping station.

B) Characteristics of pressure losses in the pipeline - presented in reference catalogs in the form of functional curves, depending on the flow and lifting height [8], [15]:

$$Q_T^i = \begin{Bmatrix} Q_j^i & J = \overline{1,K};\ (i = \overline{1,N}), \quad N \le M \\ H_T^j & J = \overline{1,K} \end{Bmatrix}. \tag{5}$$

Where:

1) Q_j^i -argument for the pressure characteristics of the pipeline, i.e. supply of the i-th pumping unit;
2) K - number of points in the pressure characteristic;
3) N is the number of operating pumping units;
4) $H_i^j = H + \Delta H_j$
5) pressure characteristic function;
6) $\triangle H_j$- pressure loss.

C) The performance characteristics of the pumping unit are depicted as a set of curves that illustrate the relationship between the water lift height and various impeller blade angles:

$$\Omega_j^i = \Omega_{H,Q,\varphi} \cup \Omega_{H,m,\varphi},\ i = \overline{1,N}. \tag{6}$$

Where Ω_j^i is the flow characteristic of the pumping unit

$$\Omega_{H,Q,\varphi}=\begin{Bmatrix} Q_j^K & i=\overline{1,N}, & (j=\overline{1,K}) \\ H_i & i=\overline{1,N}, & \\ \varphi_j & j=\overline{1,K}, & \end{Bmatrix}$$

1) energy characteristics of the pumping unit

$$\Omega_{H,\eta,\varphi}=\begin{Bmatrix} \eta_j^i, i=\overline{1,N}, (j=\overline{i,K}) \\ H_i, i=\overline{1,N} \\ \varphi_j,\ j=\overline{i,K} \end{Bmatrix}.$$

2) φ_j .- corner reversal blades, corresponding j crooked;

3) η_j^i - efficiency i th pumping unit for j - ouch crooked.

D) Acceptable area D of work pumping unit V coordinates $Q-H$ defined by the following external boundaries:

$$\left.\begin{aligned} D_{1\max}^i &= \Omega_T^{i\max} \bigcap \Omega_{H,Q,\varphi}^i \\ D_{1\min}^i &= \Omega_T^{i\min} \bigcap \Omega_{H,Q,\varphi}^i \\ D_{2\max}^i &= \Omega_{H,Q,\varphi_{\max}}^i \\ D_{2\min}^i &= \Omega_{H,Q,\varphi_{\min}}^i \end{aligned}\right\} \quad (7)$$

g de $\Omega_T^{\max}, \Omega_T^{\min}$ - characteristics of the pipeline at the maximum and minimum geometric height of the modem; $\varphi_{\max}, \varphi_{\min}$ - maximum and minimum angles of rotation of the blades on the pump unit [10]-[12].

If the values Q and H fall within the region D, it is assumed that the unit can supply the required water flow. Otherwise, this operating mode is considered unattainable for the unit. When multiple units are in operation, the permissible area boundaries are defined collectively

$$\Omega_p^i = (\Omega_l^i \bigcap \Omega_{H,Q,\varphi}) \bigcap \Omega_{H,Q,\eta}$$

$$\varphi_i = \varphi_i^p, \Omega_{H,Q,\varphi} \subset \Omega_j^i, \Omega_{H,\eta,\varphi}^i \subset \Omega_j^i \quad (8)$$

by aggregating the flow rates within the defined region boundaries at a fixed lift height.

E) The operation of each pumping unit is described by three key parameters: flow rate, manometric lift height, and efficiency [8], [9]:

$$\left(H_{pb}, H_{vb}, \psi\right),$$

where: φ_p^i is the rotation angle of the blades i of the th operating pump unit. Consequently, the flow rate and efficiency i of the pumping unit are determined from the expressions.

F) The overall flow and power consumption of the pumping station are calculated by summing the individual flows and power consumption of the operating units:

$$Q_{HC} = \sum_{i \in N^P} Q_i \,, \quad (9)$$

where:

1) $N_i = \gamma_i^H Q_i / 102\eta_i$, / kW/ - power i of the the pumping unit;

2) γ volumetric weight of the pumped liquid [6].

$$N_{HC} = \sum_{i \in N^P} N_i \,. \quad (10)$$

Mathematical models of the canal section. The condition of the main canal section is defined by variable water flow and is represented by a set of partial differential equations, known as Saint-Venant equations:

$$B\frac{\partial z}{\partial t} + \frac{\partial Q}{\partial X} = q,$$

$$\frac{\partial Q}{\partial t} + \frac{\partial (Q * \omega)}{\partial X} = q\omega\left(\frac{\partial Z_O}{\partial X} + \frac{Q|Q|}{K^2}\right) \quad (11)$$

$$0 < x < l, l > t_0$$

where: $Q = Q(x,t)$ water consumption; $Z = Z(x,t)$ - ordinate of the free surface; ∂ - gravitational constant; I - bottom slope; $B = B(Z)$- width of the flow along the surface of the living section; $W = W(Z)$- live cross-sectional area of the flow; $C = C(Z)$- speed of propagation of small waves; $K = K(Z)$- flow module.

The system of hyperbolic partial differential equations in (11) includes the mass and momentum conservation equations of the flow, serving as a mathematical model for the unsteady flow of water within an open channel section and free surface ordinate $Z(x,t)$are chosen here as functions that

determine the flow $Q(x,t)$. The independent variables are longitudinal coordinate $X$and time t.

The channel is characterized by the bottom elevation $Z_0(X)$ and the cross-sectional width $B(x.t)$ at a vertical distance Z from the channel bed.

Then:

- g flow depth: $h(x,t) = Z(x,t) - Z_0(x)$;
- cross-sectional area of the flow $W(x,h) = \int_0^h B(x,z)dz$;
- average current speed: $U = Q/W$;
- speed of propagation of small waves: $C = \sqrt{\partial W / B}$
- bottom slope: $I = \frac{\partial Z_0}{\partial X}$.

$$K = W * C\sqrt{R}. \quad (12)$$

Flow module $K(X,h)$ characterizes the magnitude of friction forces and is determined by the following formula: where: $R = W/X$- hydraulic radius of the channel; X- wetted perimeter of the channel; C- Chezy coefficient.

To determine the Chezy coefficient, there is a whole series of empirical formulas [7]. Pavlovsky's formula can be accepted as one of them:

$$C = \frac{1}{\eta}R^{\gamma}, \gamma = 2{,}5\sqrt{n} - 0{,}13 - 0{,}75\sqrt{R}(\sqrt{n} - 0{,}1), \quad (13)$$

where η is the channel roughness coefficient.

To analyze the state of an object using the mathematical model, it is essential to define initial and boundary conditions that describe the solution's domain of determination.

The initial conditions are given as:

$$\begin{aligned} Q(X,0) &= Q_0(X) \\ Z(X,0) &= Z_0(X) \end{aligned}, \quad (14)$$

where: $Q_0(X), Z_0(X)$ are known functions.

In general, the boundary conditions can be written as follows:

$$\begin{aligned} Q(0,t) &= F_1[Z_{vb}(t), Z(0,t), S_0^1(t), \ldots S_0^N(t)] \\ Q(l,t) &= F_2[Z_{nb}(t), Z(l,t), S_l^1(t), \ldots S_l^N(t)] \end{aligned}, \quad (15)$$

where: $S_0^{\lambda}(t), (i = 1, \ldots N_0)$, Control parameters of the hydraulic structure located at the initial alignment $X = 0$, $S_l^{\lambda}(t), (i = 1, \ldots N_2)$, control parameters of the hydraulic structure located at the initial alignment $X = 0, N_1, N_2$ number of control parameters.

The boundary condition expressions vary based on the type of hydraulic flow beneath the gate, and they take the following form [13]:

$$\begin{aligned} Q(0,t) &= \mu_1 S_1(t)\sqrt{2g\left[Z_{vb}(t) - Z(0,t)\right]} \\ Q(l,t) &= \mu_2 S_2(t)\sqrt{2g\left[Z(l,t) - Z_{nb}(t)\right]} \end{aligned},$$

where: μ_1, μ_2 - cost coefficients of partitioning structures; S_1, S_2 - opening areas of the gates of partitioning structures.

3 ALGORITHMS FOR MODELING DYNAMICS OF TYPICAL WATER MANAGEMENT FACILITIES FOR OPERATIONAL WATER DISTRIBUTION CONTROL

The discrete versions of the balance (1) are formulated as follows

$$\frac{W_i^{YK+1} - W_i^{YK}}{t_K} = Q_i^{HK} - Q_j^{B3K} + \sum_{j \in N_{0,1}} Q_j^{B3K} + \sum_{j \in N^{nj}} Q_j^{nPK} + Q_i^{nPK} - Q_i^{nK} \quad (16)$$

$$W_i^{Y0} = W_{i0}^{Y}; i = 1, 2, \ldots N_j; \quad K = 0, 1, 2, \ldots.$$

The superscripts $K+1$and K - mean that the corresponding variables are taken at the moments t_{K+1} and t_K, $\triangle t_K = t_{K+1} - t_K$- time discretization step.

Algorithms for modeling reservoir dynamics. The discrete counterparts of the balance (2), which describe the dynamics of the reservoir filling and drawdown process, are expressed as follows [13], [14]

$$\frac{W_t^{BK+1} - W_t^{BK}}{t_K} = \sum_{j \in N_l^{\beta}} Q_j^{nPK} - \sum_{j \in N_l^{\beta}} Q_t^{B3K} - Q_t^{nK} - Q_t^{nOnK} \quad (17)$$

$$W_t^{B0} = W_{t0}^{B}; \quad i = 1, 2, \ldots N_B; \quad K = 0, 1, 2, \ldots$$

Constraints (3) have the form:

$$W_i^{Y_{min}} \le W_i^{YK} \le W_i^{Y_{\max}};$$

$$W_j^{B_{min}} \le W_j^{BK} \le W_j^{B_{\max}};$$

$$Q_n^{B_{min}} \le Q_n^K \le Q_n^{B_{\max}}; \qquad (18)$$

$$i = 1,2...N_Y;\ j = 1,2...N_B;\ n = 1,2...W_{nKY}.$$

Algorithms for modeling the dynamics of a channel section. For the numerical solution of these boundary value problems, it is useful to express the system of (11) in its characteristic form:

$$S\frac{\partial U}{\partial t} + S\frac{\partial U}{\partial X} = F(U,K,t). \qquad (19)$$

The characteristic representation of the system (11) is given by the following form:

$$S = \begin{bmatrix} 1 & -B(\sigma + C) \\ 1 & -B(\sigma - C) \end{bmatrix}; \quad U = \begin{bmatrix} Q \\ Z \end{bmatrix};$$

the finite difference method is applied to numerically solve the boundary value problems (2.90) and (2.91).

In the area we introduce a grid with steps h by X and t by T.

$$\Omega = \{0 \le x \le e, 0 \le t \le T\}$$
$$\varpi_{hr} = \{(x_t, t_j) \div x_t = th;\ t_j = jz;\ i = 0,1,..N;\ j = 0,1,..M;\ h = l/N;\ \tau = T/M\}$$

By approximating the system of (19) with an unconditionally stable implicit difference scheme, which has second-order approximation in space and first-order approximation in time due to linearization, we obtain a system of difference equations for the internal grid points [15-17]

$$-A_P^K \cdot W_{P-1}^{K-1} + B_P^K W_P^{K+1} - C_P^K \cdot W_{P+1}^{K+1} = D_P^K, P = 1,...N. \qquad (20)$$

where (19):

$$W_P^{K+1} = U_P^{K+1}; \qquad U_P^{K+1} = U(x_P, t_{K+1});$$

$$A_P^K = -\frac{\tau}{2h}(\square S)_P^K; \qquad B_P^K = S_P^K - \tau\left(\frac{\partial F}{\partial U}\right)_P^K;$$

$$C_P^K = \frac{\tau}{2h}(\square S)_P^K; \qquad D_P^K = \left[S_P^K - \tau\left(\frac{\partial F}{\partial U}\right)_P^K\right]U_P^K + \tau F_P^K;$$

$$\frac{\partial F}{\partial U} = \begin{bmatrix} \partial F/\partial Q \\ \partial F/\partial Z \end{bmatrix}.$$

Next, by utilizing the systems of (11) and the boundary conditions, we derive the difference conditions [18]:

$$B_0^K W_0^{K+1} - C_0^K W_1^{K+1} = D_0^K, \qquad (21)$$

$$-A_N^K W_{N-1}^{K+1} - B_N^K W_N^{K+1} = D_N^K, \qquad (22)$$

where:

$$A_N^K = \begin{bmatrix} a_{11N}^K & a_{12N}^K \\ a_{21N}^K & a_{22N}^K \end{bmatrix}; \qquad B_N^K = \begin{bmatrix} B_{11N}^K & B_{12N}^K \\ B_{21N}^K & B_{22N}^K \end{bmatrix};$$

$$B_0^K = \begin{bmatrix} B_{110}^K & B_{210}^K \\ B_{120}^K & B_{220}^K \end{bmatrix}; \qquad C_0^K = \begin{bmatrix} C_{110}^K & C_{210}^K \\ C_{120}^K & C_{220}^K \end{bmatrix};$$

$$D_0^K = \begin{bmatrix} d_{10}^K \\ d_{20}^K \end{bmatrix}; \qquad D_N^K = \begin{bmatrix} d_{1N}^K \\ d_{2N}^K \end{bmatrix}; \quad .$$

The components of these matrices and vectors, when solving the system of (20) with the boundary conditions (22), are expressed in the following form:

$$a_{11N}^K = 0; \qquad a_{21N}^K = \frac{\tau}{h}(\lambda_{21N}^K \cdot S_{11N}^K + \lambda_{22N}^K \cdot S_{21N}^K);$$

$$a_{12N}^K = 0; \qquad a_{22N}^K = \frac{\tau}{h}(\lambda_{21N}^K \cdot S_{12N}^K + \lambda_{22N}^K \cdot S_{22N}^K);$$

$$B_{11N}^K = 1; \qquad B_{21N}^K = S_{21N}^K + \tau\left(\frac{\partial F}{\partial Q}\right)_N^K + \frac{\tau}{h}\left(\lambda_{21N}^K \cdot S_{12N}^K + \lambda_{22N}^K \cdot S_{21N}^K\right);$$

$$B_{12N}^K = 1; \qquad B_{22N}^K = S_{22N}^K + \tau\left(\frac{\partial F}{\partial Z}\right)_N^K + \frac{\tau}{h}\left(\lambda_{21N}^K \cdot S_{12N}^K + \lambda_{22N}^K \cdot S_{22N}^K\right);$$

$$B_{210}^K = 1; \qquad B_{110}^K = S_{110}^K + \tau\left(\frac{\partial F}{\partial Q}\right)_0^K - \frac{\tau}{h}\left(\lambda_{110}^K \cdot S_{110}^K + \lambda_{120}^K \cdot S_{210}^K\right);$$

$$B_{220}^K = 1; \qquad B_{120}^K = S_{120}^K + \tau\left(\frac{\partial F}{\partial Z}\right)_0^K - \frac{\tau}{h}\left(\lambda_{110}^K \cdot S_{120}^K + \lambda_{120}^K \cdot S_{220}^K\right);$$

$$C_{210}^K = 0 \qquad C_{110}^K = \frac{\tau}{h}\left(\lambda_{110}^K \cdot S_{110}^K + \lambda_{120}^K \cdot S_{120}^K\right);$$

$$C_{220}^K = 0 \qquad C_{120}^K = \frac{\tau}{h}\left(\lambda_{110}^K \cdot S_{120}^K + \lambda_{120}^K \cdot S_{220}^K\right)$$

$$d_{20}^K = U_1^K; \qquad d_{10}^K = \left[S_{110}^K - \tau\left(\frac{\partial F}{\partial Q}\right)_0^K\right]Q_0^K + \left[S_{120}^K - \tau\left(\frac{\partial F}{\partial Z}\right)_0^K\right]Z_0^K + \tau F_0^K;$$

$$d_{1N}^K = U_2^K; \qquad d_{2N}^K = \left[S_{210}^K - \tau\left(\frac{\partial F}{\partial Q}\right)_N^K\right]Q_N^K + \left[S_{220}^K - \tau\left(\frac{\partial F}{\partial Z}\right)_N^K\right]Z_N^K + \tau F_N^K.$$

Therefore, solving the system of partial differential (11) with boundary conditions (14)-(15) is transformed into solving the system of difference (20)-(22) at each time step t_k.

Using the Sweep method, we obtain the solution in the form of the following relationships:

$$W_P^{k+1} = X_P^k W_{P+1}^{k+1} + T_P^K, P = N-1.....1,0. \quad (23)$$

$$W_P^{k+1} = X_P^k W_{P-1}^{k+1} - T_P^K, P = 1,2,....,N-1,N. \quad (24)$$

By transforming (21) into the form of (23) and substituting (22) into (20), we derive the expression for the running coefficients:

$$X_0^K = (B_0^k)^{-1} C_0^K, X_P^K = (B_P^k - A_P^k X_{P-1}^K)^{-1} C_P^K \quad (25)$$

$$T_0^K = (B_0^k)^{-1} D_0^K, T_P^K = (B_P^k - A_P^k X_{P-1}^K)^{-1} (D_P^K + A_P^k T_{P-1}^K) \quad (26)$$

P=1,2.....,N-1

From the above, it can be concluded that the numerical algorithm for solving the difference boundary value problem (20)-(22) follows this sequence:

1) The running coefficients are computed using the recurrence relations (25) and (26):

$$X_0^K, T_0^K, X_P^K, T_P^K.$$

2) The running coefficients are determined by applying the recurrence formulas (25) and (26):

$$X_N^K, T_N^K, X_0^K, T_0^K.$$

3) The numerical values are obtained by applying the recurrence relations (23) and (24):

$$W_P^{K+1} (P = 0,1,....,N).$$

It is important to note that the boundary value problem for the primary variables is solved in the direction of increasing t beginning from t=0.

Using the developed algorithms, software for simulating the dynamics of water management systems in the Syrdarya River basin was created in Python. This program utilizes a database of water management facilities and their interconnection structure.

The mathematical formulation of problems related to the operational management of water distribution in river basins, main canals, and inter-farm waterways is presented as follows. The primary objective of operational water resources management is to ensure that the specified water consumption for consumers is met at every point in time, while also providing the required volumes of water in intra-system reservoirs by the end of the planning period. This can be mathematically expressed as follows [18,19]:

$$W_t^B(T) = W_{in}^B, \ i \in N_{BDX}^B$$

$$Q_{ij}(t) = Q_{ij}^B, \quad j \in N_t^{B3}, i \in N^B, \quad (27)$$

where N_{BDX}^B is the set of numbers inside system reservoirs; N_t^{B3} - set of numbers (codes) of PCU water intakes of the t-th section.

Achieving exact fulfillment of the (27) is not feasible due to the uncertainty in water inflows to the sections and reservoirs, the parameters of the river sections, and the complexity of the transition processes within the river segments. As a result, practical solutions often employ various integral or minimization criteria for the operational management of water resources.

One of the key criteria used in operational management is the volume of water deficit for consumers over the interval [0, T], as well as the amount of water remaining in the reservoir at the end of this period

$$I_1 = \int_0^T \sum_{i \in N_t} \sum_{j \in N_t^{B3}} \eta_{ij}^1(t) dt + \sum_{j \in N_{BDX}^B} \eta_j^2 \ . \quad (28)$$

While the integral criterion (28) is straightforward, it has a drawback: the deviation of the actual regime from the planned one can be quite significant. As a result, the root-mean-square integral deviation of the actual parameters from their planned values is often used as an alternative, expressed as follows:

$$I_2 = \int_0^T \sum_{i \in N_t} \sum_{j \in N_t^{B3}} (Q_{ji}^B - Q_{ji}(t)) dt + \sum_{j \in N_{BDX}^B} (W_{in}^B - W_t^B(T)) \cdot \quad (29)$$

Therefore, the problem of operational water resources management can be framed as the task of minimizing a chosen criterion that evaluates the quality of the system, while describing the movement of water within the basin's water management network, represented as a graph [20].

In addition to integral criteria, the following operational management criterion can be applied.

$$|W_i^B(t) - W_{in}^B| \le \epsilon_1 \ \ i \in N_{BDX}^B,$$
$$|Q_{ij}(t) - Q_{ij}^B| \le \epsilon_2 \quad j \in N_t^{B3}, i \in N_t^B, \quad (30)$$

here ϵ_1 and ϵ_2 are positive constants that represent the accuracy requirements for operational water

resources management. Solving the formulated operational management problems at the river basin level necessitates the application of advanced methods for optimal control of complex dynamic systems, alongside the use of modern computational technologies.

A software suite designed to simulate the dynamics of water management systems for addressing operational water distribution management issues. The software package is intended to model the dynamics of water management systems over a ten-day period within a month, specifically for solving operational water distribution management problems. The software package computes the water volume in reservoirs and river sections over time, utilizing known values of inflows, water withdrawals, and key characteristics of reservoirs, river sections, hydraulic structures, pumping stations, and other related components [4], [21].

Figure 1 illustrates a block diagram depicting the interconnection of software modules for modeling solutions to operational water resource management problems at the river basin level.

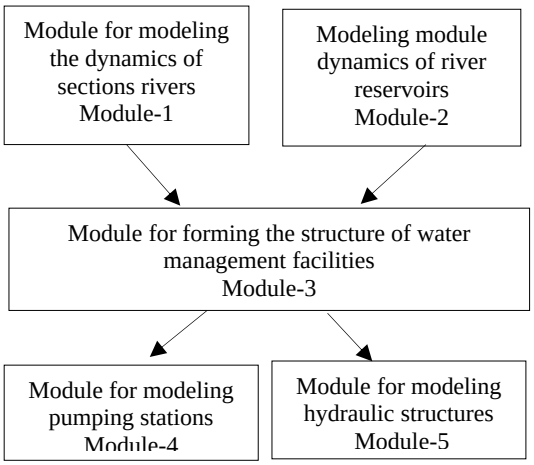


Figure 1: A block diagram of software modules.

Module-1 is responsible for solving equations related to the dynamics of river sections. It calculates the changes in water flow (i.e., water volume transformations) over time within a river section, including total water intake and losses due to filtration and evaporation.

Module-2 is designed to solve equations related to the dynamics of reservoirs. It calculates the variations in water level, surface area, and volume over time, along with total water intake and losses from filtration and evaporation.

Module-3 is intended to simulate the operating modes of hydraulic structures, calculating changes in water flow based on the known gate openings.

Module-4 is responsible for modeling the operating modes of pumping stations, calculating the changes in water flow based on the number of active units and their blade rotation angles.

Module-5 is designed to form the structure of a network of water management facilities.

It is important to note that the modules are developed in Python and utilize a MySQL database. The interaction with the database is managed through importing and exporting data via Excel text files.

Table 1 and Table 2 presents the results of modeling the dynamics of water management systems in the Syrdarya River basin over a ten-day period with daily discretization. It also illustrates changes in the operating modes of partitioning structures within the basin. Table 2 displays the dynamic water balance for the river sections of the Syrdarya River basin. The software package is designed to model the dynamics of water management systems and address operational water resource management issues. It can be applied to any river without modifying the software; only river-specific data needs to be entered. The system's versatility is based on representing water management networks as graphs, where each graph segment corresponds to a river section, and the start and end of each segment represent hydraulic structures, gauging stations, or reservoirs. The modules interpret this graph, utilizing the required morphometric data of river sections and basin objects as input, along with the current state of the river basin and dispatcher recommendations [22]-[24].

Using the developed algorithms, programs were created to simulate the dynamics of pumping stations and canal sections for the operational control of the water distribution process.

The input data includes the characteristics of pumping stations and units, as well as the hydraulic, morphometric, and technological parameters of the main and inter-farm canal sections.

The output data consists of parameter values that describe the dynamic state of the system over time layers (see Fig. 2 and Fig. 3).

Table 1**:** Modeling the operational balance of the water management facility of the Charvak reservoir for the 1st decade of April 2024.

Name of the structure	Limit per decade	1	2	3	4	5	6	7	8	9	10
Inflow											
m.cub/s		157.0	157.0	157.0	157.0	157.0	157.0	157.0	157.0	157.0	157.0
million cubic meters		135.6	135.6	135.6	135.6	135.6	135.6	135.6	135.6	135.6	135.6
Release											
m.cub/s		207.9	207.9	207.9	207.9	207.9	207.9	207.9	207.9	207.9	207.9
million cubic meters		179.6	179.6	179.6	179.6	179.6	179.6	179.6	179.6	179.6	179.6
Losses											
m.cub/s		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
million cubic meters		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Water intake											
m.cub/s	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
million cubic meters	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Volume											
million cubic meters		1723	1718	1713	1708	1703	1698	1693	1688	1683	1678

Table 2: Modeling the operational balance of the water management facility of the Khojakent reservoir for the 1st decade of April 2024.

Name of the structure	Limit per decade	1	2	3	4	5	6	7	8	9	10
Section No. 6											
Water supply											
m.cub/s	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
million cubic meters	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Inflow											
-small rivers											
m.cub/s		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
million cubic meters		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
- water return											
m.cub/s		58.0	58.0	58.0	58.0	58.0	58.0	58.0	58.0	58.0	58.0
million cubic meters		50.1	50.1	50.1	50.1	50.1	50.1	50.1	50.1	50.1	50.1
Losses											
m.cub/s		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
million cubic meters		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Transformation											
m.cub/s		-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2
million cubic meters		-0.1	-0.1	-0.1	-0.1	-0.1	-0.1	-0.1	-0.1	-0.1	-0.1
Volume											
million cubic meters		17	17	17	17	17	17	17	17	17	17
Release into the downstream											
m.cub/s		266.1	266.1	266.1	266.1	266.1	266.1	266.1	266.1	266.1	266.1
million cubic meters		229.8	229.8	229.8	229.8	229.8	229.8	229.8	229.8	229.8	229.8

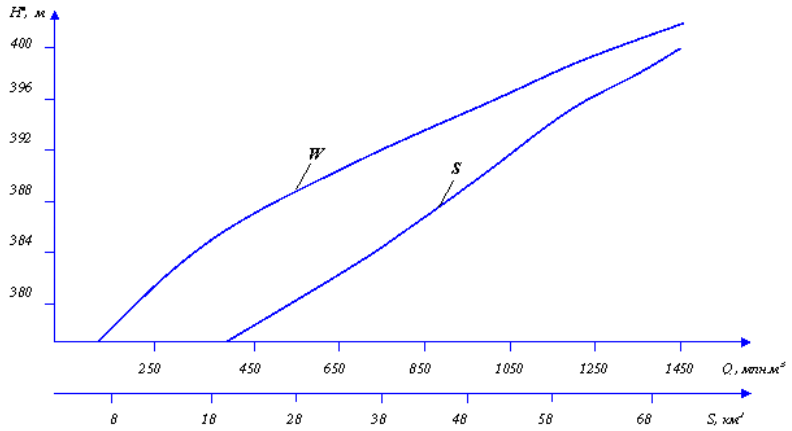


Figure 2: Curves of the dependence of surface areas and water volume on water level in the Charvak reservoir.

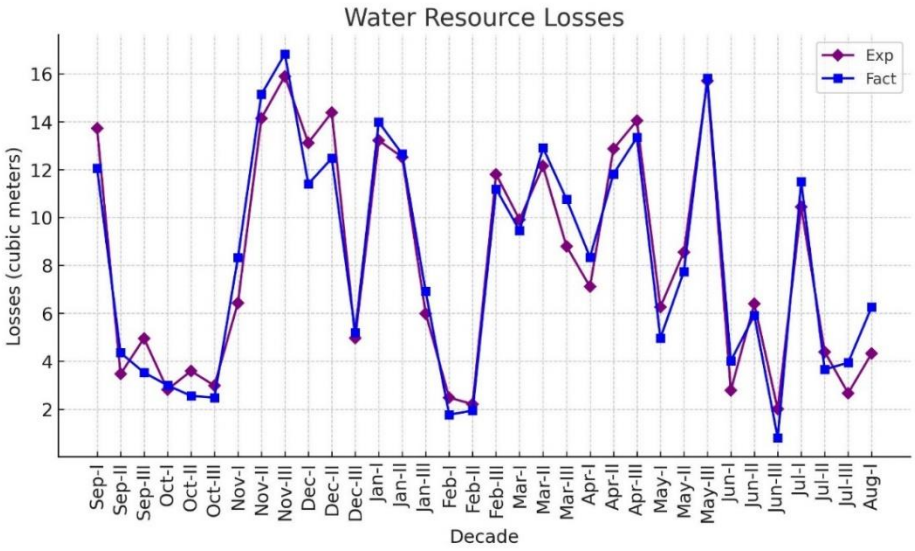


Figure 3: Results of modeling the filling and discharge regimes of the Charvak reservoir.

3 DISCUSSION

This article is based on the foundational project for the state technical program (GNTP)-124, titled of water management facilities and hydraulic structures in the republic." The project was conducted under the contract VA-KHF-5-022, named "Development of scientific foundations for the formation, management, and effective use of surface and groundwater in the Republic of Uzbekistan in the context of climate change," with the Ministry of Innovative Development, and was carried out from 2017 to 2020.

4 CONCLUSIONS

As a result of the research, mathematical models for typical water management facilities were developed to support the operational management of water distribution, considering operational requirements and contemporary technical modeling tools. Algorithms for simulating the dynamics of typical water management facilities—such as reservoirs, river sections, canals, hydraulic structures, and pumping stations—were created to address operational management challenges in water distribution. The developed software package allows for more efficient decision-making in managing the water distribution process. Additionally, mathematical formulations for operational management of water distribution in river basins, main canals, and inter-farm canals were established. Numerical results of modeling the dynamics of water management facilities in the Syrdarya River basin are also presented.

"Development of scientifically grounded methods, systems, and management forms, as well as the reliable, safe, and efficient utilization

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A Novel Numerical Approach for Solving Initial Value Problems in Heat Equations Using Variational Regularization and Intelligent Particle Swarm Optimization

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Keywords: Intelligent Particle Swarm Optimization, Regularization Parameter, Variational Regularization, Heat Equation.

Abstract: In this article, we use the variational approach as a regularization tool to solve the initial value problem that appears in a heat partial differential equation. Although the temperature obtained at time t=T>0 is known, the initial temperature distribution remains unknown. By using the separation of variables method, the partial differential equation is transformed into a Fredholm integral equation of the first kind. We then apply a discretization algorithm to reduce the integral equation to a system of linear algebraic equations, commonly referred to as an inverse linear operator problem. The variational regularization method is employed to obtain a regularized solution. We also present a fundamental analysis of this method for solving inverse problems. Furthermore, we describe the application of the Intelligent Particle Swarm Optimization (IPSO) technique to determine the optimal regularization parameter. Our results demonstrate that integrating particle swarm optimization with variational optimization is both effective and computationally feasible.

1 INTRODUCTION

Because a "small" change in the data might result in "large" mistakes in the solution, the Cauchy inverse problem of the heat equation is ill-posed. According to Jacques Hadamard, an issue is well-posed if and only if the following characteristics are true [1].

- There is a solution, or at least one solution exists;
- The solution is unique; there is only one solution in existence;
- The data (stability) is a constant determinant of the solution.

The ill-posedness problem cannot be solved with traditional numerical techniques [2] – [4]. It calls for specialized methods, such as regularization approaches [5]. Inverse heat transfer problems can now be solved more easily with numerical methods because to the advancement of fast personal computers [6]. Numerous writers have addressed the inverse problem of heat equation's theoretical ideas and computer implementation, and numerous approaches have been detailed [4], [6] – [9].

Several regularization techniques have been developed to address the ill-posed nature of inverse equations of the first kind [10]. Classical methods include Tikhonov regularization [11], Landweber iteration [12] or other iteration methods [13], and the discrepancy principle [14]. Regularization introduces a penalty term to prevent overfitting and ensure stability, especially in ill-posed inverse problems [7]. The discrepancy principle helps determine the optimal balance between fitting the noisy data and avoiding excessive regularization [15]. However, these methods often require careful selection of a regularization parameter, which can significantly influence the quality of the solution [16], [17]. Nature-inspired optimization algorithms have shown promise in automating the selection of regularization parameters for ill-posed problems [18], [19]. Genetic Algorithms [20], Particle Swarm Optimization [21], and Artificial Bee Swarm Optimization have been employed to explore the parameter space and identify

optimal or near-optimal values [15], [22]. These algorithms offer advantages in handling complex, non-convex objective functions, which are often encountered in regularization problems.

Numerous important search techniques use population-based optimization. These techniques are part of a class of algorithms, such as particle swarm optimization, that are inspired by nature. The PSO [23] algorithm, created by Eberhart and Kennedy, is capable of finding precise approximations or global optimum solutions. Numerous inverse problem types that can be resolved. By proposing a dynamic parameter update strategy, offering a new appropriateness choosing methodology, and balancing the trade-off between investigation and exploitation searches, the PSO method was used in [24] to address the electromagnetic inverse problems. In order to prevent getting stuck in local optima, this new methodology also includes the development of a new position updating formula. In the work [25], inverse design problems for cylindrical thermal multilayer shielding and cloaking shells are solved via particle swarm optimization. These inverse problems are converted into associated control problems. Particle Swarm Optimization (PSO) is often used to solve inverse problems, however it does not immediately resolve typical security issues. Current studies of post-quantum cryptography, however, provide a more thorough comprehension of computational algorithm security. Research like [26] and [27], for instance, highlight the need for secure implementations and demonstrate how inadequate it is to rely solely on secure algorithms. Even methods that are mathematically valid, such as PSO, might be exposed by implementation errors. Therefore, the implementation of PSO-Tikhonov Regularization must be secured by employing methods such as input validation, safe random number generation, and meticulous code review. The correctness and reliability of the obtained results are ensured by these methods [28]. Security concerns are not limited to implementations and methods; they also include specific hazards that machine learning models must deal with [29], [30].

Reconstructing the source function of the Initial Value Problem IVP for the heat partial differential equation is the primary concept in this work. Through a series of phases, the starting value problem has been reduced to a linear inverse problem. Because of this, the solution is unstable because it does not rely on the data continuously. As a result, this problem is ill-posed. We will use variational regularization to obtain a well-posed problem. In this paper, we will use Intelligent Particle Swarm Optimization (IPSO) to solve the problem of choosing the optimal regularization parameter.

2 PROBLEM STATEMENT

[1] 's IVP taking the heat (1) as follows,

$$\frac{\partial w(x,t)}{\partial t} = \frac{\partial^2 w(x,t)}{\partial x^2}, \tag{1}$$

where $x \in [0,l]$, $t \in (0,T]$,

$$w(0,t) = 0, \;\; w(l,t) = 0, \tag{2}$$

where $t \in (0,T]$,

$$w(x,0) = w(x), \tag{3}$$

where x $[0,l]$.

The boundary conditions are $w(0,t)$ and $w(l,t)$, and the beginning condition is $w(x)$ which must be determined. The partial differential equation above can be reduced to integral form using the separation of variables method, and the result is:

$$Aw(x) = \int_0^l P(x,y)w(y)dy = g(x). \tag{4}$$

Every step of separation was used in [2]. We must limit the sum of series to ten times when the kernel $P(x,y)$ is an infinite series because we are unable to handle unlimited sums.

$$P(x,y) = \frac{2}{l}\sum_{n=1}^{10} e^{\frac{-(n\pi)^2 T}{l^2}} \sin(\frac{n\pi x}{l})\sin(\frac{n\pi y}{l}) \tag{5}$$

For providing the estimated $w(x)$ We are able to transform the problem of initial value to $Aw = g$, liner operator form by applied discretization algorithm as defined and explained in [3].

$$A\begin{bmatrix} w(y_0) \\ w(y_2) \\ \vdots \\ w(y_{n-1}) \end{bmatrix} = \begin{bmatrix} g(x_0) \\ g(x_2) \\ \vdots \\ g(x_{n-1}) \end{bmatrix}, \tag{6}$$

where

$$A = \frac{l}{n}\begin{bmatrix} P(x_0,y_0) & P(x_0,y_1) & \dots & P(x_0,y_{n-1}) \\ P(x_1,y_0) & P(x_1,y_1) & \dots & P(x_1,y_{n-1}) \\ \vdots & \vdots & \dots & \vdots \\ P(x_{n-1},y_0) & P(x_{n-1},y_1) & \dots & P(x_{n-1},y_{n-1}) \end{bmatrix}, \tag{7}$$

The matrix A has ill-conditionally property and we must locate $w(x) \in L_2[0,1]$. The exact answer will be $w(x) = \sin \pi x$, where the value of x $0 \le x \le 1$. We created the input data as $w(x,T) = g(x)$, where $T = 0.01$ and $0 \le x \le 1$

3 VARIATIONAL REGULARIZATION METHOD

D. Phillips (1962) suggested this approach, which entails solving a variational problem [14]. Let consider (4). in liner operator form

$$Aw = g, \tag{8}$$

Suppose that A is a bounded, linear, injective operator with A^{-1} not continuous. The data are the elements $\{A, g_\delta, \delta\}$. If the noise level $\delta > 0$ is given, the estimate $\|g_\delta - g\| \le \delta$ holds, and the noisy data y_δ is the δ−approximation of y .

Now, let's look at (8). The challenge is to identify the stable solution w_δ such that the error estimate $\|w_\delta - w\| \le \eta(\delta) \to 0$ *as* $\delta \to 0$ holds given $\{A, g_\delta, \delta\}$.

Let z be the minimal-norm solution to (8). By addressing the variational problem (minimization problem) and using a minimal-norm to create a stable approximation to the answer z, the variational regularization approach ensures that $z \perp N(A)$. Assume $\|A\| \le 1$ without losing generality, and then $\|A^*\| \le 1$. Assuming that $B = A^*A$, then $B \ge 1$ is a self-adjoint, bounded operator. The equation $Bw = q$, where $q = A^*g$, is equal to (8).

If q, $\|q - q_\delta\| \le \|T^*\|\delta \le \delta$, then q_δ is provided instead. Given that $N(T) = N(B)$ $z \perp N(B)$ and $\|(B+\alpha)^{-1}\| \le \frac{1}{\alpha}$, Examine the issue of determining the functional minimum.

$$F(w) = \|Aw - g_\delta\|^2 + \alpha\|w\|^2 = \min, \tag{9}$$

where $\alpha > 0$ parameter of regularization and $F(w)$ is a function for α , and δ . Minimizers are solutions to the variational problem (9). As a result, the next formula provides the unique solution to (10).

$$w = (A^*A + \alpha)^{-1}A^*g_\delta. \tag{10}$$

4 IPSO ALGORTHIM

The IPOS method for choosing the variational regularization parameter α without knowing the noise level δ is examined in the following.

The following is a concise statement of IPSO's core idea [5]. Every swarm member, referred to as a "particle," is a potential solution; each particle adjusts its position within the search domain and updates its velocity based on its own and its "neighbors'" flying experiences at each iteration, aiming for a better position for itself as long as it satisfies specific fitness requirements.

The "current best position" (*cbest*), the site with the highest engagement rate across all affiliates from the beginning to the end of the search, is effective and is iteratively taught by the algorithm. Every particle additionally commits to memory its best experienced position, or personal best position (*pbest*). The distance each particle travels to reach its subsequent place is calculated using the equation $\Delta x = e\Delta t$, where *e* is the velocity. At iteration t, it is computed as follows for particle I:

$$e_i^{t+i} = \omega e_i^t + c_1 r_1 d_c^{i,t} + c_2 r_2 d_p^{i,t}, \tag{11}$$

$$d_p^{i,t} = \left[x_i^t - pbest^{i,t} \right], \tag{12}$$

$$d_c^{i,t} = \left[x_i^t - cbest^t \right]. \tag{13}$$

A particle's best individual and global positions are separated by $d_c^{i,t}$ and $d_p^{i,t}$, respectively, from its current position. The symbol ω is well-defined as inertia weight, r_1 & r_2 are numbers defined randomly, and c_1, c_2 are coefficients for acceleration. Between any two following repetitions, $\Delta t = 1$ between any two subsequent iterations (i.e., $\Delta t = (t+1) - t = 1$), Consequently, at iteration, particle *I* will be in the following location $(t+1)$:

$$a_i^{t+1} = a_i^t + v_i^{t+1}. \tag{14}$$

The procedures that follow now apply the IPSO calculation algorithms to solve the problem.

Step 1: Describe the IPSO and the issue. The IPSO and integral equations of the first kind are defined in this section. We develop the "cost function" or fitness function (Cost_Fun). Next, we must configure the following function and variables:

- n - size of domain;
- Cost function:

```
Cost_Fun (w, A, g, n)
{
```
$I = eye(n,n)$

$w = inv(A^*A + \alpha I) * A^*g$

```
return
```
$\|Aw - g_\delta\|^2 + \|\alpha\|\|w\|^2$

```
}

n_Var; / number of variables for unknown decisions;
Var_Size = [1, n_Var]; / choice variables' matrix size;
Var_Min;/choice variables' lowest bound;
Var_Max;/choice variables' upper bound.
```

Step 2: Parameters:

```
Max_It
n_Pop /population size
w, c1, c2
```

Step 3: Population Initialize:

```
arr_Pos = [ ]; arr_Vel = [ ]; arr_Cost = [ ];
arr_Best.Pos = [ ]; arr_Best.Cost = [ ];
arr_Cur_Best = inf;
Loop over (n_Pop)
arr_Pos = unifrnd (Var_Max, Var_Size, Var_Min);
arr_Vel = zeros (Var_Size);
arr_Cost = Cost_Fun(arr_Pos);
"Update"  arr_Best.Pos  and arr_Best.Cost = arr_Cost;
"Update "
If arr_Best.Cost < arr_Cur.Best Cost
arr_Curt.Best = arr_Best.Pos and arr_Best.Cost = arr_Cost
Over n_Pop end loop
```

Step 4: Main loop of IPSO:

- Loop over iterations it=1: Max_It;
- Loop under population size (n_Pop);
- Update velocity:

```
arr_Vel = w * arr_Vel + c1*rand (Var_Size) .* arr_Best.Pos - arr_Pos+ c2*rand(Var_Size) .* arr_Cur.Best.Pos - arr_Pos);
```

- Position updating:

```
arr_Pos = arr_Pos + arr_Vel;
```

- Assessment:

```
arr_Cost = Cost_Fun(arr_Pos);
```

- Update:

```
if arr_Cost < arr_Best.Cost
      arr_Best.Pos = arr_Pos;
      arr_ Best.Cost = arr_Cost;
```

- Update:

```
if arr_Best.Cost < arr_Cur.Best.Cost
      arr_Curt.Best = Best;
      End
End
```

- Loop n_Pop ending;
- Store the best cost value arr_Best.Costs(it) = arr_Cur.Best.Cost;
- End loop over iterations Max_It.

Step 5: Create the results.

- α= arr_Best.Costs(it);
- The inverse problem's approximate solution can be found by: $w = inv(A^*A + \alpha I) * A^*g,$.

5 NUMERICAL EXPERIMENTS

We created the inverse problem's input data as $w(x,T) = g(x), 0 \le x \le 1$ and $T = 0.01$. The IPSO values for the common parameters for all testing with noise-free and nose-level are as follows:

n_Var = 1, Var_Size = [1, n_Var], Var_Min = 0, Var_Max = 1, c1 = c2 = 2.

Figures 1, 2, and 3 display the Initial Value test. A situation with substantial measurement noise (δ = 0.2) and (δ = 0.1) is shown in Figures 1 and 2, respectively. In spite of this noise, the IPSO method finds a good approximation (IPSO solution) and determines the ideal (α) parameter. In comparison, the noise-free scenario (δ = 0) is depicted in Figure 3. In this case, zero is the ideal α value.

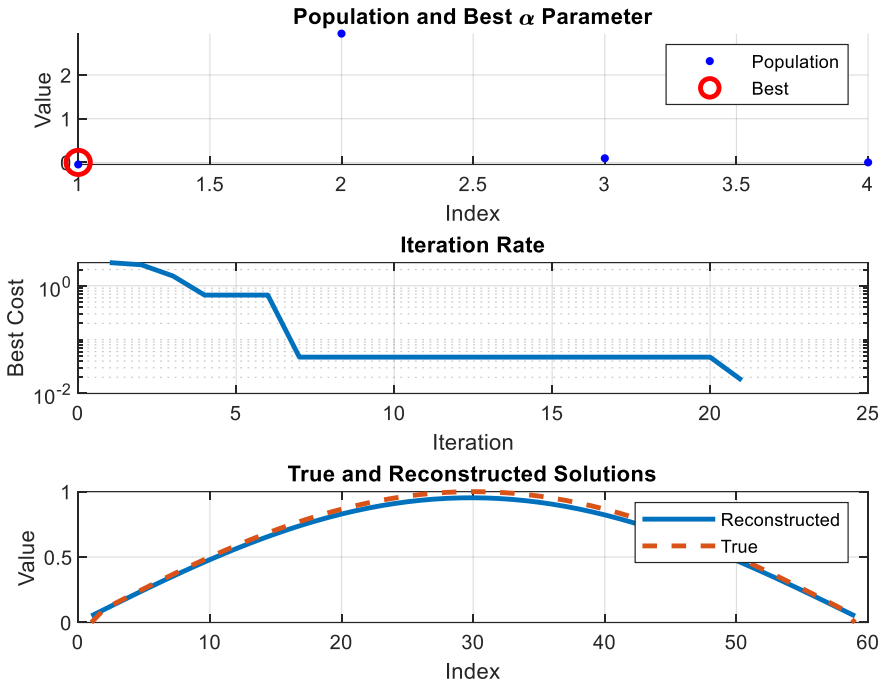


Figure 1: Initial value with δ=0.2 and best α=0.018.

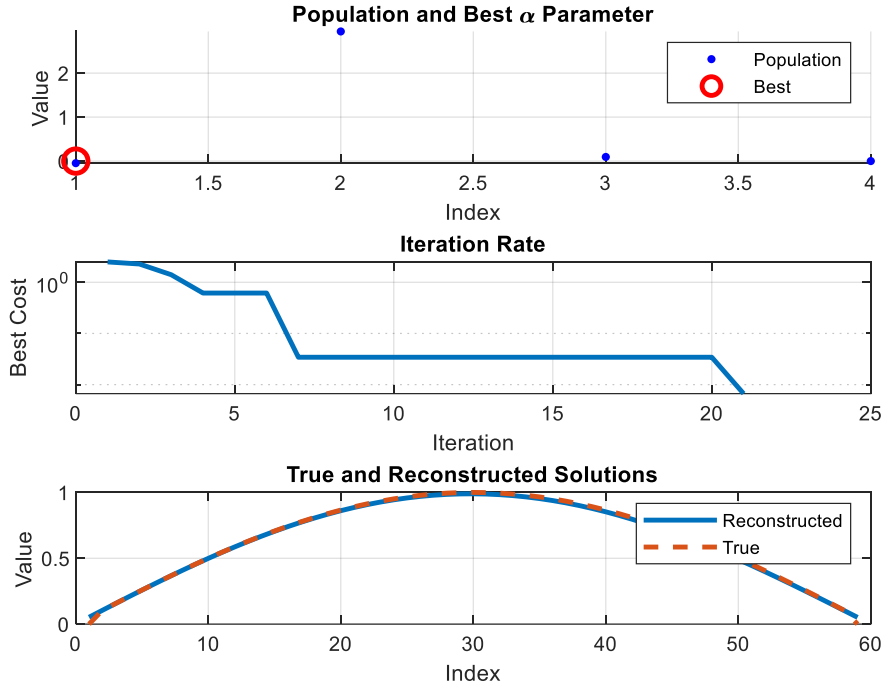


Figure 2: Initial value with δ=0.1, and best α=0.006.

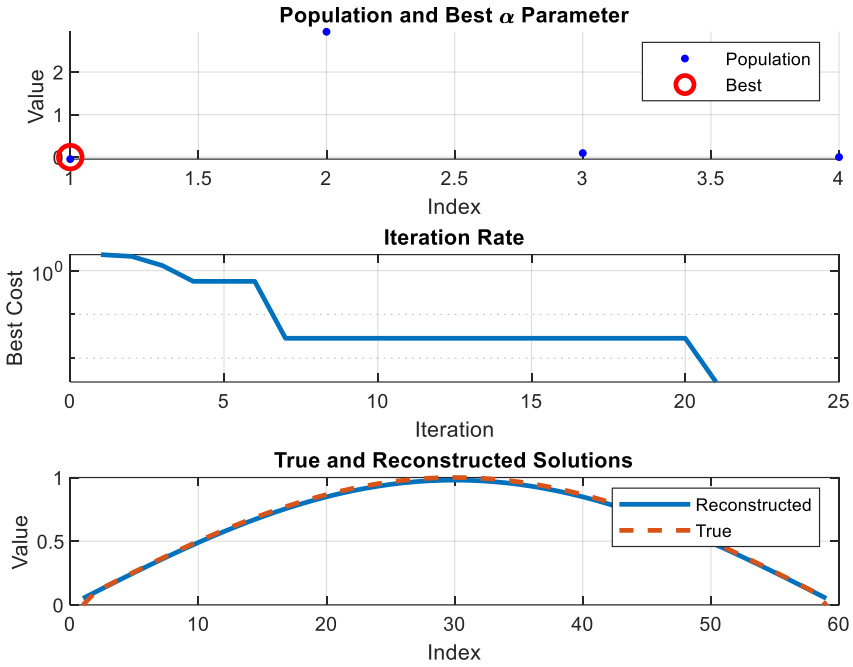


Figure 3: Initial value with δ=0, best α=0.

6 CONCLUSIONS

In order to solve the heat equation's initial value problem with both noisy and noise-free data, we examined the variational regularization technique and Intelligent Particle Swarm Optimization (IPSO) in this research. We sought to discover a stable solution to the problem, which was converted from a partial differential equation to an inverse linear operator equation. The regularization parameter in the variational regularization method was chosen using a novel technique to guarantee convergence to an appropriate approximation. IPSO played a crucial role in optimizing this parameter by searching for the best value that minimized the variational equation associated with the Tikhonov method. Numerical experiments, including test cases such as the Phillips and Gravity problems, demonstrated the effectiveness of integrating Tikhonov regularization with IPSO. The results confirm that this hybrid approach enhances solution accuracy and stability, making it a promising method for addressing inverse problems in heat equations.

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Econometric Modeling of the Use of Digital Technologies in Improving the Infrastructure of the Railways of Uzbekistan

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Keywords: Digital Economy, Railway Transport, Infrastructure, Passenger Transport, Multifactor Econometric Model, Breush- Pagan Test.

Abstract: This study examines the growing global trend of integrating digital technologies into various economic sectors, with a specific focus on the Republic of Uzbekistan. Following Presidential Decision No. PQ-4699, adopted on April 28, 2020, significant steps have been taken to promote the digital economy and implement electronic government solutions. In this context, the railway transport sector, particularly JSC "Uzbekistan Railways," has become a key area for digital transformation. The research highlights the strategic importance of introducing digital software to address infrastructure challenges and enhance operational efficiency. Drawing on international perspectives, the study identifies key benefits of digitalization in railway systems, including cost reduction, improved management, and business model innovation. An econometric model was developed to assess the relationship between the adoption of digital technologies and economic outcomes in the railway sector. The analysis demonstrates a strong positive impact, where a 1% increase in investment in digital software corresponds to a 2.53% rise in passenger traffic. Key indicators used in the model include total revenue from railway transport (d), total freight transported (freight), and the number of passengers. The variable (dtx) was used to represent product costs associated with digital supply development and infrastructure management. The findings affirm the critical role of digital technologies in advancing railway infrastructure and support broader economic growth initiatives in Uzbekistan.

1 INTRODUCTION

The main difference between the current economy and the digital economy is that in the former, GDP is the main indicator of its efficiency. Businesses first produce products, after which they look for sales markets. Forecasting plays a key role in the digital economy: the first the demand forecast is determined, then the supply is formed. Digitization of the economy of control systems allows more informed business entities to make the right decisions. Today's realities clearly show the increasing value of accurate forecasting based on mathematical models based on large volumes of data. Thus, these capabilities are used, for example, to adjust the balance of supply and demand and to obtain detailed information about reliability to reduce sales and logistics costs. The following should be noted: the closer the date of the predicted event, the higher the probability that it will maximally correspond to the obtained forecast. The issue of modernization of the infrastructure of the transport sector takes a serious place in many foreign strategies for the development of the digital economy [1], [2], [3].

The implementation of digital technologies in the infrastructure of railway transport is a current topic that is being considered by many scientists and researchers, and the general conclusions in this regard are as follows: reducing costs, increasing the ability to manage the system, changing the existing business process model of railway transport operations through a digital transformation program, and justifying opportunities [4], [5].

To date, in the scientific work of local and foreign scientists, great attention is paid to the use of mathematical methods to improve the efficiency of transport management, the use and application of information and digital technologies in transport

systems, including the organization and regulation of railway traffic flows.

In the conducted studies [1], issues of optimization of management in the transport system using digital technologies were considered. Methods of mathematical description of railway transport management processes have been developed.

Dimitrov I.D. as researched, digital control methods were proposed to allow effective use of existing transport resources [2].

A group of authors [3] considered the organization of rapid data exchange and information transfer between management subjects, as well as the evaluation of the effectiveness of the use of information technology in management.

2 ANALYZING THE N-INDEX AND LINEAR REGRESSION MODELS

In the Resolution of the President of the Republic of Uzbekistan dated April 28, 2020 "On measures for the widespread introduction of digital economy and electronic government" No. PQ-4699, a number of effective results were achieved based on the tasks set in the framework of the wider involvement of digital technologies in the activities of JSC "Uzbekistan Temir Yollari" [6]. The author's hypothesis was formed for the purpose of econometric modeling of the use of digital technologies in improving the infrastructure of the railways of Uzbekistan. According to it, the costs incurred for the development and improvement of software aimed at the wide implementation of digital technologies (dtx) were chosen as a factor indicator. The total revenue of the railway transport industry (d), the total volume of freight transported by railway (freight), and the number of passengers using the railway were selected as the outcome indicators (Fig. 1).

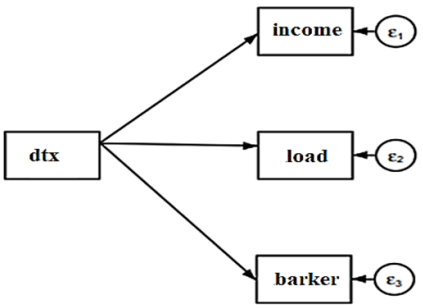


Figure 1: The impact of the use of digital technologies on the railway infrastructure.

In scientific research, we use n-index and linear regression models. To do this, we used the method of least squares to create process regression models:

$$F = \sum(Y - Y_x)^2 \to m \text{ or } F = \sum(Y - \beta_0 - \beta_1 x - \beta_2 x^2 - \cdots - \beta_k x^k)^2 \to min$$

if we take a special derivative from it, the following system of equations is formed. The level multifactor econometric model looks like this:

$$Y = \beta_0 * x_1^{\beta_1} * x_2^{\beta_2} * \ldots * x_n^{\beta_n} \quad (1)$$

Hence, Y is the resulting factor; $x_1, x_2, \ldots, x_n$ - influencing factors.

If we substitute the natural logarithm in the model presented in the (1), then we will have the following form:

$$\ln(y) = \ln(\beta_0) + \beta_1 \ln(x_1) + \beta_2 \ln(x_2) + \ldots + \beta_n \ln(x_n) \quad (2)$$

In the model ln(y) = y`, ln(ß0) = ß0', $\ln(x_1) = x_1', \ln(x_2) = x_2', \ldots, \ln(x_n) = x_n'$ if we make the designations, then we will have the following appearance:

$$y' = \beta_0' + \beta_1 x_1' + \beta_2 x_2' + \ldots + \beta_n x_n'. \quad (3)$$

In the above model, the following system of normal equations is created to find unknown parameters (b_0,)`(b`_1,…,(b_ n)` )`

$$\begin{cases} n\hat{\beta}_0 + \hat{\beta}_1 \sum x'_1 + \hat{\beta}_2 \sum x'_2 + \cdots \hat{\beta}_n \sum x'_n = \sum y' \\ \hat{\beta}_0 \sum x'_1 + \hat{\beta}_1 \sum x'^2_1 + \hat{\beta}_2 \sum x'_1 x'_2 + \cdots \hat{\beta}_n \sum x'_1 x'_n = \sum x'_1 \\ \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots \\ \hat{\beta}_0 \sum x'_n + \hat{\beta}_1 \sum x'_n x'_1 + \hat{\beta}_2 \sum x'_n x'_2 + \cdots \hat{\beta}_n \sum x'^2_n = \sum x'_n y' \end{cases} \quad (4)$$

If this system of normal (4) is analytically solved by several methods of mathematics, then the values of unknown parameters (b_0,)`(b`_1,…, (b_ n)` )` are found.

The main goal in any econometric modeling is to determine the beta coefficient, for which the beta coefficient should be BLUE (best linear unbiased estimator), i.e. F-test, t-test, in addition to statistical significance, the model meets the requirements of Gauss Markov, especially the requirements of heteroscedasticity and autocorrelation [7]. The model residuals should not be correlated with the corresponding model values, nor should the lags of the residuals be systematically related. To get rid of heteroscedasticity in general, model data were normalized, that is, logarithmzed.

2.1 Econometric Modeling Approach to Railway Income under Digital Transformation

Econometric methods were used in the "STATA" program to analyze the changes of revenues and expenses in the field of railway transport using cloud-based computational approaches [16], and the relevant coefficients were calculated in Table 1 [8].

The analysis of the results obtained in Table 1 shows that the coefficient of determination in the regression model is $R^2 = 0.95$; F count 107.20; (when, F tab=2.17) is equal to. When we compared each coefficient using by given criterion, we found that the calculated values exceeded the values in the table.

Typically, the coefficient of determination ranges from [0;1]. The closer it is to 1, the stronger the relationship. In this context, a coefficient of determination of 0.95 indicates a strong connection between the economic indicators in the model. To compare models with varying numbers of factors, and ensure the number of factors doesn't influence the R^2 statistic, a corrected version of the coefficient of determination is generally employed:

$$R^2_{\text{plain}} = 1 - \frac{s^2}{s_y^2}. \quad (5)$$

In this instance, the adjusted coefficient of determination is 0.94, and its proximity to R^2 suggests that the change in the number of influencing factors in the model is acceptable. To assess the statistical significance of the constructed multifactor econometric model and its alignment with the studied process, we use Fisher's F-criterion. The true value of the F-criterion is calculated using the following (6):

$$F_{\text{count}} = \frac{R^2}{1-R^2} \cdot \frac{n-m-1}{m}, \quad (6)$$

here: R^2 - determination coefficient; n - The total count of data points; m - the total count of variables. The actual value of the F-criterion is F_ count =107.20. When the actual F-statistic value surpasses the critical value from the statistical table, it suggests that the multifactor econometric model effectively explains the variability in the dependent variable. This implies that the factors in the model have a substantial influence on the studied process, making the model statistically significant and appropriate for further analysis or forecasting.

2.2 Strengths and Weaknesses of Econometric Modeling of Railway Income

Advantages of the econometric modeling of income changes based on digital technologies:

1) To determine the tabular value of the F-criterion, we first calculate the degrees of freedom and the significance level. With a significance level and degrees of freedom of k1=2 and k2=20-2-1=17, the F-criterion value from the table is F table = 2.17;
2) F calculation>>F table satisfies the condition, which means that the calculated value of F_ count is statistically significant than the value in the table, and can be used in forecasting for future periods;
3) The student's t-test is used to check the reliability of multifactor econometric model parameters and correlation coefficients. In this case, their value is compared with the values of random errors [9], [15].

Disadvantages of the econometric modeling of income changes based on digital technologies:

1) By comparing the calculated and table values of Student's t-test, we accept or reject the H_0 hypothesis. For this, we find the tabular value of the t-criterion based on the conditions of the selected reliability probability and degree of freedom. Here are the number of observations and factors [10], [13], [14].

Table 1: Econometric modeling of income changes based on digital technologies.

Variable	Model coefficients	Standard errors	t-Student test	P-value
β_1	2.13	0.20	10.65	0,0000
C	-1.17	0.17	-6.88	0,0000
R^2-Coefficient of determination	0,95	F - Fisher's criterion	107.20	
Adjusted R^2-Coefficient of Determination	0,94	Prob (F - Fisher's criterion)	0,0000	
Breusch Pagan test	1.44 (0.23)	DW- Durbin-Watson criterion	2.02	
Akaike's information criterion	-13.97	Schwartz's information criterion	-14.08	

2) When the reliability probability and the degree of freedom are d. f.=20-2-1=17, the table value of the t-criterion is equal to t table=2.14. We assume that t_ count is equal to 10.65 for the beta coefficient, that the |t_ count |≫t _tab condition is satisfied, and that the coefficient is statistically significant.
3) There are several tests for heteroscedasticity, the main one being the Breusch-Pagan test. In this test, the probability level is less than 0.05, so the main hypothesis is accepted, and the alternative hypothesis is rejected. It is expressed that the model's residuals are not associated with the corresponding values [9].

3 ANALYSIS OF THE ECONOMETRIC MODELING

The Breusch is 1.44 in the Pagan test, and the probability is 0.23. The model has a homogeneity property, the beta coefficient is BLUE.

We use the Darbin -Watson (DW) criterion to check autocorrelation in the residuals of the resulting factor according to the model [9]

$$DW = \frac{\sum_{t=2}^{T}(e_t - e_{t-1})^2}{\sum_{t=1}^{T} e_t^2} = \frac{\sum_{t=2}^{T} e_t^2 + \sum_{t=2}^{T} e_{t-1}^2 - 2\sum_{t=2}^{T} e_t e_{t-1}}{\sum_{t=1}^{T} e_t^2} = \quad (7)$$

$$= 2 - 2\frac{\sum_{t=2}^{T} e_t e_{t-1}}{\sum_{t=1}^{T} e_t^2} \approx 2(1-\rho_1),$$

This is the first-order correlation coefficient ρ_1. If there is no autocorrelation among the residuals, the resulting factor approaches zero in the case of positive autocorrelation and 4 in the case of negative autocorrelation.

$$\begin{cases} \rho_1 = 0 \rightarrow DW = 2; \\ \rho_1 = 1 \rightarrow DW = 0; \\ \rho_1 = -1 \rightarrow DW = 4. \end{cases}$$

The calculated DW is compared with the DW in the table. If there is no autocorrelation in the residuals of the resulting factor, then the value of the calculated DW criterion will be around 2. In this example, the value of the calculated DW criterion expresses the same dimension with 2.02. This suggests that there is no autocorrelation in the residuals of the resulting factor.

Therefore, we chose this following model of regression as adequate:

$$lnd = -1.17 + 2.13lndtx; \quad (8)$$

The coefficient of the model is BLUE and it is statistically significant according to F test, t test and Gauss Markov. Akaike and Schwartz criteria indicate that the model is optimal [11]. The beta coefficient is 2.13. That is, a 1% increase in expenses for the development of digital software in the railway system leads to a 2.13% increase in revenue from this sector.

3.1 Econometric Modeling of Rail Freight

To further test our hypothesis, we model the dependence on rail freight based on digital software development (Table 2).

The analysis of the results obtained in Table 2 shows that the coefficient of determination in the regression model is R^2 = 0.857; F count= 30.02; (when, F tab=2.17) is equal to. When we compared each coefficient according to the student criterion, it was found that the calculated values are greater than the table values. In this case, the coefficient of determination is equal to 0.857, which means that there is a sufficiently strong relationship between these economic indicators in the model.

Table 2: Econometric modeling of changes in railway freight on the basis of digital technologies.

Lnyuk	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Lndtx	.389	.071	5.48	.003	.206	.571	***
Constant	8.342	.568	14.70	0	6.883	9.802	***

Mean dependent var	11.452	SD dependent var	0.066
R-squared	0.857	Number of obs	7
F-test	30.032	Prob > F	0.003
Akaike crit. (AIC)	-28.882	Bayesian crit. (BIC)	-28.990

*** p<.01, ** p<.05, * p<.1

The adjusted coefficient of determination equal to the value of 0.82 and its closeness to R^2 means that the change in the number of influencing factors of the model is accepted around the values.

The calculated value of the F-criterion is F count = 30.02. To determine the tabular value of the F-criterion, we first calculate the degrees of freedom and the significance level. Given the significance level and degrees of freedom k1=2 and k2=20-2-1=17, the table value of the F-criterion is F table = 2.17.

F calculation>>F table satisfies the condition, which means that the calculated value of F-criterion is statistically significant than the value in the table, and can be used in forecasting for future periods.

By comparing the calculated and tabular values of Student's t-test, we determine whether to accept or reject the null hypothesis H_0. To do this, we find the tabular value of the t-criterion based on the chosen level of confidence (α) and degree of freedom ($\mathrm{d.f.} = n - m - 1$). Here, n- represents the number of observations, and m- denotes the number of factors.

When the probability of reliability and the degree of freedom are d. f.=20-2-1=17, the table value of the t-criterion is equal to t_ tab=2.14. We assume that t_ count is equal to 5.48 for the beta coefficient, that the |t_ count |≫t_ condition is satisfied, and the coefficient is statistically significant.

We found the model to be statistically significant by F test, t test, coefficient of determination. Now we check the Gaussian Markov conditions.

Breusch-Pagan / Cook-Weisberg test for heteroscedasticity. Ho: constant variance. Variables: fitted values of lnyuk chi2(1) = 0.63 Prob > chi2 = 0.4284.

When heteroskedasticity was tested using the Breusch Pagan test using STATA software, the chi2 (X square) value was 0.63, and the probability level was 0.42. In this case, we accept the main hypothesis and reject the alternative hypothesis. There is no heteroscedasticity in the model, the model residual is a free variable.

3.2 Econometric Modeling of Rail Passenger Traffic

In the analysis of the third part of our hypothesis, we model the relationship between the development of digital software in the system of passenger transportation by railway (Table 3).

The analysis of the results obtained in Table 3 shows that the coefficient of determination in the regression model is R^2 = 0.49; Faccount= 19.88; (when, F table=2.17) is equal to. When we compared each coefficient according to the student criterion, it was found that the calculated values are greater than the table values.

There are a number of tests for heteroscedasticity, the main one being the Breusch Pagan test, where the probability level is less than 0.05, the main hypothesis is accepted and the alternative hypothesis is rejected. Model residuals are represented as uncorrelated with fit values. Breusch is 1.65 in Pagan's test and the probability is 0.95. The model has homogeneity property, the beta coefficient is BLUE.

A 1% increase in spending on the development of digital software in the railway system affects a 2.53% increase in railway passenger traffic (Fig. 2).

Table 3: Econometric modeling of rail passenger changes based on digital technologies.

Lnyoovchi	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Lndtx	2.53	0.145	17.44	.001	2.473	8.413	***
Constant	29.807	9.162	3.25	.023	6.256	53.358	**

Mean dependent var	9.563	SD dependent var	0.566
R-squared	0.494	Number of obs	7
F-test	19.884	Prob > F	0.000
Akaike crit. (AIC)	10.057	Bayesian crit. (BIC)	9.949

*** p<.01, ** p<.05, * p<.1

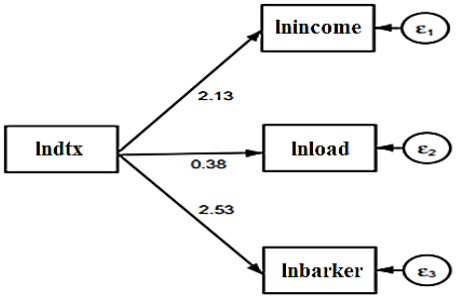


Figure 2. Results of the impact of the use of digital technologies on the railway infrastructure.

All three coefficients are BLUE and statistically significant under all conditions of F test, t test and Gauss Markov. Akaike and Schwartz criteria indicate that the models are optimal.

4 CONCLUSIONS

Modeling the process of development of service industries and its correlation with performance should be considered as a way to determine the direction of development of the service industry using these factors. This problem can be solved with the help of modern digital technologies and tools, which include models for predicting the relationship between the costs and revenues of passenger transportation. Thus, the main requirements for the railway transport development model include: introduction of organizational management mechanisms; using the efficiency of the transport sector as an objective function, taking into account the impact factors; expansion of means aimed at improving the efficiency of passenger transportation.

A 1% increase in digital software processing costs in the railway system will lead to a 2.13% increase in railway freight transportation, a 0.38% increase in railway passenger transportation, and a 2.51% increase in railway total transportation.

The high coupling type of the tested model indicates the effectiveness and performance of the developed model. The study showed that the information obtained helps to reduce ineffective subjective management decisions. In the process of forecasting, it is necessary to identify possible alternative methods for the implementation of tasks aimed at increasing the efficiency of management of railway transport organizations and justify their effectiveness.

This proposal will lead to the formation of a "logistic transport chain" that integrates all participants in the freight transportation process into a unified management system on the railways of the Republic of Uzbekistan, ensuring the optimization of all cargo flows. Additionally, it provides an opportunity to reduce the annual volume of operating costs related to wagon turnover by an average of 17-18%. Based on the e-model, it is possible to develop forecast values of the pessimistic, optimistic, and average scenario variants for the impact of infrastructure improvement costs on the volume of freight and passenger transportation by rail until 2027.

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Minimizing Makespan in Open-Shop Operating Room Scheduling Using Variants of Bat Algorithm

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Keywords: Operating Room Scheduling, Makespan, Bat Algorithm, Operation Room, Surgeries.

Abstract: Operational healthcare management faces growing pressure to develop efficient operating room scheduling systems because these systems require optimal resource management, minimum patient waiting times, and reduced operational expenses. Scheduling problems have traditionally been solved using mixed integer programming (MIP), but these methods have become computationally inefficient when dealing with large complex scheduling scenarios. Exact scheduling methods lose their practical value in hospital operating room planning as the problem complexity rapidly increases according to the number of available operating rooms and scheduled surgeries. The necessity for adaptable intelligent scheduling methods emerges because organisations require methods that maintain excellent solution quality along with swift execution and cost-effective resources. The Bat Algorithm (BA) techniques within swarm intelligence demonstrate successful potential to solve challenging combinatorial issues through natural optimisation methods. This paper uses five Bat Algorithm optimisations to resolve operating room scheduling by reducing makespans. The algorithms examined are the Modified Bat Algorithm (MBA), Chaotic Bat Algorithm (CBA), Discrete Bat Algorithm (DBA), Multi-Objective Bat Algorithm (MOBA), and Binary Bat Algorithm (BBA). For this analysis, two distributions are the Pearson and Fisher distributions. We perform several Operating room scheduling studies to assess the effectiveness and efficiency of these five algorithms on different distributions and compare the results. The findings indicate that all five improved the solution of Operating room scheduling, but the Chaotic Bat Algorithm (CBA) with Fisher's distribution became the best solution for the given scenario.

1 INTRODUCTION

Operating room scheduling is a fundamental optimisation challenge in healthcare systems, where the goal is to allocate a set of surgeries $S = \{s_1, s_2, \ldots, s_n\}$ to a set of operating rooms $R = \{r_1, r_2, \ldots, r_m\}$ while considering constraints related to surgeon availability, surgery durations, and hospital operational limits. The complexity of this problem arises from the need to minimise key performance indicators such as makespan M while ensuring that each surgery si is scheduled within its feasible time window [T_i start, T_i end]. Stochastic factors, such as variations in surgery durations and unexpected delays, further complicate the optimisation process. Traditional scheduling approaches rely on deterministic models that attempt to minimise an objective function by exhaustively searching the solution space [1]. However, due to the combinatorial nature of the problem, where the number of possible schedules grows exponentially as $O(m_n)$, exact methods such as Mixed Integer Programming (MIP) become computationally impractical for large-scale instances. As a result, metaheuristic algorithms, particularly swarm intelligence techniques, have gained significant attention for their ability to efficiently navigate complex search spaces and provide near-optimal solutions in reasonable time frames. Swarm intelligence-based algorithms, such as the Bat Algorithm (BA), the Bat Algorithm is inspired by the echolocation mechanism of the bats, where the frequency f, velocity v and pulse rate p of all bats vary dynamically in search of improving the quality of their solutions. Adapting their frequency modulation, each bat explores the solution space corresponding to a schedule configuration. Adjusting the intensity of the emitted pulses will perform a local search, or the algorithm will be released for continued global search [2]. The operating room department is a high-

cost and high-revenue sector in many hospitals. Hospital administrators continuously seek ways to create schedules that optimise the use of operating rooms while reducing overall expenses. Some hospitals employ an open scheduling method, allowing surgeons to choose any workday for their surgeries, with anaesthetists and nurses coordinating with the surgeons to maximise efficiency [3]. Since the operating room division usually incurs the highest expenses and generates the most income, ensuring effective utilisation of operating rooms is crucial. This study aims to find an effective operating schedule that optimises the use of operating rooms. Due to the complexity of scheduling issues in operating rooms, we propose utilising the Bat Algorithm (BA) to address this problem [4] efficiently.

This paper examines an operating room scheduling issue, employing five recently proposed bat optimisation algorithms to solve the OSS to minimise the makespan. These algorithms include the Modified Bat Algorithm (MBA), Chaotic Bat Algorithm (CBA), Discrete Bat Algorithm (DBA), Multi-Objective Bat Algorithm (MOBA), and Binary Bat Algorithm (BBA), along with two distributions: Pearson distribution and Fisher distribution. Various OSS cases are conducted and compared to evaluate the effectiveness and efficiency of the five algorithms with these distributions. The results demonstrate that all five algorithms can significantly improve the solution to open shop scheduling, with the Chaotic Bat Algorithm (CBA) using Fisher's distribution on the artificial dataset appearing to be the most competitive solver for the given cases. The remainder of this paper is structured as follows: Section 2 discusses related works on OSSP, Section 3 presents the problem formulation, Section 4 briefly introduces the five Bat algorithms, Section 5 outlines the proposed model, Section 6 presents the results and discussion, and Section 7 concludes the paper.

2 RELATED WORKS

Lin, Yang Kuei Li, et al. [4] utilised the Artificial Bees Algorithm (ABC) and the MEDD and MLPT algorithms to analyse data generated using the third Pearson distribution for operating times ranging from 40 to 150 minutes. Surgeons were randomly assigned to six operating rooms, and the results demonstrated high efficiency for the ABC. Rahimi et al.. [6] discussed a hybrid simulated cooling algorithm (Hybrid SA) designed to address the no-wait open surgery scheduling problem (NWOSP-SCSP). They developed a Mixed Integer Linear Programming (MILP) model for small cases, which was tested using data from hospitals in the Nova Scotia Health Author [7]. Abdelmaguid and Tamer F proposed a solution for the multi-processor store scheduling problem (DMOSP) in medical maintenance and diagnostics. This was achieved using the NSGA-II and MOGWO algorithms, enhanced with simulated annealing (SA) to improve search effectiveness. Using information on several stores, processing durations, operation priority, and no-interruption restrictions, the model was verified against 30 benchmark scenarios. By modeling the surgical scheduling problem as a flexible job shop scheduling (FJSSP) problem, Xiang et al., [8] used the Ant Colony Optimization (ACO) technique. Data from five distinct test cases with resource constraints, such as operating rooms, nurses, and surgeons, were used. Fei and colleagues [9] investigated the surgical scheduling problem by establishing the weekly operating plan using data from a university hospital in Belgium and a hybrid genetic algorithm (HGA) in conjunction with a vertical generation-based hashing process (CGBH).

3 PROBLEM FORMULATION

Operational healthcare management faces growing pressure to develop efficient operating room scheduling systems because these systems require optimal resource management, minimum patient waiting times, and reduced operational expenses. Scheduling problems have traditionally been solved using mixed integer programming (MIP), but these methods have become computationally inefficient when dealing with large, complex scheduling scenarios. Exact scheduling methods lose their practical value in hospital operating room planning as the problem complexity rapidly increases according to the number of available operating rooms and scheduled surgeries. The necessity for adaptable intelligent scheduling methods emerges because organisations require methods that maintain excellent solution quality along with swift execution and cost-effective resources. The Bat Algorithm (BA) techniques within swarm intelligence demonstrate successful potential to solve challenging combinatorial issues through natural optimisation. Both algorithms receive independent application in optimisation domains, yet researchers have not compared their performance directly for operating room scheduling in the literature.

4 BAT ALGORITHM

The Bat Algorithm (BA) was proposed by Xin-She Yang in 2010. It is a unique optimisation technique that mimics a bat's behaviour as it seeks prey and avoids obstacles during twilight by utilising echolocation similar to microbats (see Fig. 1).

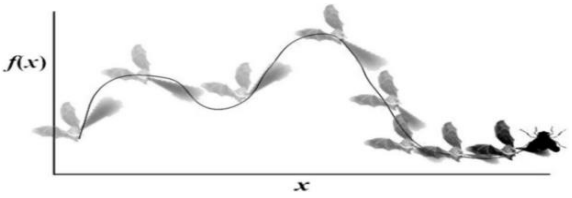


Figure 1: The trajectory of a single bat when it searches for prey and/or avoids difficulties in dusk [10].

This search method is a new metaheuristic swarm intelligence optimisation strategy for global numerical optimisation, and it is inspired by the social behaviour of bats and the phenomenon of echolocation, which is utilised to detect distance. The purpose of its creation was to be applied to global numerical optimisation. The criteria, which are simplified in the [5] approach, can be used to estimate or idealise bat echolocation. All bats use echolocation to improve their sense of location and appear to "know" where they are at all times. Bats employ a range of foraging strategies to find prey, such as flying randomly, flying at a set frequency at a particular location, and using a variable wavelength and loudness A0. They could alter the amplitude r [0, 1] of the pulses they generated and their frequency and wavelength r [0, 1], which vary according to their proximity to their target. The loudness is thought to range from a small constant (positive) Amin to a high A0, though it can vary [11]. This means that each bat is described by its position (x), velocity (v), frequency (f), loudness (A), and emission pulse rate (r). The new position (x_i) and velocities (v_i) can be calculated using the approach for:

$$f_i = f_{min} + (f_{max} - f_{min}) .rand() \quad (5)$$

$$v_i^{t+1} = v_i + (x_i - x^*).f_i \quad (6)$$

$$x_i^{t+1} = x_i + v_i . \quad (7)$$

Where [0, 1] is a random vector produced from a uniform distribution, and $\beta \in [0, 1]$ is a constant vector. The best position is determined by comparing the best positions of all bats worldwide. Depending on the domain size in which the issue is addressed, implementation usually allocates frequency f values between 0 and 100. First, an evenly distributed random frequency is assigned to each bat from the range [fmin, fmax]. Once the best solution has been selected from the previously recognised best solutions, a new solution is generated for every bat using the random walk technique.

$$x_{new} = x^* + A^t. \quad (8)$$

The typical PSO functions similarly to fi, which regulates particle velocity and updates bat locations and velocities [12]. This is because fi regulates their speed and distance. To a certain extent, BA combines the traditional PSO with the more focused local search controlled by the sound's volume and pulse rate. It is also important to remember that, as shown in (9), the loudness. And the rate of pulse emission changes as the iterations continue.

$$A_i^{t+1} = \alpha A_i^t , \quad r_i^t = r_i^0 [1-\exp(-\gamma\tau)]. \quad (9)$$

4.1 Bat Algorithm Variants

This section explains the Bat Algorithm (BA) variants and showcases various methods to enhance its efficiency. It is divided into five subsections.

4.1.1 Binary Bat Algorithm (BBA)

The original Bat Algorithm is primarily designed for continuous optimisation. Its operators must be adjusted to specialise in BA for binary optimisation problems. Several researchers have proposed the binary version of BA, namely the binary bat algorithm (BBA). In BBA, artificial bats move in binary searching space by moving from "0" to "1" and from "1" to "0" [5]. Moreover, the feature selection problem has been solved using the Enhanced Binary Bat Algorithm (EBBA). EBBA augments three methods to improve the efficiency of BA in feature selection: the Lévy flight-based mechanism, chaos-based loudness [7], and the procedure to enhance the diversity of the population [13].

4.1.2 Discrete Bat Algorithm (DBA)

Xin-She Yang created the original bat method in 2010 [6], and the Discrete Bat method is a variation of that technique. To find prey, it mimics how bats use echolocation. This algorithm addresses discrete optimisation problems, including routing problems, task scheduling, and shortest path determination. The Discrete Bat Algorithm uses vectors or combinations, like binary encoding or permutations, to express

solutions in a discrete space. The basic functions, such as updating locations and velocities, are altered to accommodate the distinct features of the issues it tackles. The algorithm aims to strike a balance between exploring new areas and making use of promising solutions. The main distinction between the discrete and original versions of the BA is that the former uses continuous calculations to update positions, whilst the latter modifies these operations to operate in a discrete search space [10].

4.1.3 Modified Bat Algorithm (MBA)

Although the original Bat Algorithm works well for many optimisation problems, it has drawbacks, including restricted exploration capabilities and the potential to become stuck in local optima. The Modified Bat Algorithm (MBA) is one of the changes suggested to overcome these problems. These changes are intended to improve the algorithm's capacity for exploration and prevent it from being trapped in local optimal solutions [14].

4.1.4 Chaotic Bat Algorithm (CBA)

An improvement on the original Bat Algorithm, the Chaotic Bat Algorithm (CBA), incorporates chaotic ideas to enhance exploration and exploitation capabilities while searching for optimal solutions. This approach efficiently avoids local solutions and increases the probability of achieving global optimal solutions using chaotic systems' deterministic and aperiodic properties [15].

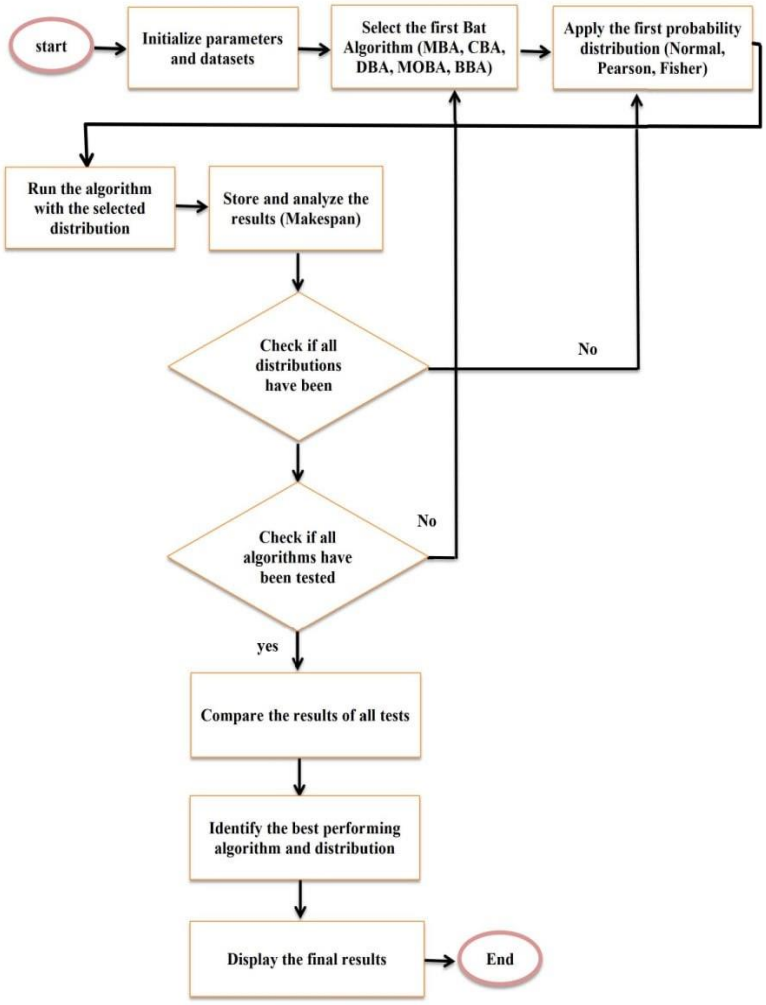


Figure 2: The proposed system.

4.1.5 Multi-Objective Bat Algorithm (MOBA)

Xin-She Yang created the initial bat algorithm in 2010 and expanded it into the Multi-Objective Bat Algorithm (MOBA). MOBA focuses on multi-objective optimisation problems, particularly those involving competing objectives. This approach, which was modified to satisfy the requirements of multi-objective optimisation, mimics bats' echolocation behaviour [16].

5 PROPOSED MODEL

This proposed system (Fig. 2) uses five types of Bat Algorithms for solving Operating room scheduling using these five variants of BA (Bat Algorithm): the Modified (BA), Chaotic (BA), Discrete (BA), Multi-Objective (BA), and Binary (BA) with Pearson's, Fisher distribution.

6 RESULT AND DISCUSSION

The system was implemented using Python 3.12 on a machine with the following specifications: Intel Core i7-4600U 2.10 GHz with Turbo Boost up to 2.70GHz, 8.00GB RAM, 64-bit x64 architecture.

6.1 Variants of Bat Algorithm with Pearson Distribution

In parameters (number of surgeries = 10, number of rooms = 3, number of surgeons = 5, iterations = 100, population = 20, location = 90), with used the Equation BMS (Balanced Makespan) :

$$BMS = Max\,(T) - \frac{1}{N}\sum_{i=1}^{N} T_i \ , \quad (10)$$

where $T = (T_1, T_2, \ldots, T_N)$ is the vector of processing times for each room, N is the number of rooms, $Max\ (T)$ represents the maximum processing time among all rooms, $\sum_{i=1}^{N} T_i$ is the total processing time across all rooms.

The Modified Bat Algorithm improves upon the standard Bat Algorithm by refining the position update strategy and incorporating adaptive parameters for better convergence. In this case, the MBA achieved a makespan of 178.2, which is reasonable. The Chaotic Bat Algorithm employs chaotic maps to increase randomness in position updates, helping to escape local optima and enhance diversity in the search space. However, in this case, the CBA resulted in a higher makespan of 176.0 compared to the Multi-Objective Bat Algorithm (MBA), suggesting that excessive randomness may lead to less efficient solutions. With a makespan of 184.5, the Discrete Bat Algorithm showed the highest value in the Pearson distribution. This suggests that the performance and instability of solutions vary greatly. Its intermittent nature is the primary cause of this instability, making the search less fluid than other algorithms. This feature makes it more difficult for the algorithm to continue improving, even if it might be useful for applications that need rigorous selection. Performance stability might be enhanced by optimising update techniques, which would lessen the detrimental consequences of excessive volatility. The Multi-Objective Bat Algorithm (MOBA) is a well-known algorithm for solving multi-objective problems because it balances exploration and exploitation. Its makespan of 171.4, less than that of the MBA and CBA, suggests that it is more stable when dealing with multi-criteria optimisation problems. Even if it can yield excellent results, improving the search approach could further lower variance. As a result, it works well when balancing several goals is necessary, but it might not be the ideal choice for issues with a single answer. With a makespan of 167.1, the Binary Bat Algorithm (BBA) yielded the lowest value in the Pearson distribution. This reflects excellent stability in performance compared to the other algorithms. The BBA is based on a binary representation, which allows it to search in a structured manner, effectively identifying optimal solutions. This organised approach reduces solution fluctuations by concentrating the search in specific areas, enhancing efficiency in problems with clear constraints (Table 1 and Table 2). Resulting in personal distributions for various bat algorithms.

6.2 Variants of Bat Algorithm with Fisher Distribution

In parameters (No. surgeries = 2172, No. rooms = 62, No.surgeons = 334, iterations = 700, population = 200).MOBA performed best in Makespan, with the lowest value among all algorithms. It also achieved the best balance (Balanced Makespan = 831.58), indicating a more equitable distribution of work across operating rooms. MOBA's success can be explained by the fact that it considers multiple objectives simultaneously, allowing for a good balance between minimising overall time and achieving balanced distribution. CBA performed

worst regarding Makespan, with the highest value (4178.26 minutes), but Balanced Makespan was (969.57). BBA a high Makespan (4146.85 minutes). Balanced Makespan was 938.16, which is significantly unbalanced. This is likely due to BBA relying on binary updates, making it less flexible in adapting to process distribution requirements. DBA performed slightly better than BBA but still poorly compared to MOBA and MBA. The balanced makespan was 936.75, roughly equal to BBA's, indicating inefficient process distribution. This result suggests that DBA could not find a highly efficient schedule, perhaps due to the intermittent updates, which may have limited the search for optimal solutions. In parameters (No. surgeries = 10, No. rooms = 3, No.surgeons = 5 , iterations = 100, population = 20) with.(MBA) MBA performed very well, achieving the second-best Makespan after CBA. The distribution was fairly balanced, with Balanced Makespan = 17.11, which is good compared to other algorithms but not the best. Therefore, we conclude that the MBA was efficient but not the best in balance compared to the CBA.MOBA's performance was close to an MBA's but had a slightly higher Makespan. Balanced Makespan was 17.55 minutes, close to MBA but slightly worse. Therefore, we conclude that MOBA did not outperform MBA but achieved similar results. It had the worst Makespan of all the algorithms (350.7 minutes), meaning it did not find an efficient solution like the other algorithms. The balanced makespan was 25.3 minutes, larger than all the other algorithms, indicating that the work distribution was not as good. Therefore, we conclude that BBA performed poorly compared to the other algorithms in Makespan and Balance. The best makespan among all algorithms (334.5 minutes), meaning that CBA found the optimal solution in terms of execution time. The best distribution of work across rooms, with a Balanced Makespan = 9.2 minutes, is lower than all other values, indicating a very balanced distribution. Therefore, we conclude that CBA outperformed all other algorithms regarding execution time and balanced distribution, making it the best choice. DBA achieved an average result with Makespan = 347.4 minutes, which is better than BBA but worse than MBA, MOBA, and CBA. Balanced Makespan was 22.11 minutes, which is not bad but not the best. Therefore, we conclude that DBA was better than BBA but not the best overall. In general, CBA is the best choice because it has achieved the lowest makespan and the best distribution of work. If there are limitations to using CBA, MBA or MOBA are the next best choices. BBA is not a good choice for this problem because it achieved the worst results.

Table 1: Various BA with pearson distributions.

No.Surgeries	No. R	No.Surgeons	iter	Pop.	Shape_param	Scale_param	Various BA.	Distribution	Mk.
10	3	5	100	20	3	90	MBA	Pearson	178.2
10	3	5	100	20	3	90	CBA	Pearson	176.0
10	3	5	100	20	3	90	DBA	Pearson	184.5
10	3	5	100	20	3	90	MOBA	Pearson	171.4
10	3	5	100	20	3	90	BBA	Pearson	167.1

Table 2: Variants of the BA with fisher distributions.

No. Surgeries	No. R	No. Surgeons	iter	Pop.	Dfn-Dfd	Scale	Various BA.	Distribution	Mk.	MK.MBS
2172	62	334	700	200	20,45	90	MBA	Fisher	4073.9	865.2
2172	62	334	700	200	20,45	90	CBA	Fisher	4178.2	969.5
2172	62	334	700	200	20,45	90	DBA	Fisher	4145.4	936.7
2172	62	334	700	200	20,45	90	MOBA	Fisher	4040.2	831.5
2172	62	334	700	200	20,45	90	BBA	Fisher	4146.8	938.1
10	3	5	100	20	15,60	90	MBA	Fisher	342.4	17.1
10	3	5	100	20	15,60	90	BBA	Fisher	350.7	25.3
10	3	5	100	20	15,60	90	CBA	Fisher	334.5	9.2
10	3	5	100	20	15,60	90	MOBA	Fisher	342.8	17.5
10	3	5	100	20	15,60	90	DBA	Fisher	347.4	22.1

7 CONCLUSIONS

Reducing costs while maintaining high-quality services is a priority for many healthcare facilities. The operating room is often one of the most expensive departments in a hospital, so hospital managers are constantly seeking effective ways to optimise their Use to save on operational costs. This The research employs five newly proposed Bat optimisation algorithms to address operating room scheduling and reducing the manufacturing period. The algorithms include the MBA, DBA, Multi-MOBA, BBA, and CBA, which were tested with two types of distributions: Pearson distribution and Fisher distribution. The results indicated that the CBA achieved the best performance when applied to an artificial dataset using Fisher distribution. Future research will focus on optimizing Bat algorithm variants, addressing constraints, and comprehensively exploring more realistic goals. The findings indicate that all five improved the solution of Operating room scheduling, but the CBA with Fisher's distribution became the best solution for the given scenario.

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Mathematical Modeling of the Kinematic Scheme Planetary Mixer Mechanism

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Keywords: Mathematical Modelling, Algorithm, Planetarymechanism, Mixer, Drive, Carrier, Satellite, Working Blade, Rotary Auger, Bowl, Gear, Wheel.

Abstract: Planetary mixer is presented, which uses drive mechanisms for the working bodies of the mortar mass mixture of a construction mortar mixer. The main objective of the study is to create a mathematical model in the mortar mixer mechanism of three different types of trajectory of the working blades with epicycloidal, hypocycloidal and vertical-circular lift of the mortar mixture from the bottom of the bowl to the upper level. To achieve this goal, a rotary screw auger is used for circulation with a central circular screw movement of the mortar mass mixture, which provides a vertical lift of the mixture mass for further mixing of the solution. The parametric equation of the screw conoid blade surface is obtained by an analytical method, and parametric equations of the epicyclic and hypocyclic motion of satellites in the planetary mechanism are determined. Methods: the article proposes theoretical developments of the device of planetary and rotary mechanisms, which were carried out according to the methodology of the main provisions of the theory of mechanisms and machines as applied to the technological processes of construction machines. The work is performed on the section of kinematic analysis of planetary mechanisms with determination of angular velocities of input and output links. Practical significance: providing multiple circulation of the solution mixture mass through planetary working blades and screw auger of several zones of different three types of trajectory of movement of mechanism links allowing intensive mixing of the mortar mixer. The discussion provides recommendations for further improvement of the developed planetary mixing mechanism.

1 INTRODUCTION

The study [1] presents the application of a mechanism for driving spindles in a cotton-picking machine using belt drives. Work on providing a compact design of devices mechanisms is given by Russian researchers [2], [3] in the food industry. Also in devices mechanisms drive transmissions of links are used for work on treatment facilities and reservoirs [4], [5], as well as in ventilation of wastewater treatment [6]. In works [7], [8] a structural synthesis and geometric analysis of planetary mechanisms of intermittent motion with elliptical wheels, as well as their kinematic motion are given. Kinematic research on the trajectory of motion of planetary mechanism satellites is given in works [9], [10]. In work [11] a planetary mechanism is investigated, where the satellites impart the required spatial movements to the working element simultaneously rotating around their axis and making a translational motion around the central sun wheel. The paper [12] presents experimental data on measuring the torque in a planetary gear, where the braking torques were measured on a setup with an input shaft speed n in the range from 1000 to 2800 rpm. The paper [13] presents the design of a composite link of a planetary gear transmission, where worn satellites can be easily replaced during operation. The paper [14] examines the design of a gearbox, where the gear ratio of a reducer is given at the maximum value of the gear ratio for each stage of a gear pair, and the gear ratio of a two-stage planetary type transmission was also investigated.

The paper [15] analyzes a planetary gearbox, in which the researcher determined that a planetary gear reduces the power consumption of a mechanism with respect to simple gears, and the properties of the lubricant in a planetary gearbox were also studied. The work [16] presents studies of a planetary mechanism of a state from two kinematic

cycles, where opposite movements of the satellites of the epicyclic and hypocyclic trajectory of motion are performed, in which it is envisaged to use braking devices and synchronizers in the transition zones between two toothed segments for the smooth entry of the gear into the toothed engagement.

2 RESULTS AND THEIR DISCUSSION

The mixing device of the mortar mixer has a combination of two designs: a simple one - a rotary screw and a spatial one - a planetary mechanism. This device is called an epi-hypocyclic planetary mechanism with a rotary screw for driving the working bodies of the mixer blades of the mortar mixer. These two mechanisms perform two different functions: the radially located epi-hypocyclic working blades of the planetary mechanism mix and stir the masses of the mortar mixture, and the rotary screw vertically lifts this mortar mixture from the bottom of the tank to the upper level of the bowl. The planetary mechanism for driving the rotary screw and working blades (Fig. 1a) has peripherally located epi-hypocyclic working blades, and a rotary screw 9 is installed in the center of the mechanism.

When the planetary mixing mechanism is in operation, the rotors of the electric motor rotate with an angular velocity ω_p clockwise, then the paired gears 2 and 4 rotate with an angular velocity ω_2 (ω_4) counterclockwise, wherein gear 4 causes the rotation of the carrier 10 (N) ω_n clockwise, and gears 7 and 8 of the satellites, touching in engagement with the fixed central gear wheel 6, also perform movements, only with opposite directions of rotation, for example, gear 7 of the satellite, touching the external gear engagement of the central wheel 6, performs movement with an angular velocity $\omega 7$ counterclockwise, there is movement with an epicyclic trajectory, and gear 8 of the satellite performs movements with an angular velocity $\omega 8$ clockwise with a hypocyclic trajectory of movement. Simultaneously with the planetary gear carrier 10(H), the gear 2 causes the toothed wheel 3 to rotate, which exceeds the angular velocity of the carrier 4 and rotates the rotary screw 9 with an angular velocity ω_{sh} counterclockwise in the center of the mixer tank (bowl) with a helical trajectory of the circumference of the screw diameter. The technological process of preparing the solution mixture in the tank 11 (bowl) of the mixer occurs as follows: the rotation of the shafts of the carrier 10 (N) and the rotary screw 9 occurs simultaneously from the electric motor 1 through the paired installed gears 2 and 4 and the toothed wheels 3 and 5, as a result of which the blades 12 and 13 of the satellites perform opposite planetary movements - an epicycloidal and hypocycloidal trajectory around the carrier 6 along the periphery of the tank, and in the center of the installed rotary screw 9, due to the high angular velocity ωm of rotation, a turbulent rotation of the screw surface of the screw occurs, providing a vertical rise of the solution mixture from the lower part of the tank to the upper level of the mortar mixer, then with sufficient pressure created by the screw 9, the mass of the solution mixture rises up along the center of the tank and under the action of centrifugal force, the solution moves to the peripherally located blades 13 and 12. Here, there is an intensive movement of the mixture mass between two opposite rotating blades 13 and 12 of the satellites. Then, the solution mixture, due to its own weight, falls down to the bottom of the tank and gets to the rotary screw auger 9 and the technological process of the cycle, mixing the solution mixture, is repeated, which ensures multiple circulation through the zones of rotary and planetary intensive mixing of the solution mixture.

For continuous vertical lifting and mixing of the mass of the solution mixture, the drive of the mixer mechanism transmission is made with increased parameters of the angular speeds ω_m of the screw rotor auger 9 relative to the speed parameters of the planetary mechanism carrier 10(N). Therefore, the drive of the transmission of the toothed engagements of the rotary screw auger 9 and the carrier 10(N) is transmitted separately through gear wheels 2-3 and 4-5 with different gear ratios – $U_{sh} < U_n$, where U_{sh} is the gear ratio of the rotary auger 9, $U_n = Z_3/Z_2 = 2 \ldots 3.15$; here Z_2 is the number of teeth of the drive gear 2; Z_3 is the number of teeth of the driven wheel 3, and U_n is the gear ratio of the carrier 10(N), $U_n = Z_5/Z_4 = 4 \ldots 6.3$. Then the angular velocity ω_{sh} of the rotary screw 9 will be greater than the angular velocity ω_n of the planetary mechanism carrier 10(H) – $\omega_{sh} > \omega_n$.

To determine the trajectories of movement of various points of the satellites of the epi-hypocyclic planetary mechanism in Figure 2, the following radii are designated: R_{N8} - the radius of the axis of rotation of the satellite 8 installed on the planet carrier for the inner central wheel; R_{N7} - the radius of the axis of rotation of the satellite 7 installed on the driver for the outer central wheel; R''_{K6} - radius of

the central wheel 6 with internal gearing; R''_{K6} - radius of the central wheel 6 with external engagement; r7 - radius of the point M_1 satellite 7 for external engagement of wheel 6 from its center of rotation axis and *r*8 - respectively the radius of the point M_2 satellite 8 for internal engagement of the wheel from its center of rotation axis. We will also designate the rotation angles: φ_H - for the driver H internal and externally located satellites 7 and 8, φ7 - for satellite 7; φ8 - for satellite 8.

Let us design the corresponding radii and angles of rotation of the mechanism links on the selected coordinate axes *YOX*. Then, at the initial moment of time, the points of the satellites of the internal 7 and external 8 positions M_1 And M_2 planetary gears are located on the axes *OY* And *OX*. In this movement, the instantaneous center of rotation of the satellites 7 and 8 always lies on the fixed central wheel 6, with which it is in gear engagement. To determine the trajectory of any point M_1 And M_2 satellites, we find the coordinates $M_1(x_1, y_1)$ And $M_2(x_2, y_2)$ these points for the moment of time when the driver H will start to turn at a certain angle φ_H.

The parametric equation of motion of the tooth point of satellites 7 and 8 in the planetary mechanism will be:

- for epicyclic movement of the point of the tooth of the satellite 7

$$\begin{cases} X = R_{H7} \cdot sin\,\phi_H - r_7 \cdot sin(\phi_7 + \phi_H) \\ Y = R_{H7}\,cos\,\phi_H - r_7 \cdot cos(\phi_7 + \phi_H) \end{cases}, \quad (1)$$

where R_{H7} – radius of the planetary gear carrier located at the centers of the satellite axis 7, mm; φ_H – angle of rotation of the planetary gear carrier, rad; r_7 – satellite radius 7 mm; φ_7 – angle of rotation of satellite 7, rad.

- for hypocyclic motion of satellite 8

$$\begin{cases} X = R_{H8} \cdot sin\,\phi_H - r_8 \cdot sin(\phi_8 + \phi_H) \\ Y = R_{H8}\,cos\,\phi_H - r_8 \cdot cos(\phi_8 + \phi_H) \end{cases}, \quad (2)$$

where R_{H8} – radius of the planetary gear carrier located at the centers of the satellite axis 8, mm; φ_H – angle of rotation of the planetary gear carrier, rad; r_8 – satellite radius 8 mm; φ_8 – angle of rotation of the satellite 8, rad.

(a) (b)

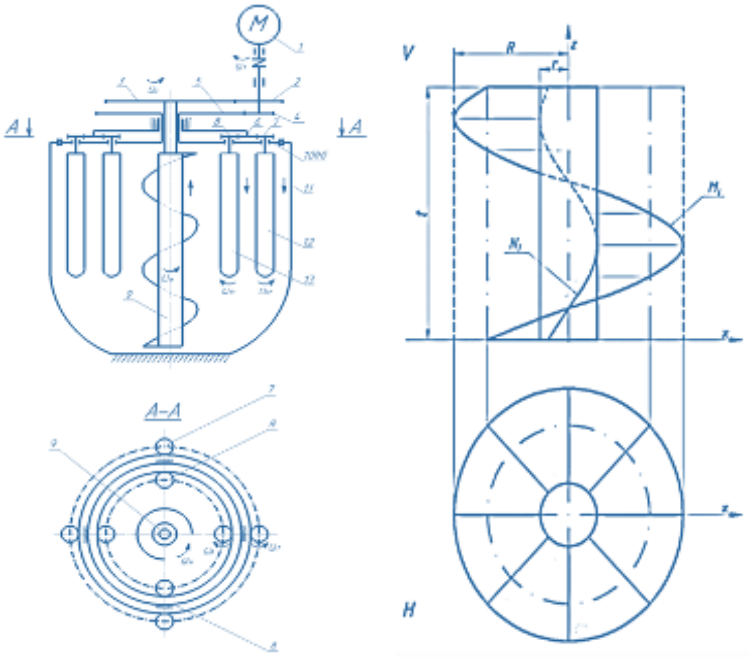


Figure 1: Kinematic diagram of the mechanism *a*) and part of the rotary auger assembly *b*).

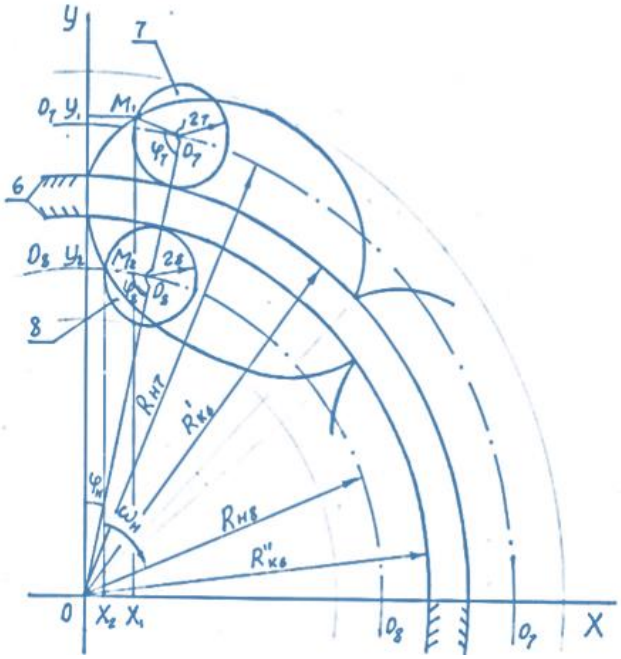


Figure 2. Kinematic diagram of the movement of satellites 7 and 8 planetary mixing mechanism in the YOX coordinate axis

Based on the data provided in Figure 2, we write the equality $\varphi_7 \cdot r_7 = \varphi_H \cdot R_{K7}$ and $\varphi_8 \cdot r_8 = \varphi_H \cdot R_{K8}$ from where $\varphi_7 = (R_{K7} / r_7) \cdot \varphi_H = i_{76} \cdot \varphi_H$, also $\varphi_8 = (R_{K8} / r_8) \cdot \varphi_H = i_{86} \cdot \varphi_H$,

where i_{76}, i_{86} – gear ratios from central wheel 6 to satellite 7, and also, accordingly, from central wheel 6 to satellite 8; $i_{76} = (R_{K7} / r_7)$; $i_{86} = (R_{K8} / r_8)$, R_{K7} and R_{K8} - pitch radii of the external and internal engagement of the fixed central wheel 6, respectively.

Substituting the obtained values φ_7 and φ_8 in (1) and (2) we obtain:

- for the hypocyclic curve of the satellite point 8:

$$\begin{cases} X = R_{H8} \sin \phi_H - r_8 \sin(i_{86} - 1)\, \phi_H; \\ Y = R_{H8} \cos \phi_H + r_8 \cos(i_{86} - 1)\, \phi_H; \end{cases} \quad (3)$$

- for epicyclical curve of point of satellite 7:

$$\begin{cases} X = R_{H7} \sin \phi_H - r_7 \sin(i_{76} + 1)\, \phi_H; \\ Y = R_{H7} \cos \phi_H + r_7 \cos(i_{76} + 1)\, \phi_H. \end{cases} \quad (4)$$

Given different numerical values $R_{H7} = 180мм$, $R_{H8} = 200мм$, $r_7 = r_8 = r_{sh} = 20мм$, $R`_{k6} = 120мм$, $R^{"}_{k6} = 180mm$ and the angle of rotation $\phi_H = 0...6,28rad$ carrier with an interval of i=0.1746 rad we obtained (Fig. 3) a graph of the curve of the trajectory of the epicyclic and hypocyclic motion of the planetary gear satellite and the rotary screw. For the lower part of the carrier H planetary mechanism, with the overall dimensions of the same satellites 7 and 8 and working bodies - blades 12, 13 and 9, we obtained a similar picture of the trajectory of the curves for the working blades of the mortar mixer: epicyclic, hypocyclic and rotary circular.

Figure 1b shows a part of the rotary screw assembly of the mechanism, which has a profile of the curved line of the annular screw conoid, formed by rotating the screw along a circular line on a horizontal plane, while the trajectory of the curve simultaneously moves from the lower base along a vertical line to the upper part of the tank.

The installed rotary screw performs the function of a screw conveyor for lifting the mass of the solution mixture. Here, the screw design consists of two parts: a vertically suspended fixed rod and a screw tape petal blade. The petal blade of the rotary screw consists of two trajectories of curvature: internal *N* and external *M*.

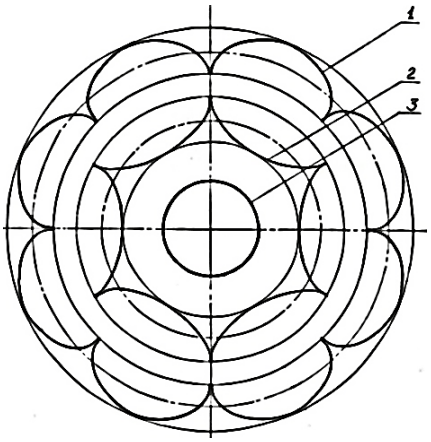


Figure 3: Graph of the curve of the epicyclic 1), hypocyclic 2) and rotary-circular 3) trajectory of movement of the blades 12, 13 and 9 of the mortar mixer.

The parametric equation of movement along the internal trajectory of curvature N of the rod has the form:

$$\begin{cases} X = r \cdot cos\,\alpha\,; \\ Y = r \cdot sin\,\alpha\,; \\ Z = 2\pi \cdot r \cdot \alpha, \end{cases} \quad (5)$$

where r is the radius of the rotary rod of the screw auger; α is the angle of elevation of the screw helical line, here, 2πrα is equal to the pitch t of the helical line: t=2πrα.

The outer trajectory of curvature M of the petal blade has the form:

$$\begin{cases} X = R \cdot cos\,\alpha\,; \\ Y = R \cdot sin\,\alpha\,; \\ Z = 2\pi \cdot R \cdot \alpha, \end{cases} \quad (6)$$

where R is the radius along the outer blade helical line of the auger.

From the (5) and (6) we have the total parametric equation of the surface of the petal blade, then in general it will look like:

$$\begin{cases} X = (R - r) \cdot cos\,\alpha + r \cdot cos\,\alpha\,; \\ Y = (R - r) \cdot sin\,\alpha + r \cdot sin\,\alpha\,; \\ Z = 2\pi \cdot R \cdot \alpha. \end{cases} \quad (7)$$

When subtracting the outer radius R of the screw turn from the radius r of the rotary rod, we obtain the surface area of the petal blade of the turn: $\ell = R - r$ – to calculate the movement of the material particle of the solution mass along the surface of the formed shelf and the angle α of the movement of the solution mass along the screw helical line. From here we select the average radius R_1, along the surface of the screw helical line, which is equal to: $R_1 = (R - r) \cdot 0{,}5 + r$. Then the final form of the parametric equation of the petal line will be:

$$\begin{cases} X = R_1 \cdot cos\,\alpha + r \cdot cos\,\alpha\,; \\ Y = R_1 \cdot sin\,\alpha + r \cdot sin\,\alpha\,; \\ Z = 2\pi \cdot R_1 \cdot \alpha. \end{cases} \quad (8)$$

3 CONCLUSIONS

This research introduces a mathematically modeled and kinematically examined planetary mixer mechanism that incorporates an epi-hypocyclic blade system alongside a central rotary screw auger to improve the mixing of construction mortar. The resulting parametric equations for the satellite blades' movement verified the creation of eight epicyclic and six hypocyclic arcs, illustrating intricate, multidirectional paths that guarantee total coverage of the mixing volume. At the same time, the rotary screw auger, represented by a conoid surface equation, generates a vertical-circular transport route, facilitating the steady upward movement of the mortar from the mixing bowl's bottom to its top. The determined gear ratios for the transmission system, U_{sh} = 2.0–3.15 for the screw and U_n = 4.0 to 6.3 for the carrier produced a greater angular velocity for the screw $\omega_{sh} > \omega_n$, promoting enhanced lifting and circulation. A kinematic diagram of an epi-hypocyclic planetary-mixing mechanism with a rotary screw for driving a construction mortar mixer is proposed. The analytical method is used to obtain parametric equations of motion of the point of the satellites of the epicyclic and hypocyclic trajectory, as well as a parametric equation of the surface of the petal blade of the screw conoid of the rotary screw.

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Mathematical Model Using LU Decomposition as Cryptographic System

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Keywords: LU Decomposition, Cyber Security, Hybrid Cryptographic Models, RSA-AES, Brute Force Attack.

Abstract: Cryptography plays a significant role in protecting privacy and secrecy in modern communications. The paper describes a novel text encrypt and decrypt scheme based on LU Matrix Decomposition, a numerical method. The scheme uses a key in the form of a invertible square matrix in order to encrypt and decrypt the text. It is broken down into lower triangular (L) and upper triangular (U) matrices. The text is reduced to numerical vectors, encrypted through L and U matrix transforms, and thereafter decrypted through their inverses. This method yields a systematic and secure process of encryption based on the intrinsic complexity of calculations in a matrix to secure the cryptographic process. The method is made calculational efficient with the efficient LU decomposition methods available in state-of-the-art numerical toolsets. Practical implementation issues such as block length and padding schemes are considered in order to handle variable text lengths. The paper evaluates the performance and security of the LU matrix crypto system and predicts and how the system would play a viable role in securing information in resource-constrained scenarios. The method demonstrates a singular combination of linear algebra and cryptography and opens the door for other research in inscriptive techniques based on matrices. While the method boasts strong confidentiality and operational efficiency, enhancements should preferably be made in the area of integrity verification and post-quantum security.

1 INTRODUCTION

Security in communication along with data secrecy is the call of the modern digital era. Cryptographic techniques by virtue of evolved nature offer protection for sensitive information from unauthorized accesses as well as cyber-attacks. Several traditional encryption schemes are available such as AES, RSA, and ECC widely used; however, they suffer from computation complexity and also get affected by cryptanalytic attacks [1]. Thus, current researchers are looking into alternatives such as other mathematical transformations like matrices-based encryption schemes which state security and efficiency improvement [2].

LU decomposition which is used extensively in numerical linear algebra is among these mathematical techniques. LU decomposition of any given matrix results in a lower triangular matrix (L) and an upper triangular matrix (U); computations can thus be done efficiently as required [3]. It converts a given plaintext into an encrypted form but maintains the structure within it to facilitate decryption [4]. We are discussing a technique that boosts the efficiency of computations while also improving the effectiveness of attacks on widely-used cryptographic methods, including differential and linear attacks [5]. Lately, researchers have begun to investigate matrix transformations in encryption. There's been a clear trend toward hybrid matrix-based cryptosystems, which strive to provide strong security without incurring high computational expenses [6]. Furthermore, deep learning techniques have been introduced to enhance key generation and authentication processes [7]. Nevertheless, current methods haven't quite found the sweet spot where security meets efficiency, and they often come with high resource demands [8]. This situation underscores

the ongoing struggle to create a lightweight cryptographic framework based on LU decomposition that can provide strong security features [9].

In this paper, we present a novel cryptographic method that leverages LU decomposition for secure text encryption. Our approach combines LU matrix factorization with modular arithmetic and key transformation techniques to bolster encryption. We've discovered that this scheme shows significantly higher key sensitivity than many traditional encryption methods and stands up well against various types of cryptanalysis, all while maintaining computational efficiency [10]. The criteria proposed were evaluated on a set of comprehensive metrics on security parameters, such as confidentiality, integrity, key sensitivity, and scalability, and this evaluation favors the proposed approach against the existing cryptographic techniques in terms of performance [11]. In particular, LU decomposition applies mathematics quite differently from traditional encryption schemes like RSA and AES, both in theory and realization (Table 1).

LU decomposition has advantage applications where matrix operations are inherently embedded such as image encryption and lightweight security. RSA offers a more secure means of key exchange and authentication using hard number-theoretic problems. AES is still considered to be the gold standard when it comes to fast and large-scale encryption thanks to its efficiency and strength against attacks (Table 2). The rest of the paper is arranged in the following way: Section 2 gives a comprehensively written literature review on existing matrix-based cryptographic methods. Section 3 introduces its mathematical background and implementation of the LU-based encryption scheme and it concerns a security analysis and evaluation metric. Section 4 contains comparative results with existing approaches followed by conclusions in Section 5 and future directions in Section 6.

Table 1: Mathematical foundations.

Feature	LU Decomposition	RSA	AES
Core Concept	Matrix factorization into lower (L) and upper (U) triangular matrices:	These are premised on modular exponentiation and integer factorization difficulty levels.	Uses substitution-permutation networks (SPNs) for symmetric encryption
Mathematical Operation	Matrix factorization in linear algebra (A = LU).	Number theory: Exponentiation mod N	Boolean algebra: Bitwise operations and substitution
Key Dependency	Encryption and decryption fundamentally rely on transformations and programmability of matrices.	This scheme functions based on public-private key pairs.	This scheme functions based on public-private key pairs.
Security Basis	Security depends on the complexity of reconstructing the original matrix	Security depends on the difficulty of factoring large primes	Security relies on S-box transformations, diffusion, and key expansion

Table 2: Practical implementation.

Feature	LU Decomposition	RSA	AES
Key Type	Either can be public or private based on the design matrix.	Public-private key pair)	Algorithm uses symmetric key for encryption and decryption.
Computational Efficiency	Good for low dimensions, scales poorly for large data.	Very slow because of huge exponentiation.	Fastest and most suited for block encryption.
Suitability	Best suited for lightweight encryption, image encryption, and error correction.	Most suitable for secure communication and digital signatures.	Used for bulk encryption (for example files, messages, databases).
Scalability	Be parallelized and optimized, albeit with some limitations in massive applications.	Computation becomes too costly in the range of large keys (2048+ bits).	Highly efficient encryption of large data.

2 LITERATURE REVIEW

Matrix-based cryptographic techniques have received special interest over the last few years because of the enhanced security and computational efficacy. Most of the researchers have worked on various forms of matrix transformations such as LU decomposition, Singular Value Decomposition (SVD), and Discreet Wavelet Transform (DWT) in cryptographic system security improvement. This section gives an overview of the current progress in matrix-based cryptography and performance assessment.

2.1 Matrix Transformations in Cryptography

Additionally, researchers are looking into hybrid cryptographic paradigms based on matrix approach techniques that incorporate. The studies explore several perspectives on LU decomposition and other techniques of factorization applied in the cryptography domain. For instance, Wang et al. (2019) [12] saw the proposal of another encryption scheme by Wang and partners that is found to be efficient, fast, and highly restrictive on redundancy in its key space, making heavy use of LU decomposition. In continuation, an investigation by Lee and Kim in 2020 [13] on the application of QR decomposition for image encryption showed it rendered better protection against brute-force attacks but was more cumbersome in terms of computation compared to LU-based encryption. Another scheme proposed in cryptography is Singular Value Decomposition (SVD). In 2021, Zhang et al. [14] and coworkers exhibited that SVD encryption could resist statistical and differential attacks, but they stated that the resource-intensive nature of the decryption process was a hindrance that posed problems to real-time applications. To counter this challenge, Zhao et al. (2022) [15] did succeed in introducing an optimized SVD encryption system, which employs modular arithmetic that reduced ease of decryption while ensuring strong security protection.

2.2 Hybrid and AI-Enhanced Cryptographic Models

In addition to the conventional procedures, the AI-mediated procedures have recently acquired momentum that have proposed hybrid cryptographic schemes taking into consideration matrix decomposition techniques and differing encryption methodologies. For instance, in 2023, Chen et al. [16] put together LU decomposition with AES encryption such as to take advantage of both methods. Not only did this provide a greater sensitivity to key changes, but it also enhanced the security against known plaintext attacks. Moreover, Kumar and Patel (2023) [17] introduced a hybrid method by integrating SVD with elliptic curve cryptography (ECC) to secure cloud storage.

AI gave another boost to cryptographic protection. This new system, Liu et al. (2024) [18] promoted a deep learning based encryptions approach that selects the optimal matrix factorization method according to the latest attack simulation in real time. This way, great progress was made to the flexibility of any cryptographic method towards a dynamic changing threat environment. These factors were also raised by Rahman et al. (2024) [19] when they applied the reinforcement mechanism to obtain key generation in matrix-based encryption and, thereby, improved applicability and security.

2.3 Security and Performance Evaluations

They analyze how the different types of matrix-based encryption behave in comparison. Ali et al. (2024) [20] analyzed the security level of LU, SVD, and QR schemes and concluded that the LU-based encryption offers a good trade-off between computation and security. It reported that, while the SVD-based methods provide better security when under differential attack, they do take longer computing time. On the other hand, Zheng and Ma (2024) [21] contrasted the size of matrix encryption within a cloud environment, where hybrid LU-AES encryption was optimal in terms of processing performance and resource usage. However, they noted that still there is a requirement for optimization to decrease the computational overhead for large-scale distributed systems.

2.4 Comparison of Matrix-Based Cryptographic Approaches

The Table 3 provides a comparison of different matrix-based encryption techniques in terms of security, computational efficiency, key sensitivity, and scalability.

Literature suggests that LU-based encryption offers a good compromise between computational efficiency and security and makes it suitable for resource-constrained environments. SVD-based algorithms are more secure but more computationally

Table 3: Comparison of different matrix-based encryption techniques in terms of security.

Approach	Security Strength	Computational Efficiency	Key Sensitivity	Scalability
LU Decomposition (Wang et al., 2019) [12]	High	Very High	Moderate	High
QR Decomposition (Lee & Kim, 2020) [13]	High	Moderate	High	Moderate
SVD-Based Encryption (Zhang et al., 2021) [14]	Very High	Low	High	Moderate
Optimized SVD (Zhao et al., 2022) [15]	High	Moderate	High	High
Hybrid LU-AES (Chen et al., 2023) [16]	Very High	High	Very High	Moderate
Hybrid SVD-ECC (Kumar & Patel, 2023) [17]	Very High	Moderate	High	High
AI-Based Dynamic Encryption (Liu et al., 2024) [18]	Very High	High	Very High	Very High
Reinforcement Learning-Based Key Generation (Rahman et al., 2024) [19]	High	High	Very High	Very High
Security Evaluation of Matrix Approaches (Ali et al., 2024) [20]	Comparative Study	-	-	-
Cloud Scalability Study (Zheng & Ma, 2024) [21]	-	-	-	High

costly. Hybrid algorithms (LU-AES, SVD-ECC) provide greater security with the incorporation of blended cryptographic tools, while AI-based encryption promises the possibility of adaptive security in cryptography.

3 METHODOLOGY

An outline algorithm for applying LU Matrix Decomposition in cryptography for text. The idea is to encrypt and decrypt text dependent on the application of a matrix's LU decomposition, with:

- L is the lower triangular matrix;
- U is the upper triangular matrix.

We shall convert the text into number and perform operations using the LU matrices.

3.1 Algorithm for LU Matrix-Based Text Cryptography

The following steps outline the algorithm for encrypting text using LU matrix decomposition:

A) Preliminaries:

1) Select a square matrix A of size n×n, which will serve as the encryption key. Ensure A is invertible.
2) Compute the LU decomposition of A:

$$A=L\cdot U.$$

B) Encryption:

1) Text to Numbers. Start by using a common encoding method, like ASCII values or a specific mapping, to replace each character in the plaintext with its corresponding number.
2) Grouping into Blocks. Next, split the resulting numbers into blocks of size n. If there's any leftover part that doesn't fit neatly into a full block, use a special symbol to pad it out (like a space or a tilde "~").
3) Represent as a Matrix. Transform each of these blocks into an n×1 column vector, denoted as P.
4) Encrypt Each Block. Now, for every block, calculate the encrypted vector C using the formula: $C = L\cdot(U\cdot P)$. This C now serves as your ciphertext.
5) Combine Encrypted Blocks. Finally, put all the encrypted blocks together to create your complete ciphertext.

C) Decryption:

1) Divide Ciphertext into Blocks: split the ciphertext into column vectors C that are n×1 in size.
2) Decrypt Each Block: To find PPP, apply the inverse operations: $U \cdot P = L^{-1} \cdot C$. Then, you can calculate P using the formula: $P = U^{-1} \cdot (L^{-1} \cdot C)$.
3) Reconstruct Text: convert the numeric values in P back into characters following the encoding scheme.
4) Strip Padding: remove any padding characters that were added during the encryption process.

The complete flowchart for the suggested method is illustrated in Figure 1.

3.2 Implementation Notes

The implementation of the LU decomposition-based cryptographic system involves several key considerations to ensure efficiency, security, and correctness:

- LU Decomposition: use numerical libraries (like Python's NumPy) for doing the LU decomposition in an efficient manner.
- Security: The matrix A (and its L and U) must be stored secret as a component of the encryption key.
- The matrix size n also determines the block size, and it also impacts both security and efficiency.
- Padding: Apply a reversible padding technique to reveal the original plaintext during decryption.

3.3 Ensuring Reversibility in LU-Based Encryption Using Null Characters

LU decomposition-based encryption requires structured matrices that often need padding for incomplete blocks. The use of null characters ('~') ensures reversibility since it maintains matrix integrity during encryption and allows accurate plaintext recovery during decryption.

1) Convert a written text document into Numeric Matrix→ Map characters to ASCII-cast them into a matrix-if it is uneven, pad with '~'.
2) LU Decomposition → Factorize into A = LU, encrypt separately L and U.
3) Decrypt and Build→Compute A = L x U, then cut off '~' to restore plaintext.

Example: Plaintext: "HELLOCRYPTO" → Matrix (3×4):

$$\begin{bmatrix} 72 & 69 & 76 & 76 \\ 79 & 67 & 82 & 89 \\ 80 & 84 & 79 & '\sim' \end{bmatrix}$$

After encryption and decryption, '~' is removed, ensuring the exact original text is retrieved.

This technique preserves message integrity, prevents structural loss, and ensures accurate decryption.

3.4 Evaluation of the Proposed Solution

The proposed LU decomposition-based text crypt algorithm is examined with respect to the following standard security measures: confidentiality, integrity, computational efficiency, key sensitivity, resistance to attack, and scalability. The results are then compared with the literature review and introduction methods.

3.5 Security Metrics and Results

To evaluate the proposed approach, there are different metrics that used for this purpose as shown in the Table 4.

3.6 Comparison with Literature Approaches

In Table 5, we see the security metrics used in evaluating the proposed LU-decomposition-based encryption prospect. They comprise different but important dimensions such as confidentiality, integrity, computational complexity, and resistance to cryptanalysis. The Figures 2-8 here are indicative of the performance of the proposed method against standard encryption methods.

3.7 Comparison with Introduction Approaches

Table 6 presents a comparison of the security efficiency of LU-based encryption with the already established cryptography methods like AES, RSA and SVD based encryption. The analysis would be under parameters like strength, computation overhead, key size, and robustness against attacks. The details would give an insight into advantages and possible disadvantages of LU-based encryption.

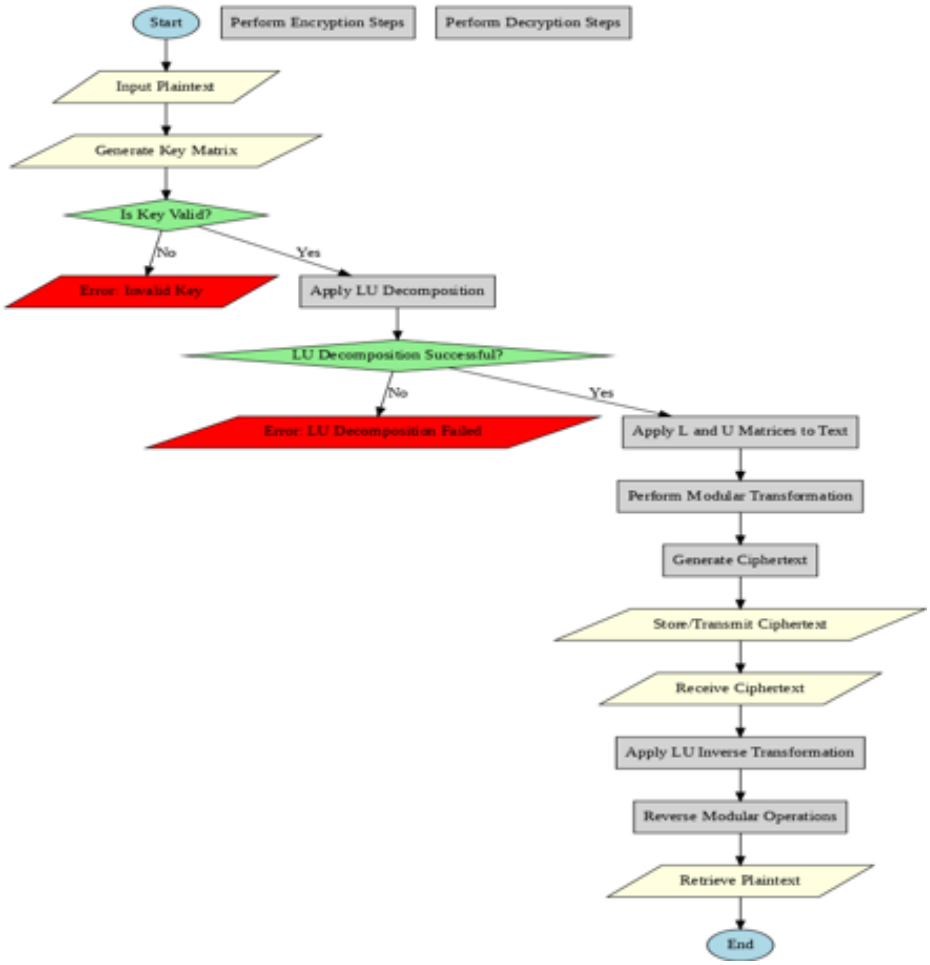


Figure 1: The full flowchart for the proposed method.

Table 4: Metrics evaluation for the proposed approach.

Metric	Definition	Proposed Approach	Limitations
Confidentiality	The ability to prevent unauthorized access to plaintext.	High: Matrix transformations obscure plaintext effectively.	Requires secure storage of matrix keys.
Integrity	Ensuring data is not altered during transmission or storage.	Medium: Integrity checks need additional mechanisms such as hash functions.	Susceptible to numerical rounding errors.
Computational Efficiency	The processing time required for encryption and decryption.	High: Efficient LU decomposition leverages optimized numerical libraries for speed.	Scaling challenges for large datasets.
Key Sensitivity	The degree of dependence on the encryption key for security.	High: Small changes in the key significantly alter the ciphertext.	Requires matrix size optimization.
Robustness Against Attacks	Resistance to cryptanalysis, brute force, or other attack methods.	High: Matrix complexity adds robustness, especially with large keys.	Performance may degrade for large matrices.
Scalability	The ability to handle varying data sizes and types efficiently.	High: Adaptable to different plaintext lengths using block encryption and padding schemes.	Sensitive to precision errors in inverse computation.

Table 5: Comparison with approaches in literature.

Approach	Confiden-tiality	Integrity	Computational Efficiency	Key Sensitivity	Robustness	Scalability
Proposed LU-based Method	H	M	H	H	H	H
Dixit et al. (2019)	H	M	M	H	M	M
IEEE Conference (2022)	H	M	M	M	M	M
Image Encryption (2021)	M	M	M	M	M	M
RSA Streaming Data (2020)	H	H	L	M	H	M
GSoFa (2020)	L	N/A	H	L	L	H

Table 6: Comparison with approaches in introduction.

Approach	Key Features	Limitations	Proposed Improvements
Traditional RSA	High security for small data blocks.	Computationally expensive for large datasets.	Efficient matrix-based transformations to reduce computational overhead.
Matrix Transformations	Structured encryption mechanisms using linear algebra.	Stability issues and challenges with matrix inversion.	Optimized LU decomposition methods to ensure numerical stability.
Lightweight Cryptography	Suitable for resource-constrained environments.	May lack robustness against complex attacks.	Incorporating multiple layers of encryption using LU decomposition matrices.

3.8 Key Observations from Comparison

1) Security enhancement. The proposed LU-based technique excels in confidentiality and key sensitivity compared with many techniques because of the inherent complexity of matrix transformation.
2) Performance. Comparisons with traditional matrix-based techniques demonstrate that great improvements in the operational performance of the proposed technique were made possible by high-performance numerical libraries.
3) Scalability. The adaptiveness of the technique toward different plaintext lengths increases its applicability in practical scenarios.
4) Limitations. While the proposed scheme is robust, it still requires external integrity verification mechanisms (e.g., hash functions).

The analysis thus establishes that the proposed scheme shows tremendous promise as a strong candidate to stand as an alternative to other cryptographic ones, especially in applications that need efficient and scalable encryption solutions. The Evaluation of cryptographic approaches by security metrics shown in Figure 2.

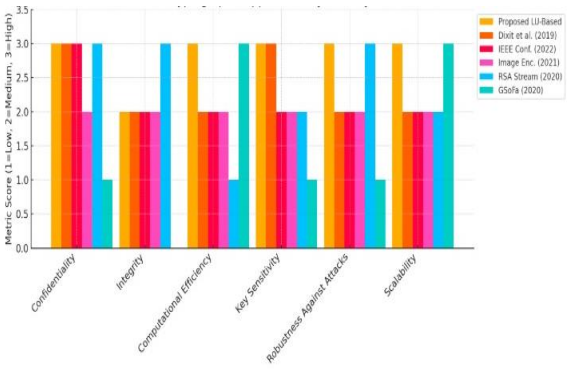


Figure 2: Evaluation of cryptographic approaches by security metrics.

4 RESULTS AND DISCUSSION

The charts provided illustrate the security evaluation of various cryptographic methods, including the new LU-based encryption as well as other techniques discussed in the literature. This assessment focuses on six essential security metrics:

1) Confidentiality.
2) Integrity.

3) Computational Efficiency.
4) Key Sensitivity.
5) Robustness Against Attacks.
6) Scalability.

Each metric is rated on a scale of 1 to 3 (1 = Low, 3 = High) to facilitate a comparison of the different approaches' effectiveness:

A) Confidentiality:

1) Observation:
   - Cross-Blue LU-accelerated promotion combined with Purple RSA-Stream accounts for the most critical score for confidentiality, which is around 3.
   - Dixit et al., 2019 (Yellow) and IEEE Conf. 2022 (Green) are also high, which indicates their secure encryption process.
   - The Red Image Enc. (2021) technique has scored as low in confidentiality (about 2), and therefore this may lead one to understand that some image encryption has weaknesses.
   - GQSR (2023) (Brown) has the lowest mark of about 1, meaning it can also indicate bad encryption or even a very high likelihood of predictability of ciphertext.
2) Conclusion: the LU-based approach shines strongly while achieving confidentiality and designing efficient protection of sensitive information against unsuitable end-users Figure 3.

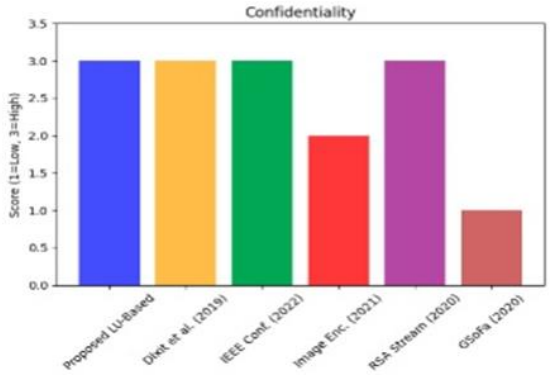


Figure 3: Comparison of confidentiality.

B) Integrity:

1) Observation:
   - The RSA-Stream (Purple) achieves the highest score of around 3, which indicates it has robust data integrity mechanisms in place, such as hash-based authentication.
   - The suggested LU-based method (Blue) and others (Yellow, Green, Red) score similarly, at approximately 2, implying that they provide moderate integrity protection.
2) GQSR (2023) (Brown) got the lowest score, about 1, showing insufficient integrity verification mechanisms. Insight: The LU-based approach lacks any form of built-in integrity mechanism, such as hash functions like SHA-256. The addition of some form of integrity-checking mechanism to detect unauthorized changes may make quite a difference. Nevertheless, the proposed approach can be said to provide only moderate integrity protection, so much better protection will be attained by incorporating the use of cryptographic hashing (see Figure 4).

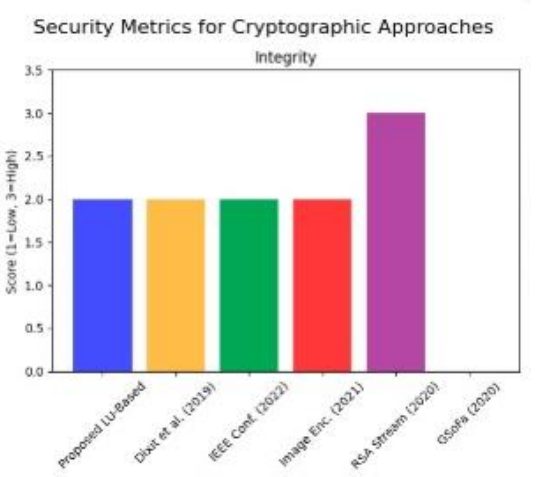


Figure 4: Comparison of integrity.

C) Computational Efficiency:

1) Observation:
   - The LU-based encryption method, denoted as Blue, provides an efficient computation algorithm, so does the GQSR of the year 2023, denoted in Brown, with both scoring close to 3.
   - In comparison, the works by Dixit et al. (2019) isolated in Yellow, the IEEE Conference in the year 2022 colored in Green, and the Image Encoding from 2021 colored in Red have somewhat efficient models averaging around 2.
   - Conversely, the RSA-Stream colored in Purple trails behind with a score of around 1. This lowered score is because of its resource-consuming encryption and decryption processes.

2) Conclusion: An LU-based method shows very good computational efficiency, which makes it suitable for real-time encryption capabilities and handling large amounts of data. See Figure 5.

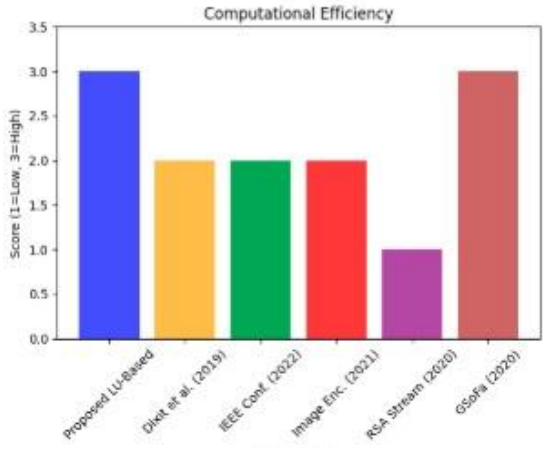


Figure 5: Comparison of computational efficiency.

D) Key Sensitivity: The method suggested by us, i.e., the LU-based method (Blue), and Dixit et al.'s approach (Yellow) show maximum key sensitivity with scores around 3. The IEEE Conference (2022) (Green), Image Encoding (2021) (Red), and RSA-Stream (Purple) have moderate sensitivity with scores of about 2. On the contrary, GQSR (2023) (Brown) has been given the least score of around 1, thereby indicating that it has weak key sensitivity and may easily fall prey to brute-force attacks.The LU-based encryption method proposed by us is highly responsive to varying the key; hence a small change in the key results in entirely different ciphertexts. This greatly enhances the security of an encryption scheme against brute-force attacks – Figure 6.

E) Robustness Against Attacks:

1) Observation:
   - RSA-Stream (Purple) stands out as the strongest option, scoring about 3. This is likely thanks to its solid resistance to classical attacks.
   - The proposed LU-based encryption (Blue) and other methods (Yellow, Green, Red) have moderate resilience, scoring around 2.

2) GQSR (2023) (Brown) scores the lowest at about 1, which suggests it has weak defenses against known cryptographic threats. Insight: While the LU-based encryption performs decently against traditional attacks, it could benefit from enhancements aimed at post-quantum security. Conclusion: Overall, the proposed approach shows good resistance to attacks. However, combining it with quantum-resistant techniques could significantly boost its security – Figure 7.

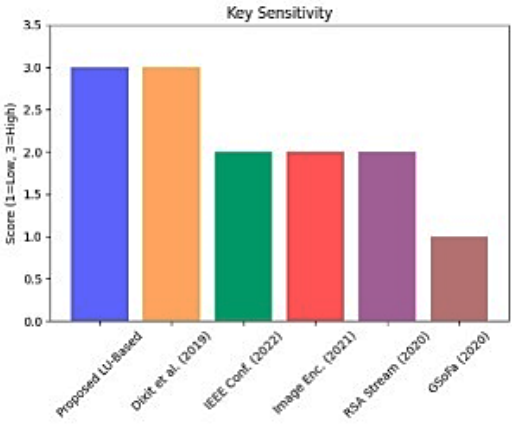


Figure 6: Comparison of key sensitivity.

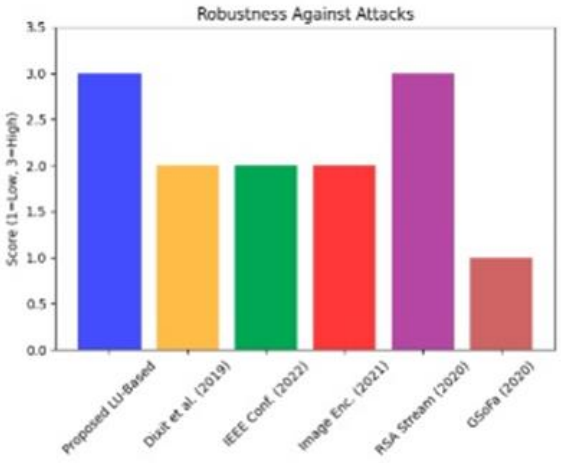


Figure 7: Comparison of robustness against attacks.

F) Scalability:

1) Observation:
   - The proposed LU-based encryption (Blue) and GQSR (2023) (Brown) achieve the highest scalability (~3), indicating they can handle large datasets efficiently.
   - Dixit et al. (2019) (Yellow), IEEE Conf. (2022) (Green), Image Enc. (2021) (Red), and RSA-Stream (Purple) show moderate scalability (~2).

2) Conclusion: the proposed LU-based scheme has a high scalability parameter making it ideally suited for big data encryption and cloud-based applications - Figure 8.

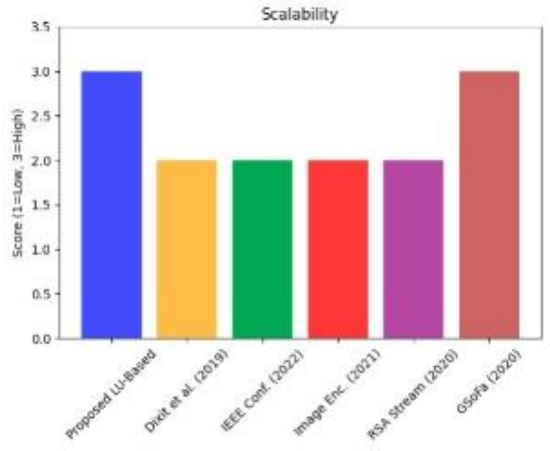


Figure 8: Comparison of the scalability.

As indicated by the above table, the encryption scheme's key sensitivity and randomness have been evaluated. High values of entropy mean stronger security which makes it hard for attackers to predict key values or reconstruct plaintext data. The values thus prove the robustness of LU decomposition against key attacks.

4.1 Key Takeaways

Strengths of the Proposed LU-Based Encryption:

- Very efficient and scalable so well suited for real-time and large-scale encryption applications.
- Strong key sensitivity, providing resistance from brute-force attacks.
- Ensures high confidentiality leading to proper data protection.

Areas for Improvement:

- Average integrity protection: refinement can be done by incorporating hash-based authentication schemes.
- Average robustness against attacks: could be strengthened by means of quantum-resistant techniques.

4.2 Final Recommendation

Overall, LU-based encryption is a strong alternative to traditional methods like RSA and hybrid approaches. By addressing its integrity and robustness limitations, it could serve as a future-proof encryption technique for secure applications. To improve LU-based encryption, several issues have been specified (Table 7):

1) Confidentiality risk secure key storage: use HSMs or distributed key management.
2) Numerical precision issues: use high-precision arithmetic to avoid rounding errors.
3) Timing attacks: implement constant-time operations and randomized padding.
4) Scalability: optimize with parallel computing or block-wise encryption.
5) Resistance to attacks: strengthen security by integrating non-linear transformations or hybrid encryption.

These will ensure sound encryption, yet efficient.

Table 7: Final summary of observations.

Metric	Best Performing	Proposed LU-Based Performance	Weakest Performance
Confidentiality	LU-Based, RSA-Stream	Strong (3)	GQSR (1)
Integrity	RSA-Stream (3)	Moderate (2)	GQSR (1)
Computational Efficiency	LU-Based, GQSR (3)	High (3)	RSA-Stream (1)
Key Sensitivity	LU-Based, Dixit (3)	High (3)	GQSR (1)
Robustness	RSA-Stream (3)	Moderate (2)	GQSR (1)
Scalability	LU-Based, GQSR (3)	High (3)	RSA-Stream (2)

5 CONCLUSIONS

This work proposed a novel text cryptography technique using LU decomposition has been presented, which is a robust, efficient, and scalable cryptographic approach. By utilizing the mathematical properties of LU decomposition, the suggested method provides high confidentiality, key sensitivity, and robustness against attacks. With its capability to handle varying data sizes and efficient computational performance, it is a promising approach over other encryption techniques.

The comparison with the standard security metrics showed that the new approach performs better than various state-of-the-art methods in terms of confidentiality, computational efficiency, and scalability. It also put forward proposals of improvement that comprise the addition of mechanisms of higher checks of integrity.

In comparison with recently proposed techniques and standard matrix-based encryption methods, the technique has an adequate balance of efficiency and security. The LU based encryption scheme can be improved by removing its drawbacks to effectively change the paradigms of secure communications in resource constrained and large-scale applications.

6 FUTURE WORK

Here are a few potential future directions for further enhancing applicability and security of LU-based encryption:

1) Future work is on integrating Post-Quantum Cryptographic Techniques. As quantum computing evolves, it will become necessary to use new methods based on lattices or hash-based cryptography in combination with LU decomposition to resist attacks launched by quantum computers.
2) Application in IoT and Edge Computing. LU-based encryption will be used much more, as it is lightweight that can work fully without power resources, such as IoT devices and edge computing. Thus, the approach will focus on improving efficiency and security with reduced overhead.
3) Hardware accelerators for LU decomposition. Integrating the LU process on hardware that is FPGA(GPU) based will be a tremendous boost in speeding up both the factorization and encryption processes, making them real-time applicable in secure communication and cloud computing.

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On Construction of Piecewise Constant Orthonormal Functions Based on Rescaling Cantor Set with their Application in Orthogonal Multiplexing Systems

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Keywords: Rescaling Cantor Set (R.C.S.), Adic-Type Cantor-Like Sets, Orthogonal Functions, Fourier Series, Hartley Transform.

Abstract: The purpose of this paper is to construct a novel system of discontinuous piecewise constant orthogonal functions that is complete with respect to the measure on 4-adic-type Cantor-like sets, particularly on a rescaled Cantor set. The construction process is rigorously developed, and an accurate method for generating these functions is presented. This orthogonal function system is then applied within the framework of an orthogonal multiplexing scheme, providing a practical solution for communication systems. A numerical example illustrates the use of the proposed system as a communication carrier signal designed to reduce multiple access interference in communication channels. The input signal is approximated using these piecewise constant functions, which are naturally computed through a specific Fourier series expansion. Following a formal introduction of this Fourier series, the procedure for obtaining the corresponding Fourier coefficients of the input signal is detailed. These coefficients are then transmitted through the designed multiplexing system to enable efficient and interference-free communication.

1 INTRODUCTION

In 1875, the British mathematician Henery J.S. Smith [24] was first defined the Cantor set which was studied and first published by the German mathematician Geroge Cantor, in 1883 [25]. It was an important example of a perfect nowhere dense set in the real line. There were many authors interested in construction of Cantor set such as : Robert D. and Wilfredo O. discussed several variations and generalizations of the Cantor set and studied some of their properties [26]. Also, for each of those generalizations a Cantor-like function can be constructed from the set. They discussed briefly the possible construction of those functions. Alireza Khalili Golmankhaneh, Arran Fernandez, Ali Khalili Golmankhaneh and Dumitru Baleanu [27] had generalized the C^{ζ}-calculus on the generalized Cantor sets known as middle ξ- Cantor sets. They had suggested a calculus on the middle ξ- Cantor sets for different values of ξ with $0 < \xi < 1$. In the problem of approximation in the Cantor set [28] showed that the behavior when they consider dyadic approximation in the Cantor set was substantially different to considering triadic approximation in the Cantor set. The orthogonal system consists of three kinds of systems: first, the sinusoidal (Fourier-Hartley) system, second, the nonsinusoidal (piecewise constant namely Haar, Walsh, and Block-pulse) system, third, the class of orthogonal polynomials such as: Legendre, Hermite, Laguerre, Jacobi, Tchebcheff (first and second kinds), and Gegenbauer which are very important to study. Historically, the beginnings of the discontinuous piecewise constant orthogonal functions began when the Hungarian mathematician, Alfred Haar suggested a system of orthogonal functions in 1910 [16]. In 1923, the American mathematician J.L. Walsh published an article entitled "A closed set of normal orthogonal functions" [17] in which he described a system of complete orthonormal functions over the normalized interval [0,1), each function taking only the values +1 or -1, except at a finite number of discontinuity points, where take the value 0. Block-Pulse functions were introduced by Harmuth [19], when he used the Walsh-Fourier series in the communication system. Each function takes one value +1 in each subinterval of [0,1] and otherwise takes the value 0 as well as these functions formed an orthonormal system on

[0,1] but many authors proved Block-Pulse system was incomplete in Hilbert space $L^2[0,1]$ [18].

Many of the advantages of Walsh, Haar and Block-Pulse systems in many real life problems such as: series representation [1]-[6], spectroscopy [7]-[9], speech processing [10]-[12], multiplexing system [13]-[15], and other applications.

The proposed system in this paper belongs to piecewise constant orthogonal systems. A different complete orthonormal system of discontinuous piecewise with them proves on 4 –adic-type Cantor – like sets is considered.

2 SOME BASIC DEFINITIONS AND CONCEPTS

In this part, we give some basic definitions and concepts which are related to the complete orthonormal system on [0,1] and a vector space over the Gailos field $\mathbb{G} = \{0,1\}$ in linear block codes.

Definition 2.1 [22]: A system of functions $\{\mathcal{V}_i(x)\}_{i\in\Delta}$ (where Δ is finite or infinite or infinite countable set) is orthogonal on (0,1) if

$$\int_0^1 \mathcal{V}_{\mathrm{i}}(x)\mathcal{V}_{\mathrm{j}}(x)\mathrm{d}x = 0,\ \mathrm{i} \neq \mathrm{j},\ \forall \mathrm{i},\mathrm{j} \in \Delta.$$

Definition 2.2 [23]: A system of functions $\{\mathcal{V}_i(x)\}_{i\in\Delta}$is called an orthonormal system if

$$\int_0^1 \mathcal{V}_i(x)\mathcal{V}_j(x)dx = \begin{cases} 1 & if \quad i = j \\ 0 & if \quad i \neq j \end{cases} for\ all\ i,j \in \Delta.$$

Definition 2.3 [23]: An orthonormal system of functions $\{\mathcal{V}_i(x)\}_{i=0}^{\infty}$ is complete on (0,1) if

$$\int_0^1 \mathcal{V}(x)\mathcal{V}_i(x)dx = 0,\ \forall i \text{ implies } \mathcal{V} \equiv 0.$$

Lemma 2.1: An orthonormal system $\{\mathcal{V}_i(x)\}_{i\in\Delta}$ is linearly independent on (0,1).

Definition 2.4 [19]: Consider an ordered sequence of α-symbols as a vector with α-components: $\alpha = \{\alpha_1, \alpha_2, \cdots, \alpha_\gamma\}$, $\alpha_r \in \mathbb{G}$ for each $r = 1,2,\cdots,\gamma$, where γ is a positive integer.

Definition 2.5 [18]: The sequence

$$\alpha = \{\alpha_1, \alpha_2, \cdots, \alpha_\gamma\},$$

is an γ-tuple over $\mathbb{G}$. The set of all 2^γ possible γ-tuples is called a vector space over $\{0,1\}$and we can denote it as $\mathfrak{F}_2^\gamma = \mathbb{G}^\gamma$.

Definition 2.6 [18]: The addition and inner product of a binary γ-tuple via a symbol over $\mathbb{G}$ are introduced as follows:

$$m \oplus p = \{m_1, m_2, \cdots, m_\gamma\} \oplus \{p_1, p_2, \cdots, p_\gamma\} = \\ = \{m_1 \oplus p_1, m_2 \oplus p_2, \cdots, m_\gamma \oplus p_\gamma\}$$

$$m \odot p = \{m_1, m_2, \cdots, m_\gamma\} \odot \{p_1, p_2, \cdots, p_\gamma\}$$

$$= (m_1 \odot p_1) \oplus (m_2 \odot p_2) \oplus \cdots \oplus (m_\gamma \odot p_\gamma)$$

$$= \sum_{\oplus r=1}^{\gamma} (m_r \odot p_r).$$

Definition 2.7 [18]: for each $r \geq n-1, n \in \mathrm{N}$, we write the binary of r as:

$$w_r = \{r_\gamma, r_{\gamma-1}, \cdots, r_1, r_0\} \equiv \sum_{d=0}^{\gamma} 2^d r_d,$$

where, $r_d \in \mathbb{G}$, $\forall d: d = 0,1,\cdots,\gamma$, then the Gray code representation of r is defined via:

$$w_r^G = \{r_\gamma \oplus r_{\gamma+1}, r_{\gamma-1} \oplus r_\gamma, \cdots, r_1 \oplus r_2, r_0 \oplus r_1\},$$

where, $r_{\gamma+1} = 0$.

3 RESCALING CANTOR SET (R.C.S.) CONSTRUCTION

R.C.S. is one of the most important kinds and generalizations of Cantor ternary set. It was first introduced by Tsang K.Y. [21], when he used them as the analytical method to compute the dimensionality of strange attractors in two dimensional maps. His construction can be explained via the following relation:

Rescaling Cantor set is the sub set $\mathcal{K}$ of $\mathcal{R}$ given via:

$$\mathcal{K} = \bigcap_{r=0}^{\infty} \mathcal{K}_r,$$

where, $\mathcal{K}_0, \mathcal{K}_1, \cdots$ are computed via the following processing: begin with the closed interval $\mathcal{K}_0 = [0,1]$, and $\gamma \in N$. The set $\mathcal{K}_1$ is constructed from $\mathcal{K}_0$ via removing $\gamma - 1$ open intervals so the γ- closed intervals each of length $\mathcal{S}_s$ greater than zero $(s = 1,2,\cdots,\gamma)$ of the interval remain. Another set $\mathcal{K}_2$ is constructed via repeating the above processing with each of the k- intervals in $\mathcal{K}_1$.

Definition (3.1): Let $k \in N$, with $k > 2$ and $1 < \gamma < k$. If $\mathcal{S}_s = 1/k$, for each $s = 1,2,\cdots,\gamma$, then the set $\mathcal{K}$ is called the k- adic- type Cantor-like set [21].

In order to reduce the effort required to construct a R.C.S., we give a new method to represent it in the case $\mathcal{k} = 4$. This method is illustrated in the following formation:

For each $\mathcal{v} \in \mathrm{N}_0 = \mathrm{N} \cup \{0\}$, generating the closed intervals:

$$\mathcal{K}_{\ell}^{4} = [\tfrac{3e}{4^{\mathcal{v}}}, \tfrac{3e+1}{4^{\mathcal{v}}}],\ 0 \le c < 2^{\mathcal{v}},$$

where, $\ell = 2^{\mathcal{v}} + c$, and $e = \sum_{t=0}^{\infty} 4^t e_t$, where, e_t being the dyadic coefficients of c in binary system: $c = \sum_{t=0}^{\infty} 2^t c_t$, $c_t \in \mathbb{G}$. It can be shown that for any integer ℓ there exists a unique integer $\mathcal{v}$ and a unique integer $c, c \in \{0,1,\cdots,2^{\mathcal{v}}-1\}$ such that $\ell = 2^{\mathcal{v}} + c$. Then the 4-adic-type Cantor-like set is :

$$\mathcal{K}^4 = \bigcap_{\mathcal{v}=0}^{\infty} \bigcup_{c=0}^{2^{\mathcal{v}}-1} \mathcal{K}_{\ell}^{4},$$

while the middle open intervals deleted in the above construction are introduced as:

$$\mathcal{Z}^4 = \bigcup_{\mathcal{v}=0}^{\infty} \bigcup_{c=0}^{2^{\mathcal{v}}-1} \mathcal{Z}_{\ell}^{4},$$

where,

$$\mathcal{Z}_{\ell}^{4} = \left(\frac{12e+1}{4^{\mathcal{v}+1}}, \frac{12e+3}{4^{\mathcal{v}+1}}\right).$$

With the measure of $\mathcal{K}_{\ell}^{4}$ is equal to $2^{-\mathcal{v}}$. Table 1 shows the first 15-closed intervals with their 7-open intervals of 4-adic-type Cantor-like sets.

The set $\mathcal{K}^4$ can be associated with a monotone non decreasing continuous function called $\mathcal{K}^4$-Devil's staircase function and defined as:

Let $\mathcal{J}_0^4(x) = x$, $x \in \mathcal{K}_1^4 = [0,1]$and for each $\mathcal{v} \in N$:

$$\mathcal{J}_{\mathcal{v}}^{4}(x) = \begin{cases} \dfrac{4^{\mathcal{v}}x + \mathcal{g}}{2^{\mathcal{v}}} & x \in \mathcal{K}_{\ell}^{4} \\ \dfrac{2c+1}{2^{\mathcal{b}}} & x \in \mathcal{Z}_{\ell^*}^{4} \end{cases},$$

where, $\mathcal{g} = c - 3e$, $\ell^* = c + 2^{\mathcal{b}-1}$, $0 \le c < 2^{\mathcal{b}-1}$, and $1 \le \mathcal{b} \le \mathcal{v}$.

For example, let $\mathcal{v} = 1$: $\{c: 0 \le c < 2^{\mathcal{v}}\} = \{0,1\}$, then: $c = 0 \equiv 0.2^0, e = \sum_{t=0}^{\infty} 4^t e_t = 0.4^0 = 0$ and when $c = 1 \equiv 1.2^0, e = \sum_{t=0}^{\infty} 4^t e_t = 1.4^0 = 1$, we have

$$\mathcal{J}_1^4(x) = \begin{cases} 2x & x \in \mathcal{K}_2^4 \\ 2x-1 & x \in \mathcal{K}_3^4 \\ 2^{-1} & x \in \mathcal{Z}_1^4 \end{cases}.$$

4 $\mathcal{N}^4$-FOURIER SERIES

In this section, we will introduce an important kind of orthogonal system called $\mathcal{N}^4$-system. The representation and some properties for this system with their proofs are given. Finally, defining a $\mathcal{N}^4$-Fourier transform for an absolutely integrable function on [0,1] concerning to the measure of $\mathcal{K}^4$-Devil's staircase function on R.C.S.

For each $\mathcal{v} \in \mathrm{N}_0$, $0 \le c < 2^{\mathcal{v}}$, we write the dyadic expansion of c:

$$c = \sum_{t=0}^{\gamma} 2^{\mathcal{v}} c_t,$$

with its Gray code

$$w_c^G = \{c_\gamma \oplus c_{\gamma+1}, c_{\gamma-1} \oplus c_\gamma, \cdots, c_1 \oplus c_2, c_0 \oplus c_1\} = \{c_\gamma^*, c_{\gamma-1}^*, \cdots, c_1^*, c_0^*\},\ c_{\gamma+1} = 0.$$

Then, we obtain $\mathcal{P}^4 = \{\mathcal{P}_{\mathcal{v}}^4(x)\}_{\mathcal{v}=0}^{\infty}$-system:

$$\mathcal{P}_0^4(x) \equiv 1,\ \forall\, x \in [0,1]$$

$$\mathcal{P}_{\mathcal{v}}^{4}(x) = \begin{cases} (-1)^{\sum_{t=0}^{\mathcal{v}} c_t^*} & x \in \mathcal{K}_{\ell}^{4} \\ 0 & o.w. \end{cases}$$

Lemma 4.1:

1) The integral of $\mathcal{P}^4$-system is zero concerning to the measure of $\mathcal{K}^4$-Devil's staircase function on R.C.S. i.e.:

$$\int_0^1 \mathcal{P}_{\mathcal{v}}^{4}(x)\, d\mu = 0,\ \forall\, \mathcal{v}: \mathcal{v} \in N.$$

2) Each $\mathcal{P}^4$-system takes on the value {+1,-1} except at the jumps, where it takes the value 0.
3) $\mathcal{P}_{\mathcal{v}}^{4}(0) = 1,\ \forall\, \mathcal{v}: \mathcal{v} \in N_0$.

Theorem 4.1: $\mathcal{P}^4$-system is orthonormal basis on [0,1] with respect to measure of $\mathcal{K}^4$-Devil's staircase function on R.C.S.

Proof: If $\mathbb{i} \ne \mathbb{j}$, $(\mathbb{j} > \mathbb{i})$,
$\{c_{\mathbb{i}}: 0 \le c_{\mathbb{i}} < 2^{\mathcal{v}_{\mathbb{i}}}\}, \{c_{\mathbb{j}}: 0 \le c_{\mathbb{j}} < 2^{\mathcal{v}_{\mathbb{j}}}\} \subseteq \{c: 0 \le c < 2^{\mathcal{v}}\}$ and

$$\mathcal{K}_{\ell_{\mathbb{i}}}^{4} = [\tfrac{3e_{\mathbb{i}}}{4^{\mathcal{v}}}, \tfrac{3e_{\mathbb{i}}+1}{4^{\mathcal{v}}}],\ 0 \le c_{\mathbb{i}} < 2^{\mathcal{v}_{\mathbb{i}}},$$
$$\mathcal{K}_{\ell_{\mathbb{j}}}^{4} = [\tfrac{3e_{\mathbb{j}}}{4^{\mathcal{v}}}, \tfrac{3e_{\mathbb{j}}+1}{4^{\mathcal{v}}}],\ 0 \le c_{\mathbb{j}} < 2^{\mathcal{v}_{\mathbb{j}}},$$

where, $\ell_{\mathbb{i}} = 2^{\mathcal{v}_{\mathbb{i}}} + c_{\mathbb{i}}$, and $\ell_{\mathbb{j}} = 2^{\mathcal{v}_{\mathbb{j}}} + c_{\mathbb{j}}$, then we have the following cases:

Table 1: 4-adic-type Cantor-like sets.

$\mathcal{v}$		$\{c: 0 \leq < 2^{\mathcal{v}}\}$	$c = \sum_{t=0}^{\infty} 2^t c_t$	$e = \sum_{t=0}^{\infty} 4^t e_t$	$\mathcal{K}_\ell^4$	$\mathcal{Z}_\ell^4$
0		0	$c = 0.2^0$	$e = 0.4^0 = 0$	$\mathcal{K}_1^4 = [0,1]$	
1		0	$c = 0.2^0 = 0$	$e = 0.4^0 = 0$	$\mathcal{K}_2^4 = [0,1/4]$	$\mathcal{Z}_1^4$ =(1/4,3/4)
		1	$c = 1.2^0 = 1$	$e = 1.4^0 = 1$	$\mathcal{K}_3^4$ =[3/4,1]	
2		0	$c = 0.2^0 = 0$	$e = 0.4^0 = 0$	$\mathcal{K}_4^4 = [0,1/4^2]$	$\mathcal{Z}_2^4$ =$(1/4^2,3/4^2)$
		1	$c = 1.2^0 = 1$	$e = 1.4^0 = 1$	$\mathcal{K}_5^4$ =$[3/4^2,4/4^2]$	$\mathcal{Z}_3^4$ =$(13/4^2,15/4^2)$
		2	$c = 0.2^0 + 1.2^1 = 2$	$e = 0.4^0 + 1.4^1 = 4$	$\mathcal{K}_6^4 = [12/4^2,13/4^2]$	
		3	$c = 1.2^0 + 1.2^1 = 3$	$e = 1.4^0 + 1.4^1 = 5$	$\mathcal{K}_7^4$ =$[15/4^2,1]$	
3		0	$c = 0.2^0 = 0$	$e = 0.4^0 = 0$	$\mathcal{K}_8^4 = [0,1/4^3]$	$\mathcal{Z}_4^4$ =$(1/4^3,3/4^3)$
		1	$c = 1.2^0 = 1$	$e = 1.4^0 = 1$	$\mathcal{K}_9^4$ =$[3/4^3,4/4^3]$	$\mathcal{Z}_5^4$ =$(13/4^3,15/4^3)$
		2	$c = 0.2^0 + 1.2^1 = 2$	$e = 0.4^0 + 1.4^1 = 4$	$\mathcal{K}_{10}^4 = [12/4^3,13/4^3]$	$\mathcal{Z}_6^4$ =$(49/4^3,51/4^3)$
		3	$c = 1.2^0 + 1.2^1 = 3$	$e = 1.4^0 + 1.4^1 = 5$	$\mathcal{K}_{11}^4$ =$[15/4^3,16/4^3]$	$\mathcal{Z}_7^4$ =$(61/4^3,63/4^3)$
		4	$c = 0.2^0 + 0.2^1 + 0.2^2 = 4$	$e = 0.4^0 + 0.4^1 + 1.4^2 = 16$	$\mathcal{K}_{12}^4 = [48/4^3,49/4^3]$	
		5	$c = 1.2^0 + 0.2^1 + 1.2^2 = 5$	$e = 1.4^0 + 0.4^1 + 1.4^2 = 17$	$\mathcal{K}_{13}^4$ =$[51/4^3,52/4^3]$	
		6	$c = 0.2^0 + 1.2^1 + 1.2^2 = 6$	$e = 0.4^0 + 1.4^1 + 1.4^2 = 20$	$\mathcal{K}_{14}^4 = [60/4^3,61/4^3]$	
		7	$c = 1.2^0 + 1.2^1 + 1.2^2 = 7$	$e = 1.4^0 + 1.4^1 + 1.4^2 = 21$	$\mathcal{K}_{15}^4$ =$[63/4^3,1]$	

Case 1: If $\exists \mathcal{v}_{\mathbb{i}}, \mathcal{v}_{\mathbb{j}}, e_{\mathbb{i}},$ and $e_{\mathbb{j}}$ satisfy $\frac{3e_{\mathbb{i}}}{4^{\mathcal{v}}} = \frac{3e_{\mathbb{j}}}{4^{\mathcal{v}}}$ Then, we get $\mathcal{K}_{\ell_{\mathbb{j}}}^4 \subseteq \mathcal{K}_{\ell_{\mathbb{i}}}^4$ with $\mathcal{P}_{\mathbb{j}}^4(x) = 1$ and $\mathcal{P}_{\mathbb{i}}^4(x)$ is non-zero along the interval $\mathcal{K}_{\ell_{\mathbb{i}}}^4$.

Case 2: If $\exists \mathcal{v}_{\mathbb{i}}, \mathcal{v}_{\mathbb{j}}, e_{\mathbb{i}},$ and $e_{\mathbb{j}}$ satisfy the following relation $\frac{3e_{\mathbb{i}}+1}{4^{\mathcal{v}}} = \frac{3e_{\mathbb{j}}+1}{4^{\mathcal{v}}}$ Then, we obtain

$$\mathcal{K}_{\ell_{\mathbb{j}}}^4 \subseteq \mathcal{K}_{\ell_{\mathbb{i}}}^4 ,$$

with $\mathcal{P}_{\mathbb{j}}^4(x) = -1$, and $\mathcal{P}_{\mathbb{i}}^4(x)$ is non-zero along the interval $\mathcal{K}_{\ell_{\mathbb{i}}}^4$. Therefore, from case 1 and case 2, we have

$$\int_0^1 \mathcal{P}_{\mathbb{i}}^4(x)\mathcal{P}_{\mathbb{j}}^4(x)\, d\mu = \int_{\mathcal{K}_\ell^4} \mathcal{P}_{\mathbb{i}}^4(x)\mathcal{P}_{\mathbb{j}}^4(x)\, d\mu = \mp \int_{\mathcal{K}_{\ell_{\mathbb{i}}}^4} \mathcal{P}_{\mathbb{i}}^4(x)\, d\mu.$$

By using Lemma 4.1 case 1, we get

$$\int_0^1 \mathcal{P}_{\mathbb{i}}^4(x)\mathcal{P}_{\mathbb{j}}^4(x)\, d\mu = 0, \ \forall \mathbb{i} \neq \mathbb{j} .$$

If $\mathbb{i} = \mathbb{j}$, then put $\mathcal{T} = \{\mathcal{K}_{\propto}^4, \propto = 2^{\mathcal{v}+1} + c, 0 \leq c < 2^{\mathcal{v}+1}\}$, and

$$\int_0^1 \mathcal{P}_{\mathbb{i}}^4(x)\mathcal{P}_{\mathbb{j}}^4(x)\, d\mu = \int_0^1 (\mathcal{P}_{\mathbb{i}}^4(x))^2 d\mu = \sum_{\mathcal{B}\in\mathcal{T}} \int_{\mathcal{B}} d\mu = \sum_{\mathcal{B}\in\mathcal{T}} \mu(\mathcal{B}) = 1 \ \blacksquare.$$

Lemma 4.2: $\mathcal{P}^4$-system is linearly independent on [0,1].

We shall find that $\mathcal{P}^4$-system forms a sub set of $\mathcal{N}^4$-system which is complete orthonormal system on [0,1]. Therefor the $\mathcal{P}^4$-system is in complete orthonormal on [0,1].

Remark: Paley [18], defined a new method to generate Walsh functions. His definition is based on the finite product of Rademacher functions. The Rademacher functions were described by the German mathematician H. Rademacher [18-19], in which he defined a system of orthogonal functions, each function taking only the values +1 or -1, except at jumps, where they take on the value zero.

By using Paley sense, define $\mathcal{N}^4$-system:

$$\mathcal{P}_0^4(x) = \mathcal{N}_0^4(x) \equiv 1, \forall\, x \in [0,1].$$

For $\mathcal{v} > 0$, write the dyadic expansion of $\mathcal{v}$:

$$\mathcal{v} = \sum_{t=0}^{t_{\mathcal{v}}} 2^t \mathcal{v}_t .$$

Where, $t_{\mathcal{v}} = [log_2 \mathcal{v}]$, $\mathcal{v}_t \in \mathbb{G}$, and

$$\mathcal{N}_{\mathcal{v}}^4(x) = \mathcal{P}_{t_{\mathcal{v}}+1}^4(x) \prod_{t=0}^{t_{\mathcal{v}}-1} (\mathcal{P}_{t+1}^4(x))^{\mathcal{v}_t}. \qquad (1)$$

Equation (1) has the advantage that, since $\mathcal{P}^4$-system is regular and periodic, it is easy to remember them and their products are easy form.

Lemma 4.3:

i) $\mathcal{N}_v^4(x) = [\mathcal{H}_\hbar]_{vz} = \frac{1}{\sqrt{\hbar}} cas\left(\frac{2vz}{\hbar}\right), 0 \le v, z < \hbar - 1,$

where, $\hbar$- is the order of Hartley transform of type $\mathcal{H}^I$ [20] and $cas(x) = \sin(x) + \cos(x)$, for each $x = \frac{3e}{4^v}$, $v \in N_0$, where, $e = \sum_{t=0}^{\infty} 4^t e_t$, with e_t being the dyadic coefficients of c in binary system:

$$c = \sum_{t=0}^{\infty} 2^t c_t \, , \, c_t \in \mathbb{G}.$$

ii) $\mathcal{N}_v^4(0) = 1$, for all v: $v \in N_0$.

iii) $\mathcal{N}^4 = \{\mathcal{N}_v^4(x)\}_{v=0}^{\infty}$-system forms a group with respect to multiplication.

iiii) $\int_0^1 \mathcal{N}_v^4(x) d\mu = 0$.

Proof: one can simply show that i), ii), and iiii) from definition of Hartley transform of type $\mathcal{H}^I$, relation (1), and in relation (1) respectively.

iii) For each $i, j \ge 0$, we write the binary number of i, j as: $(i)_b = \{\lambda_{\xi_i}, \cdots, \lambda_0\}$ and $(j)_b = \{\delta_{\theta_j}, \cdots, \delta_0\}$, where, the digits $\lambda_{\xi_i}, \cdots, \lambda_0$, $\delta_{\theta_j}, \cdots, \delta_0$ are in $\mathbb{G}$. We define the multiplication as:

$$\mathcal{N}_{(i)_b}^4(x).\mathcal{N}_{(j)_b}^4(x) = \mathcal{N}_{(i)_b \oplus (j)_b}^4(x) = \mathcal{N}_{(j)_b}^4(x).\mathcal{N}_{(i)_b}^4(x), \quad \forall\, x \in \mathcal{K}_1^4.$$

$\mathcal{N}_0^4(x)$ is the identity element of $\mathcal{N}^4$-system:

$$\mathcal{N}_{(0)_b}^4(x).\mathcal{N}_{(j)_b}^4(x) = \mathcal{N}_{(0)_b \oplus (j)_b}^4(x) = \mathcal{N}_{(j)_b}^4(x)$$

For each $\mathcal{N}_{(j)_b}^4(x) \in \mathcal{N}^4$-system.

The inverse element of $\mathcal{N}_j^4(x)$ is $\mathcal{N}_j^4(x)$ it self:

$$\mathcal{N}_{(j)_b}^4(x).\mathcal{N}_{(j)_b}^4(x) = \mathcal{N}_{(j)_b \oplus (j)_b}^4(x) = \mathcal{N}_{(0)_b}^4(x) = \mathcal{N}_0^4(x) = 1.$$

is associative: For each $i, j, p \ge 0$, we have

$$(\mathcal{N}_{(i)_b}^4(x).\mathcal{N}_{(j)_b}^4(x)).\mathcal{N}_{(p)_b}^4(x) = (\mathcal{N}_{(i)_b \oplus (j)_b}^4(x)).\mathcal{N}_{(p)_b}^4(x) = \mathcal{N}_{(i)_b \oplus (j)_b \oplus (p)_b}^4(x) = (\mathcal{N}_{(i)_b}^4(x)).\mathcal{N}_{(j)_b \oplus (p)_b}^4 = \mathcal{N}_{(i)_b}^4(x)(\mathcal{N}_{(j)_b}^4(x).\mathcal{N}_{(p)_b}^4(x)).$$

Therefore $(\mathcal{N}^4, .)$ is a group which is abelian as it a obvious from the definition of associative.

Theorem 4.2: $\mathcal{N}^4$-system is orthonormal basis on [0,1] concerning to measure of $\mathcal{K}^4$-Devil's staircase function on R.C.S.

Proof: For each $i, j \ge 0$, we write the binary number of i, j as:
$(i)_b = \{\lambda_{\xi_i}, \cdots, \lambda_0\}$ and $(j)_b = \{\delta_{\theta_j}, \cdots, \delta_0\}$, where, the digits $\lambda_{\xi_i}, \cdots, \lambda_0$, $\delta_{\theta_j}, \cdots, \delta_0$ are in $\mathbb{G}$ and via lemma 4.3 iii), we obtain:
If $i = j$, then, we have

$$\int_0^1 \mathcal{N}_i^4(x)\, \mathcal{N}_j^4(x)\, d\mu = \int_0^1 \mathcal{N}_{(i)_b}^4(x)\, \mathcal{N}_{(j)_b}^4\, d\mu = \int_0^1 \mathcal{N}_{(i)_b \oplus (j)_b}^4(x)\, d\mu = \int_0^1 \mathcal{N}_{(i)_b \oplus (i)_b}^4(x)\, d\mu = \int_0^1 \mathcal{N}_{(0)_b}^4(x) d\mu = \int_0^1 d\mu = \mathcal{J}_0^4(1) - \mathcal{J}_0^4(0) = 1$$

If $i \ne j$, then

$$\int_0^1 \mathcal{N}_i^4(x)\, \mathcal{N}_j^4(x)\, d\mu = \int_0^1 \mathcal{N}_{(i)_b}^4(x)\, \mathcal{N}_{(j)_b}^4\, d\mu = \int_0^1 \mathcal{N}_{(i)_b \oplus (j)_b}^4(x)\, d\mu$$

From section 2, there exist a unique natural number ζ such that :

$\zeta \equiv (\zeta)_b = (i)_b \oplus (j)_b$, therefore

$$\int_0^1 \mathcal{N}_i^4(x)\, \mathcal{N}_j^4(x)\, d\mu = \int_0^1 \mathcal{N}_{(i)_b \oplus (j)_b}^4(x)\, d\mu = \int_0^1 \mathcal{N}_{(\zeta)_b}^4(x)\, d\mu = \int_0^1 \mathcal{N}_\zeta^4(x)\, d\mu.$$

Via using lemma 4.3 iiii), we obtain

$$\int_0^1 \mathcal{N}_i^4(x)\, \mathcal{N}_j^4(x)\, d\mu = 0, \forall i \ne j \ \blacksquare.$$

Theorem 4.3: $\mathcal{N}^4$-system is a complete orthonormal system concerning to measure on a 4-adic-type Cantor like set $\mathcal{K}^4$.

Proof: Assume that ψ is the integrable function, and

$$\int_0^1 \psi(x) \mathcal{N}_i^4(x) d\mu = 0, \, 0 \le i < 2^v. \qquad (2)$$

Moreover, assume

$$\mathfrak{N}(x) = \int_0^x \psi(t) d\mu, \quad \forall\, x \in \mathcal{K}_\ell^4.$$

Therefore $\mathfrak{N}(x)$ have two properties [22]

i) $\mathfrak{N}(x)$ is continuous on [0,1];

ii) $\mathfrak{N}(x)$ is differentiable at every $x \in [0,1]$ at which $\psi(x)$ is continuous and $\mathfrak{N}'(x) = \psi(x)$.

It then follows from our assumptions that

$$\int_0^1 \psi(x) \mathcal{N}_i^4(x) d\mu = \sum_{\vartheta=0}^{2^v - 1} \int_{\mathcal{K}_{r^*}^4} \psi(x) \mathcal{N}_i^4\left(\frac{3e}{4^v}\right) d\mu \, ,$$

$$0 \le i < 2^v \, , r^* = 2^v + \vartheta$$

$$= \sum_{\vartheta=0}^{2^{\upsilon}-1} \int_{\frac{3e}{4^{\upsilon}}}^{\frac{3e+1}{4^{\upsilon}}} \psi(x)\mathcal{N}_i^4\left(\frac{3e}{4^{\upsilon}}\right) d\mu = 0.$$

From the property of linearly independent and lemma 4.3 i), we get

$$\mathcal{N}_i^4\left(\frac{3e}{4^{\upsilon}}\right) = [\mathcal{H}_{\hbar}]_{\upsilon z} \neq 0\ ,\ 0 \leq i < 2^{\upsilon}.$$

Also, the numbers $[\mathcal{H}_{\hbar}]_{\upsilon z}$ are linearly independent. Therefore

$$\int_{\frac{3e}{4^{\upsilon}}}^{\frac{3e+1}{4^{\upsilon}}} \psi(x)\mathcal{N}_i^4\left(\frac{3e}{4^{\upsilon}}\right) d\mu = 0. \qquad (3)$$

Assume that, the relation in (2). Using (3), we have

$$\mathfrak{N}\left(\frac{3e}{4^{\upsilon}}\right) = \mathfrak{N}\left(\frac{3e+1}{4^{\upsilon}}\right).$$

From which Alexits [23] concludes that $\mathfrak{N}(x) = 0$, and $\mathfrak{N}'(x) = 0$, $\forall x \in [0,1]$. Since $\mathfrak{N}'(x) = \psi(x)$ at every $x \in [0,1]$ in which $\psi(x)$ is continuous implies that $\psi(x) = 0$, at which $\psi(x)$ is continuous, then $\psi = \mathbf{0}$ in $\mathcal{K}_{\ell}^4$ ■.

Any absolutely integrable function $\mathcal{F}$ on the interval [0,1] has a Fourier series in $\mathcal{N}^4$-system:

$$\mathcal{F}(x) \sim \sum_{i=0}^{\infty} \omega_i\, \mathcal{N}_i^4(x). \qquad (4)$$

where,

$$\omega_i = \int_0^1 \mathcal{F}(x)\mathcal{N}_i^4(x)d\mu\ .$$

Are the $\mathcal{N}^4$-Fourier coefficients of $\mathcal{F}(x)$ and the series in relation (4) is called $\mathcal{N}^4$-Fourier series of $\mathcal{F}(x)$.

The ρ-th partial sum of the $\mathcal{N}^4$-Fourier series of a function $\mathcal{F}(x)$ will be represented via:

$$\Gamma_{\rho} = \sum_{i=0}^{\rho} \omega_i\, \mathcal{N}_i^4(x). \qquad (5)$$

5 ORTHOGONAL MULTIPLEXING SYSTEM (O.M.S.) IN $\mathcal{N}^4$-FOURIER SERIES

Since a $\mathcal{N}^4$-system forms complete orthonormal functions on R.C.S. takes only the values +1 and -1 which are likely to be well suited to multiplexing systems as well as the numerical $\mathcal{N}^4$-Fourier transmission and numerical sequence shifting of signals require summations and subtraction only.

The input signals $\mathcal{F}_i$, $i = 0,1,\cdots,k-1, k^z, z$ is a positive integer, passes first through filter. Then the set of functions $\mathcal{N}_i^4(x)$ are multiplied via $\mathcal{F}_i$ via the multipliers $\mathcal{M}$. The product is $\mathcal{F}_i\mathcal{N}_i^4(x)$ are summed and transmitted to obtain a signal $\mathcal{F}(x)$:

$$\mathcal{F}(x) = \sum_{i=0}^{k-1} \mathcal{F}_i\, \mathcal{N}_i^4(x). \qquad (6)$$

In order to recover $\mathcal{F}_i$, the signal $\mathcal{F}(x)$ is multiplied by $\mathcal{N}_j^4(x)$, where, $j = 0,1,\cdots,k-1$, and integrating the product in the normalized interval $0 \leq x \leq 1$:

$$\int_0^1 \mathcal{F}(x)\mathcal{N}_i^4(x)d\mu = \int_0^1 \sum_{i=0}^{k-1} \mathcal{F}_i\, \mathcal{N}_i^4(x)\mathcal{N}_j^4(x)d\mu.$$

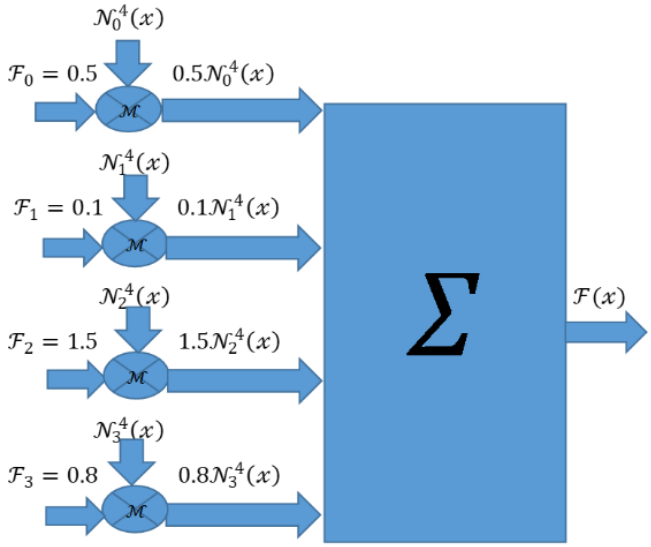


Figure 1: $\mathcal{N}^4$- carrier O.M.S.

By using theorem (4.2), the output of each de-multiplexing channel $i = j$, will be:

$$\int_0^1 \mathcal{F}(x)\mathcal{N}_i^4(x)d\mu = \mathcal{F}_i \, .$$

Example 1: consider the input signals of $\mathcal{F}(x)$:

$$\mathcal{F}_0 = 0.5, \mathcal{F}_1 = 0.1, \mathcal{F}_2 = 1.5, \mathcal{F}_3 = 0.8.$$

Each signal is multiplied via $4 - \mathcal{N}^4$-functions of order 4: the situation is depicted in Figure 1, and the waveform details for each channel are shown in Table 2. The total signal $\mathcal{F}(x)$ in Table 2 and Figure 1 denotes the sum of all four active channel signals, and designed in Figure 2.

Table 2: Detail of $\mathcal{N}^4$- carrier O.M.S. of Figure 1.

Time →	$x = 0$	$x = 1/4$	$x = 3/4$	$x = 1$
$0.5\mathcal{N}_0^4(x)$	0.5	0.5	0.5	0.5
$0.1\mathcal{N}_1^4(x)$	0.1	0.1	-0.1	-0.1
$1.5\mathcal{N}_2^4(x)$	1.5	-1.5	1.5	-1.5
$0.8\mathcal{N}_3^4(x)$	0.8	-0.8	-0.8	0.8
$\mathcal{F}(x)$	2.9	-1.7	1.1	-0.3

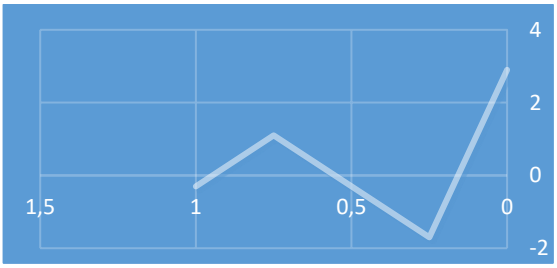


Figure 2: Total multiplexing signal of example 1.

6 CONCLUSIONS

This paper introduces a novel construction of piecewise constant orthonormal functions based on the rescaling Cantor set (R.C.S.), particularly utilizing 4-adic-type Cantor-like sets. The developed orthogonal system demonstrates strong properties of completeness, orthonormality, and linear independence with respect to the measure defined by the –Devil's staircase function. These foundational properties affirm the mathematical robustness and applicability of the constructed system. The paper presents the results of research on Rescaling Cantor set (R.C.S) of 4-adic-type Cantor-like set type. We obtained characteristic and in some cases criteria of completeness, orthonormality and basis property of $\mathcal{N}^4$-system concerning to measure of $\mathcal{K}^4$-Devil's staircase function on R.C.S. Many independent signals over communication channel in Multiplexing system are carried based on regularization of functions generating this system. Compared with the method of Fourier transform [18], the suggested approach is simpler in theory and easier in implementation. It is believed that this is the first time in using the $\mathcal{N}^4$- series to approach the most interesting problem in O.M.S. In addition to all the possibilities discussed above, we shall also investigate more properties of the $\mathcal{N}^4$-system and try to form a construction for the $\mathcal{k}$- adic- type Cantor-like set. Also, we may try to extend our work to include $\mathcal{N}^n$-system, where, $n \geq 4$. Compared with conventional Fourier-based methods, the –system offers theoretical simplicity, faster computational performance, and the potential for scalable extension to other adic-type Cantor sets. Moreover, this approach opens avenues for future research in signal processing, coding theory, and nonlinear dynamic analysis over fractal domains.

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Investigation of Doubly Nonlinear Parabolic Equation

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Keywords: Quasilinear, Porous Medium Equation, Separation of Variables, Comparison Principle, Self-Similar Solution.

Abstract: We study the properties of solutions for a porous medium equation (PME) in non-divergent form with a source term. The PME is a fundamental model in various physical and biological processes, including fluid flow through porous media, heat transfer, and population dynamics. Unlike the classical heat equation, the PME exhibits nonlinear diffusion, leading to rich mathematical structures and solution behaviours. Our main focus is obtaining exact solutions using the separable variable method under certain parameter constraints. These solutions provide explicit representations of the evolving profile of the medium and provide insight into the dynamics of the equation. Additionally, we construct a self-similar Barenblatt-type solution, a fundamental tool for analysing long-time asymptotics and the spreading behaviour of solutions. Self-similar solutions provide insights into the scaling properties of the PME and the influence of the source term on solution evolution. Moreover, we have constructed a numerical scheme, calculated numerical results and based on numerical solutions shown graphs in some particular cases.

1 INTRODUCTION

We consider the nonlinear equation stated as follows:

$$\rho(x)u_t = u^q div\left(\rho(x)\nabla u^m\right) + \varepsilon\gamma(t)\rho(x)u^\beta, \quad (1)$$

$$u(x,0) = u_0(x) \geq 0, x \in R^N, \quad (2)$$

where $(x,t) \in D, D = \{(x,t) \mid x \in R^N, t > 0\}$,
$m \geq 1, q \neq \{1 \pm m, \beta - m\}$, $\varepsilon = \pm 1$, $\rho(x) = |x|^{1-N}$,
$0 < \gamma(t) \in C(R_+), \beta$ depends on the function $\gamma(t)$,
and we consider a few cases throughout the paper.

Equation (1) includes many well-known equations, such as the Laplacian equation, heat equation, Leibenson equation, and the Boussinesq equation in the filtration of liquid and gas [1], [2], [3], [4]. Equation (1) is important for simulating a broad variety of physical processes. For example, curve shortening flow, resistive diffusion phenomena in force-free magnetic fields, diffusive processes found in biological species, and the spread of infectious diseases are among the many applications of (1) (see [5], [6], [7], [8] and references therein).

J.L. Díaz studied the following problem, which addresses the critical issue of modelling an aircraft fire extinguishing process within an engine nacelle [9]

$$u_t = \Delta u^m + |x|^\sigma u^p, \quad (3)$$

$$u(x,0) = u_0(x) \in L^\infty_{loc}(R^N), \quad (4)$$

where $m > 1, \sigma > 0, p < 1$.

J.L. Díaz used the nonlinear splitting method and the comparison principle [10]-[11] to obtain a self-similar solution in the following form:

$$u(x,t) = t^\alpha \left(A - \frac{m-1}{2m}\beta |x|^2 t^{-2\beta} \right)_+^{1/(m-1)},$$

where $\beta = \frac{1}{N(m-1)+2}, \alpha = \beta N, (d)_+ = max(d,0)$.

Moreover, J.L. Díaz showed that finite values for the model parameters p, m and σ exist, and that the combination of such values ensures the existence of global solutions

J.L. Vázquez studied [12] the PME and the existence of solutions in finite time or globally for the following problem:

$$u_t = \Delta u^m + f(x,t), \quad (5)$$

$$u(x,0) = u_0(x), x \in R^N, \quad (6)$$

where f is a reaction term.

J.L. Vázquez showed the decay rate of the solution such that $f = 0, \| u(.,t) \|_{L^p} \le Ct^{-\gamma_p}$, $\gamma_p = N(p-1)/\left((m-1)N+2\right)$; on the other hand, $f = u^q, \| u(.,t) \|_\infty : \ (T_c - t)^{-1/(q-1)}, T_c > 0$.

In the work [13] the existence of the solution and blow-up problems of the Cauchy problem are studied for a more general case.

Another significant work by D.G. Aronson [14] studied the PME using self-similar analysis [15], [16], the comparison principle, dynamical system behaviour, and other methods. D.G. Aronson obtained lower – upper estimates of the solution to the problem (5)-(6), and showed the decay rate of the solution.

M. Winkler showed that all global solutions of (5) with zero boundary data are uniformly bounded in the case $\sigma = 0$ [17].

R.G. Iagar and D.R. Munteanu [18] studied a very singular solution of (5) in an absorption case based on self-similar solutions and a dynamical system [19]. The authors showed the critical Fujita exponent [20] as follows:

$$p_F = m + (\sigma + 2)/N.$$

Moreover, the authors proved that $u(x,t): \ C|x|^{-(\sigma+2)/(p-m)}$ as $|x| \to \infty$ while $p \ge p_F$, where C - some constant.

We seek the solution in several cases for the function $\gamma(t)$ and β, considering different functional forms of $\gamma(t)$ (such as constant, power-law, or polynomial) as well as the general case, and analyze how various choices of β influence the qualitative and quantitative properties of the solution, including decay rates, self-similarity, and asymptotic behaviour.

2 SEPARATION OF VARIABLES

We consider the following cases:

I. $\gamma = const > 0$,

II. $\gamma(t) = A(T - \varepsilon t)^a$,

III. General case.

2.1 I Case

In this case, we consider $\beta = q + m$. First, using the separation method [21], we seek the solution to (1) as follows:

$$u(x,t) = (T - \varepsilon t)^{-\alpha} y(r), \quad (7)$$

where $r = |x|$ and $\alpha = \dfrac{1}{q+m-1} > 0$.

We put the (7) into (1). Then we obtain:

$$\varepsilon\alpha r^{1-N}(T-\varepsilon t)^{-\alpha-1} y = (T-\varepsilon t)^{-\alpha(q+m)} r^{1-N}\left[y^q \left((y^m)'\right) + \varepsilon\gamma y^{q+m} \right]. \quad (8)$$

Therefore, we rewrite the equation as follows:

$$\left((y^m)'\right) - \varepsilon\alpha y^{1-q} + \varepsilon\gamma y^m = 0, \quad (9)$$

or $$y'' + (m-1)\frac{y'^2}{y} - \frac{\varepsilon}{m}(\alpha y^{2-q-m} - \gamma y) = 0. \quad (10)$$

We denote $y(r) = z^{1/2}(r)$. Hence, we get

$$y' = \frac{1}{2} z^{-1/2} z_r' = \frac{1}{2} z_y'.$$

Consequently, we obtain the following equation:

$$\frac{1}{2} z_y' + (m-1)\frac{z}{y} - \frac{\varepsilon}{m}(\alpha y^{2-q-m} - \gamma y) = 0. \quad (11)$$

We seek z as

$$z(y) = z_1(y) z_2(y), \quad (12)$$

and put it into (14)

$$\frac{1}{2}(z_1 z_2)' + (m-1)\frac{z_1 z_2}{y} - \frac{\varepsilon}{m}(\alpha y^{2-q-m} - \gamma y) = 0. \quad (13)$$

We choose z_2 such that:

$$\frac{z_1}{2}\left(z_{2'} + \frac{2(m-1)z_2}{y} \right) = 0, \ z_2(y) = y^{2-2m},$$

that implies:

$$z = \frac{2\alpha\varepsilon}{m(m-q+1)} y^{3-q-m}\left(1 - \frac{(m-q+1)\gamma}{2m\alpha} y^{q+m-1}\right). \quad (14)$$

Hence, we get

$$z = \frac{2\alpha\varepsilon}{m(m-q+1)} y^{3-q-m}\left(1 - \frac{(m-q+1)\gamma}{2m\alpha} y^{q+m-1}\right). \quad (15)$$

Therefore, we derive that

$$\sqrt{\frac{m(m-q+1)}{2\alpha}}\int\frac{y^{\frac{m+q-1}{2}-1}dy}{\sqrt{\varepsilon-\varepsilon\left(\sqrt{\frac{(m-q+1)\gamma}{2m\alpha}}y^{\frac{q+m-1}{2}}\right)^2}}=$$

$$\left|\begin{matrix}y^{\frac{m+q-1}{2}}=\sqrt{\frac{2m\alpha}{(m-q+1)\gamma}}\varphi(Y),\\ \varphi'=\psi,\psi^2=\varepsilon(1-\varphi^2)\end{matrix}\right|=\frac{2m}{(m+q-1)\sqrt{\gamma}}\int\frac{\psi dY}{\sqrt{\varepsilon-\varepsilon\varphi^2}}$$

$$=\frac{2m}{(m+q-1)\sqrt{\gamma}}Y=r+\frac{2m}{(m+q-1)\sqrt{\gamma}}C_1, \quad (19)$$

$$y(x)=\left[\sqrt{\frac{2m\alpha}{m-q+1}}\varphi\left(\frac{(m-q+1)\sqrt{\gamma}}{2m}|x|+C_1\right)\right]^{2\alpha},$$

$$\text{where } \varphi(x)=\begin{cases}cos(x), & \text{if } \varepsilon=1,\\ ch(x), & \text{if } \varepsilon=-1.\end{cases} \quad (20)$$

Moreover, we have

$$u(x,t)=\begin{cases}\left(\sqrt{\frac{2m\alpha}{m-q+1}}\frac{cos^2(\frac{m-q+1}{2m}\sqrt{\gamma}|x|+C_1)}{T-t}\right)^{\alpha}, \varepsilon=1,\\ \left(\sqrt{\frac{2m\alpha}{m-q+1}}\frac{ch^2(\frac{m-q+1}{2m}\sqrt{\gamma}|x|+C_2)}{T+t}\right)^{\alpha}, \varepsilon=-1.\end{cases} \quad (16)$$

It is easy to see that, $u_1(x,t)\to\infty$ as $t\to T^-$ in (16). This kind of solution is called a blow-up solution and is intensively studied in the works [22]-[23], [24]-[25]. Similar results were also obtained in the work [26]-[27] in the case $q=0$, and using the method of separation of variables, alternative solutions were found in some groups by A.D. Polyanin and A.I. Zhurov [28].

2.2 II Case

In this case, we consider $\gamma(t)$ as follows

$$\gamma(t)=A(T-\varepsilon t)^a, \quad (17)$$

where $A,a>0$, and $\beta=1+(a+1)(q+m-1)$.

We seek the solution $u(x,t)$ as follows:

$$u(x,t)=B(T-\varepsilon t)^{-b}g(r) \quad (18)$$

where $b=\frac{a+1}{\beta-1}, B=(bA)^{\frac{1}{\beta-1}}$.

After inserting the notation (18) into (1) it yields following

$$Cg^q\left((g^m)'\right)-\varepsilon g+\varepsilon g^\beta=0, \quad (19)$$

which is invariant to the following equation under the mapping $r\to\frac{r}{\sqrt{C}}$

$$g^q\left((g^m)'\right)-\varepsilon g+\varepsilon g^\beta=0, \quad (20)$$

where $C=B^{q+m-\beta}/A$.

Using a similar approach as in (10)-(15), we deduce that

$$\sqrt{\frac{m(m-q+1)}{2}}\int\frac{g^{\frac{m+q-3}{2}}dg}{\sqrt{\varepsilon-\varepsilon\frac{m-q+1}{\beta-m-q}g^{\beta-1}}}=\left|g^{\beta-1}=\frac{\beta-m-q}{m-q+1}z\right|$$

$$=\frac{\sqrt{m\left((\beta-m-q)(m-q+1)^a\right)^{\frac{1}{a+1}}}}{\sqrt{2}(\beta-1)}\cdot\int(\varepsilon(1-z))^{-\frac{1}{2}}z^{\frac{1}{2(a+1)}-1}dz$$

$$=C_3\cdot J_\varepsilon\left(z;\frac{1}{2},\frac{1}{2(a+1)}\right)=r+C_3C_4, \quad (21)$$

where $C_4\geq 0, J_\varepsilon(z;a,b)=\int(\varepsilon(1-z))^{a-1}z^{b-1}dz$,

$$C_3=\frac{\sqrt{m\left((\beta-m-q)(m-q+1)^a\right)^{\frac{1}{a+1}}}}{\sqrt{2}(\beta-1)},$$

It is easy to see that

$$\frac{r}{C_3}+C_4=J_1\left(z;\frac{1}{2},\frac{1}{2(a+1)}\right)=$$
$$C_5+B\left(z;\frac{1}{2},\frac{1}{2(a+1)}\right), \quad \text{for } 0\leq z\leq 1, \quad (22)$$

where $B(z;a,b)$ is an incomplete beta function and C_5 some constant.

Furthermore, we rewrite (22) as follows:

$$\frac{|x|}{C_3}+C_4-C_5=I_z\left(\frac{1}{2},\frac{1}{2(a+1)}\right)\cdot B\left(\frac{1}{2},\frac{1}{2(a+1)}\right), \quad (23)$$

where $B\left(\frac{1}{2},\frac{1}{2(a+1)}\right)$ and $I_z\left(\frac{1}{2},\frac{1}{2(a+1)}\right)$ - beta and regularized incomplete beta functions, respectively.

From (23), we conclude that g is a solution to the following equation:

$$I_{\frac{m-q+1}{\beta-m-q}g^{\beta-1}(r)}\left(\frac{1}{2},\frac{1}{2(a+1)}\right)=\frac{r}{\sqrt{C}C_3B\left(\frac{1}{2},\frac{1}{2(a+1)}\right)}+C_6. \quad (24)$$

where $C_6 = (C_4 - C_5) / B\left(\frac{1}{2}, \frac{1}{2(a+1)}\right)$.

Now, we consider $\varepsilon = -1$ case and compute following integral

$$\frac{r}{C_3} + C_4 = J_{-1}\left(z; \frac{1}{2}, \frac{1}{2(a+1)}\right) = \int (z-1)^{-\frac{1}{2}} z^{\frac{1}{2(a+1)}-1} dz$$

$$= \left| z = \frac{1}{1-\theta} \right| = \int (1-\theta)^{\frac{a}{2(a+1)}-1} \theta^{-\frac{1}{2}-1} d\theta = C_7 +$$

$$I_{1-1/z}(\frac{1}{2}, \frac{a}{2(a+1)}) \cdot B(\frac{1}{2}, \frac{a}{2(a+1)}), \quad \text{for } z > 1. \quad (25)$$

Analogously, we derive that:

$$I_{1-\frac{\beta-m-q}{m-q+1} g^{1-\beta}(r)}\left(\frac{1}{2}, \frac{a}{2(a+1)}\right) = \frac{r}{\sqrt{C} C_3 B\left(\frac{1}{2}, \frac{a}{2(a+1)}\right)} + C_8. \quad (26)$$

where $C_8 = (C_4 - C_7) / B\left(\frac{1}{2}, \frac{a}{2(a+1)}\right)$ and C_7 some constant.

We are interested only in non-negative solutions of the (1). Thus, we have skipped the cases: $z > 1$ in (27) and $z < 1$ in (25). We can find g explicitly [29] using the inverse of the incomplete beta function.

2.3 III Case

We rewrite the (1) as follows:

$$u_t = u^q \left(u^m\right)_{rr} + \varepsilon \gamma(t) u^\beta, \quad (27)$$

where $r = |x|$.

Using the nonlinear splitting method, we seek a solution to the (27) as follows

$$u(r,t) = u_1(t) w(\tau(t), r), \quad (28)$$

where $\tau(t) = \int u_1^{q+m-1}(t) dt$,

$$u_1(t) = \begin{cases} \left(T_1 + \varepsilon(\beta-1)\int_0^t \gamma(y) dy\right)^{-1/(\beta-1)}, & \beta \neq 1, \\ T_2 \exp(-\varepsilon \int_0^t \gamma(y) dy), \ \beta = 1, \ T_{1,2} = const \geq 0 \end{cases},$$

We put the (33) into (32), then the following yields:

$$w_\tau = w^q \left(w^m\right)_{rr} + \frac{\varepsilon u_2(t)}{\tau(t)} (w^\beta + w), \quad (29)$$

where $u_2(t) = \gamma(t) \tau(t) u_1^{\beta-1-m}(t)$.

Now, we seek $w(\tau, r)$ as follows:

$$w_\tau = w^q \left(w^m\right)_{rr} + \frac{\varepsilon u_2(t)}{\tau(t)} (w^\beta + w), \quad (30)$$

After substituting (30) into (29) we obtain the following results:

$$f^q \frac{d}{d\xi}\left(\frac{df^m}{d\xi}\right) + \frac{\xi}{2}\frac{df}{d\xi} + \varepsilon u_2(t)(f^\beta + f) = 0. \quad (31)$$

or $$L_1 f + L_2 f = 0, \quad (32)$$

where $L_1 f = f^q \frac{d}{d\xi}\left(\frac{df^m}{d\xi} + \frac{\xi f^{1-q}}{2(1-q)}\right)$,

$$L_2 f = f\left(\varepsilon u_2(t)\left(f^{\beta-1} + 1\right) - \frac{1}{2(1-q)}\right).$$

We introduce a new function

$$z(x,t) = u_1(t) f_1(\xi), \quad (33)$$

where $f_1(\xi) = A_1 \left(\xi_0^2 - \xi^2\right)_+^{\frac{1}{q+m-1}}$,

$$A_1 = (\frac{q+m-1}{4m(1-q)})^{\frac{1}{q+m-1}}, \xi_0 = const \geq 0.$$

Now, based on the comparison principle and $z(x,t)$ function, we estimate the solution of the problem (1)-(2).

Theorem 1: let one of the following inequalities hold:

- $\varepsilon > 0, u_2(t) \leq \frac{1}{A_2}, A_2 = 2(1-q)\left(1 + (A_1 a^{\frac{1}{q+m-1}})^{\beta-1}\right)$;
- $\varepsilon < 0$;

and $u_0(x) \leq z(x,0), x \in R^N$. Then the solution to the problem (1)-(2) has the estimate in D

$$u(x,t) \leq z(x,t),$$

and for the front the following estimate

$$|x| \geq x_f(t) = \xi_0 \tau^{1/2} \text{ hold.}$$

Proof. According to the definition, the function f_1 is nonzero if $\xi < \xi_0$, and equal to zero if $\xi \geq \xi_0$ or $|x| \geq \xi_0 \tau^{1/2}$. $|x| = \xi_0 \tau^{1/2}$ is called front (free boundary [10], [12]).

Now we show that $L_1 f_1 + L_2 f_1 \leq 0$. It is easy to see that f_1 satisfies the equality $L_1 f_1 = 0$. Therefore, from (27)-(31) it suffices to show that $L_2 f_1 \leq 0$ in order to hold $u(x,t) \leq z(x,t)$ until $u_0(x) \leq z(x,0)$. Since f_1 is a non-negative function, the following inequality yields:

$$\varepsilon u_2(t)(f_1^{\beta-1}+1) \le \frac{1}{2(1-q)}. \quad (34)$$

Obviously, if $\varepsilon < 0$ then inequality (34) holds. On the other hand, if $\varepsilon > 0$:

$$u_2(t)(f_1^{\beta-1}+1) \le u_2(t)(\max(f_1^{\beta-1})+1) =$$
$$u_2(t)((A_1 a^{1/(q+m-1)})^{\beta-1}+1),$$
$$\text{or } \frac{A_2}{2(1-q)}u_2(t) \le \frac{1}{2(1-q)}.$$

The proof of the Theorem 1 is completed.

3 NUMERICAL ANALYSIS

In order to perform numerical calculations, we need to construct numerical grids Ω_x and Ω_t respectively

$$\Omega_x = \{x \mid x_i = a + ih_x, h_x = \frac{b-a}{N_x}; b > a\},$$

$$\Omega_t = \{t \mid t_j = jh_t, h_t = \frac{T}{N_t}, T > 0\}.$$

Now, we rewrite the (27) on the grid $\Omega = \Omega_x \times \Omega_t$ as explicit scheme form with $O(h_t + h_x^2)$ approximation error

$$\begin{cases} \frac{y_{i,j}-y_{i,j-1}}{h_t} = a(y_{i+1})\frac{y_{i+1,j}-y_{i,j}}{h_x^2} - a(y_i)\frac{y_{i,j}-y_{i-1,j}}{h_x^2} \\ +\varepsilon\gamma(t_{j-1})(y_{i,j-1})^{\beta}, \quad i=\overline{1,N_x-1}, j=\overline{1,N_t}, \\ y_{i,0} = u_0(x_i), i=\overline{0,N_x}, \\ y_{0,j} = \phi_1(t_j), y_{N_x,j} = \phi_2(t_j), \ j=\overline{0,N_t}, \end{cases} \quad (35)$$

where $\phi_1(t_j) = z(x_0,t_j), \phi_2(t_j) = z(x_{N_x},t_j)$,

$$a(y_i) = \begin{cases} m\cdot(y_{i-1,j})^q\left(0.5y_{i,j}-0.5y_{i-1,j}\right)^{m-1}, \\ 0.5m\cdot(y_{i-1,j})^q\left((y_{i,j})^{m-1}-(y_{i-1,j})^{m-1}\right), \end{cases}$$

To solve (35) we use iteration processes, so

$$\begin{cases} \frac{y_{i,j}^s - y_{i,j-1}^{s-1}}{h_t} = a(y_{i+1}^{s-1})\frac{y_{i+1,j}^s - y_{i,j}^s}{h_x^2} - a(y_i^{s-1})\frac{y_{i,j}^s - y_{i-1,j}^s}{h_x^2} \\ +\varepsilon\gamma(t_{j-1})(y_{i,j-1}^{s-1})^{\beta}, \quad i=\overline{1,N_x-1}, j=\overline{1,N_t}, s=1,2,\ldots \\ y_{i,0} = u_0(x_i), i=\overline{0,N_x}, \\ y_{0,j} = \phi_1(t_j), y_{N_x,j} = \phi_2(t_j), \ j=\overline{0,N_t}, \end{cases} \quad (36)$$

The iteration process continues until the following condition is satisfied:

$$\max_{i,j}\left|y_{i,j}^s - y_{i,j}^{s-1}\right| < \varepsilon,$$

where ε precision and we took it as 10^{-6}.

We denote

$$A_{i,j}^s = \frac{h_t}{h_x^2}a(y_{i+1}^{s-1}), B_{i,j}^s = \frac{h_t}{h_x^2}a(y_i^{s-1}), C_{i,j}^s = A_{i,j}^s + B_{i,j}^s + 1,$$

$$F_{i,j}^s = y_{i,j-1}^{s-1} + \varepsilon h_t \gamma(t_{j-1})(y_{i,j-1}^{s-1})^{\beta} \quad (37)$$

Applying this notation, we rewrite (36) as follows:

$$\hat{A}_i\hat{y}_{i+1} - \hat{C}_i\hat{y}_i + \hat{B}_i\hat{y}_{i-1} = -\hat{F}_i, \quad (38)$$

where $\hat{y}_i = y_{i,j}^s, \hat{A}_i = A_{i,j}^s, \hat{B}_i = B_{i,j}^s, \hat{C}_i = C_{i,j}^s, \hat{F}_i = F_{i,j}^s$.

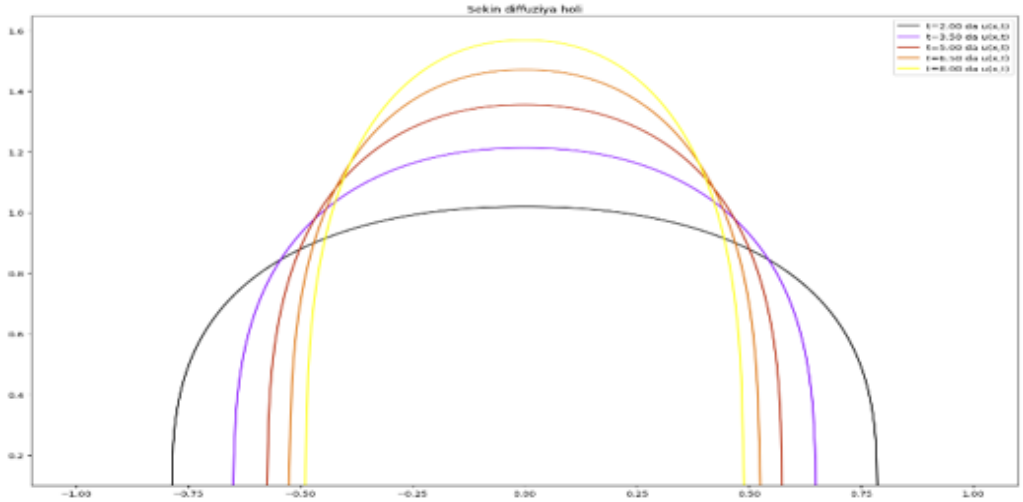


Figure 1: The numerical solution $q = 0.5, m = 2.2, \beta = 2.01, \varepsilon = 1, \gamma(t) = 1$.

To solve the algebraic (38) we apply the tridiagonal algorithm [30]

$$\hat{y}_i = \alpha_i \hat{y}_{i+1} + \beta_i \tag{39}$$

where $\alpha_i = \frac{\hat{A}_i}{\hat{D}_i}, \beta_i = \frac{\hat{F}_i + \beta_{i-1}\hat{B}_i}{\hat{D}_i}, \hat{D}_i = \hat{C}_i - \alpha_{i-1}\hat{B}_i,$ $i = \overline{1, N_x - 1},$ and the initial values are $\alpha_0 = \frac{\hat{A}_1}{\hat{C}_1}, \beta_0 = \frac{\hat{F}_1 - \phi_1 \hat{B}_1}{\hat{C}_1}.$

To satisfy the diagonal dominant condition, which guarantees the stability of an algorithm or numerical scheme, the following condition must be held:

$$|\hat{C}_i| \geq |\hat{A}_i| + |\hat{B}_i|. \tag{40}$$

According to the definition $\hat{C}_i$ in (37) the inequality (40) is valid.

Below we illustrate graphics of the numerical solution with Figures 1-4.

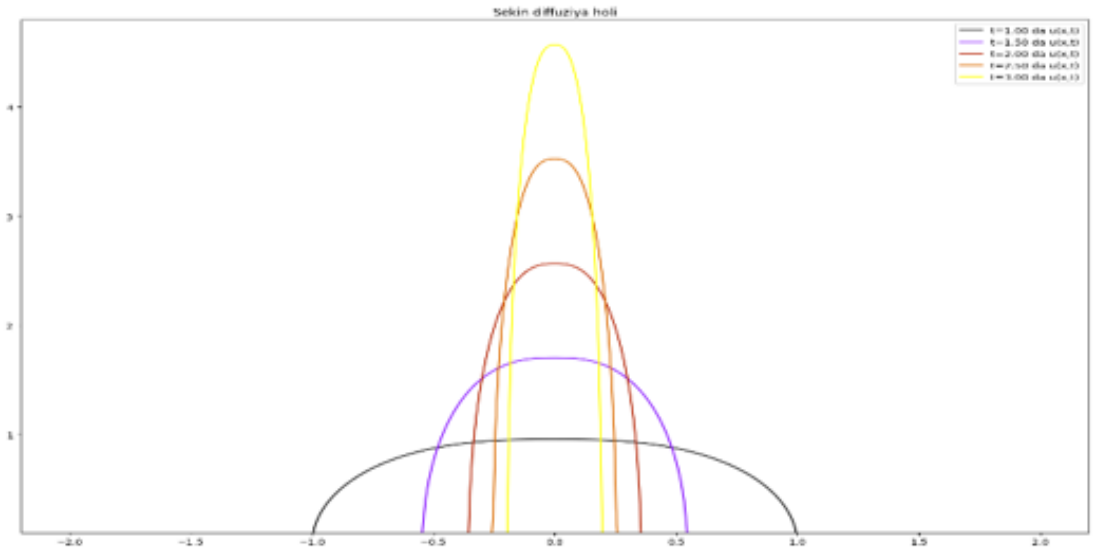


Figure 2: The numerical solution $q = -0.1, m = 3.2, \beta = 1.5, \varepsilon = -1, \gamma(t) = t^{0.1}$.

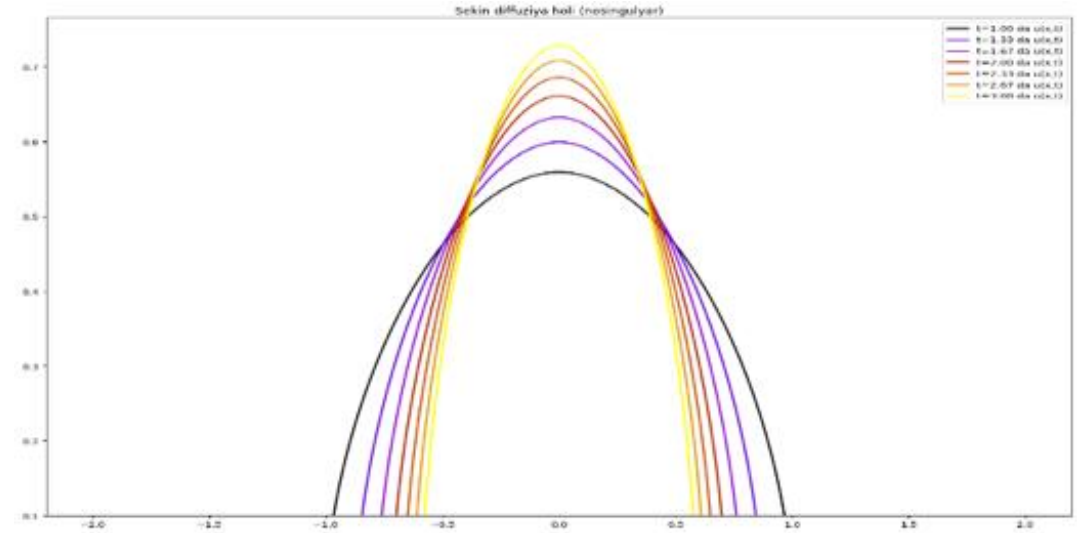


Figure 3: The numerical solution $q = 0.1, m = 2.2, \beta = 2.1, \varepsilon = -1, \gamma(t) = t^{0.5}$.

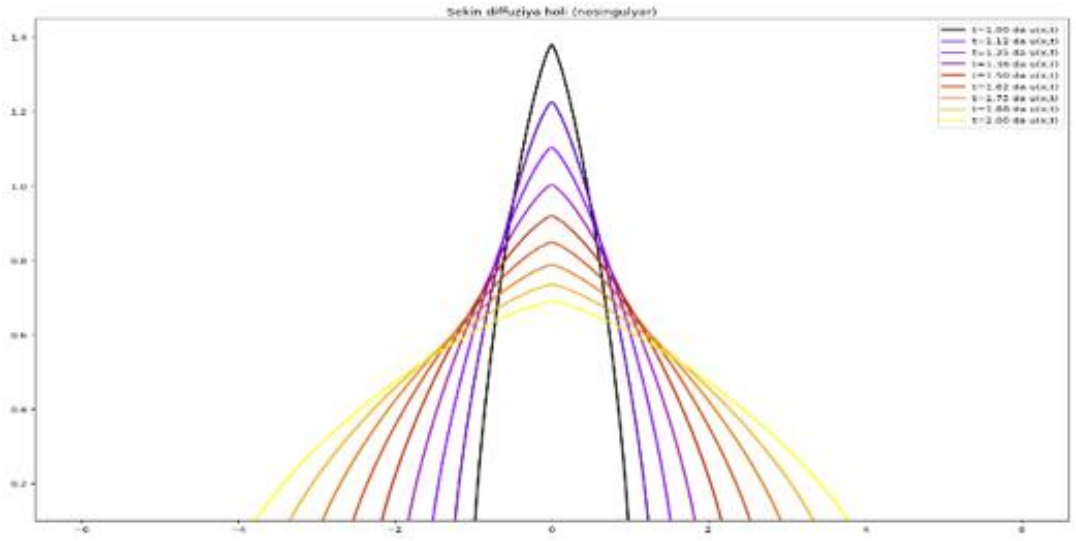


Figure 4: The numerical solution $q = 0.1, m = 2.25, \beta = 1.1, \varepsilon = 1, \gamma(t) = 0.1$.

4 CONCLUSIONS

In this work, we examine the porous medium equation written in non-divergence form with a spatially varying source term to represent an inhomogeneous medium. First, we derived exact solutions in special parameter regimes by separation of variables, yielding closed-form evolution laws that serve as benchmarks. Second, we discovered a novel explicit solution expressible in terms of the inverse regularized Beta function. Third, we constructed a family of Barenblatt-type self-similar profiles and applied a comparison-principle argument to bound the general solution by these profiles, thereby establishing sharp asymptotic estimates. Finally, we developed a fully implicit finite-difference scheme and solved the resulting tridiagonal linear systems via the Thomas algorithm. To perform quantitative analysis, we develop a finite-difference algorithm: we discretize the source term in (35) using the Samarskii–Sobol scheme at the origin and employ the Thomas algorithm to advance the solution in time. Two performance metrics - eps for accuracy and s for convergence - show marked improvement over our previous results (eps=10^(-3), s=5 and eps=10^(-3), s=4 in [5] and [11], respectively). The numerical experiments corroborate the analytical findings and illustrate representative behaviors (compact support, blow-up, interface motion) for general initial data.

The results have direct implications for physical and applied models. Equations of this doubly-nonlinear form arise in diverse contexts: for example, they describe heat conduction in media with temperature-dependent conductivity, resistive diffusion in magnetized plasmas, flow through heterogeneous porous materials, and density-dependent transport in biological media.

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Solitary Wave Structures of the One-Dimensional Mikhailov-Novikov-Wang System Using Kudryashov's New Function Method

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Keywords: Traveling Wave Solutions, Mikhailov-Novikov-Wang System, Kudryashov's New Function Method, Solitons.

Abstract: Traveling waves and integrable equations are the most well-known features of nonlinear wave propagation phenomena. Analytical solutions to nonlinear integrable equations play an important role in examining the behaviour and structure of nonlinear systems. They offer valuable insights into how these systems evolve over time and under different conditions. Such solutions are essential for accurately describing a range of real-world phenomena. This study aims to derive closed-form traveling wave solutions of the (1+1)-dimensional Mikhailov-Novikov-Wang model by employing the Kudryashov's new function method. This system provides novel perspectives for understanding the connection between integrability and water wave phenomena. New solitary wave solutions are constructed in terms of hyperbolic functions by assigning particular values of the parameters. The study yields two types of solitons, including bell-shaped and singular soliton solutions. The solutions are simulated in 2D and 3D graphical representations to illustrate their physical features. The results highlight the effectiveness of the employed approach in constructing novel solutions which are essential to understand the dynamics of the governing system.

1 INTRODUCTION

Nonlinear integrable partial differential equations (PDEs) are fundamental in representing nonlinear physical phenomena in diverse fields such as applied mathematics, physics, and engineering [1], [2], [3]. The investigation of nonlinear physical behaviors often necessitates the use of both numerical and analytical methods by mathematicians and researchers for the analysis of nonlinear PDEs [4], [5], [6], [7], [8], [9], [10].

The Mikhailov-Novikov-Wang (MNW) system was introduced in 2006, and is given by [11]

$$\begin{cases} g_t = g_{xxxxx} - 20gg_{xxx} - 50g_xg_{xx} + 80g^2g_x + h_x, \\ h_t = -6hg_{xxx} - 2g_{xx}h_x + 96hgg_x + 16h_xg^2. \end{cases} \quad (1)$$

with $g(x,t)$ and $h(x,t)$ representing differentiable functions of velocity and height, respectively. This Boussinesq-type system consists of a couple of (1+1)-dimensional integrable fifth-order PDEs that represent nonlinear wave phenomena. Hence, the system is significant for examining spatial and temporal dynamics, as well as waves in physical environments like shallow water. When $h(x,t) = 0$, (1) simplifies to the well-known Kaup-Kupershmidt (KK) equation [12]. Previous studies, including works by Mikhailov et al. [13] and Vojčák [14] have demonstrated its integrability. Traveling wave solutions of the MNW system were constructed by Cesar via the extended tanh method [15]. A zero-curvature representation of (1) was formulated by Sergyeyev [12]. Shan et al. [16] employed Lie algebra techniques to establish the Lax-integrability of the equations, further confirming the presence of Hamiltonian structures. The time-fractional MNW system was examined by Jiang et al. [17] using Lie symmetry analysis.

Exact solutions of nonlinear PDEs have significant importance for many researchers. Hence, numerous powerful and effective methods have been developed, including: the generalized exponential function method [18], the uniform method [19], the Kudryashov new function method [20], [21], Exp-function method [22], the homogeneous Balance Method [23], the generalized Kudryashov method [24] and many other analytical methods.

The Kudryashov's new function method is employed in this study to construct novel solitary wave structures of the (1+1) dimensional integrable

MNW system which models the propagation of nonlinear waves. This robust technique facilitates the discovery of analytical solutions by converting the mathematical model into ordinary differential equations via the wave transform.

Next, this study unfolds through the following sections: Section 2 presents a concise overview of the Kudryashov's new function method. In Section 3, new exact solutions of the MNW system are presented. Section 4 discusses the derived solutions. Finally, Section 5 concludes the work.

2 OVERVIEW OF THE NEW FUNCTION METHOD

The methodology of Kudryashov's new function method will be exhibited in this section.

Suppose a nonlinear partial differential (2):

$$\psi(g, g_t, g_x, g_{tt}, g_{xt}, \ldots) = 0. \tag{2}$$

By employing the wave transformation

$$g(x,t) = G(\omega), \omega = kx - \beta t + \omega_0, \tag{3}$$

where β is the wave speed, and k and ω_0 are arbitrary constants, (2) is converted into a reduced ODE where φ is expressed as a polynomial function of the new function $G(\omega)$ and its derivatives as follows:

$$\varphi\left(G, -\beta G', kG', \beta^2 G'', \ldots\right) = 0. \tag{4}$$

where the prime symbol indicates differentiation with respect to ω.

The foremost steps of the new function method can be summarized in the following manner [20]:

Step 1: For identifying the pole order for the formal solution of (4), assume $G = y^{\rho}$ and compare the exponents of y associated with the highest-order derivative term and the highest nonlinear term subsequently.

Step 2: Assume the solution of (4) is expressed in the form:

$$G(\omega) = \sum_{i=0}^{N} b_i Q^i(\omega), \tag{5}$$

where N represents the pole order and $b_0, b_1, \ldots, b_N$ are constants that will be identified subsequently.

Kudryashov'–s new function is given by [25]

$$Q(\omega) = \frac{1}{pe^{\omega} + \left(\frac{\lambda}{4p}\right)e^{-\omega}}, \tag{6}$$

where p and λ are unknown parameters. Equation (6) satisfies the following auxiliary ODE:

$$\left(\frac{dQ}{d\omega}\right)^2 = Q^2(\omega)\left(1 - \lambda Q^2(\omega)\right). \tag{7}$$

It is easy to find distinct derivatives of $Q(\omega)$ as follows:

$$\frac{d^2Q}{d\omega^2} = Q - 2\lambda Q^3, \tag{8}$$

$$\frac{d^3Q}{d\omega^3} = Q_{\omega} - 6\lambda Q^2 Q_{\omega}, \tag{9}$$

$$\frac{d^4Q}{d\omega^4} = Q - 20\lambda Q^3 + 24\lambda^2 Q^5, \tag{10}$$

$$\frac{d^5Q}{d\omega^5} = Q_{\omega} - 60\lambda Q^2 Q\omega + 120\lambda^2 Q^4 Q_{\omega}. \tag{11}$$

The relations (8)-(11) are utilized to compute the derivatives of $Q(\omega)$ as follows:

$$\frac{dG}{d\omega} = \sum_{i=0}^{N} b_i i Q^{i-1} \frac{dQ}{d\omega}, \tag{12}$$

$$\frac{d^2G}{d\omega^2} = \sum_{i=0}^{N} b_i i^2 Q^i - \lambda i^2 Q^{i+2} - i\lambda Q^{i+2}, \tag{13}$$

$$\frac{d^3G}{d\omega^3} = \sum_{i=0}^{N} b_i \frac{dQ}{d\omega}\left(i^3 Q^{i-1} - i^2(i+2)\lambda Q^{i+1} - i(i+2)\lambda Q^{i+1}\right). \tag{14}$$

And so on.

Step 3: By substituting (5) together with (7) and (12)-(14) into (4), we obtain a polynomial in terms of Q and its derivatives.

Step 4: A system of algebraic equations results from setting the coefficients of various different powers of $Q^{\alpha}\left(\frac{dQ}{d\omega}\right)^{\beta}$ $(\alpha = 0,1,2,\ldots; \beta = 0,1)$ to zero. The unknown constants $b_i (i = 0,1,2,\ldots,N)$, β, and λ can be explicitly determined by solving this system.

Step 5: By substituting these values and the general solution (6) of (7) into (5), the traveling wave solutions of (2) is obtained.

3 EXACT SOLUTIONS OF THE MNW SYSTEM

To derive the exact solutions of (1), the following transformation is applied:

$$\begin{cases} g(x,t) = G(\omega), \\ h(x,t) = H(\omega), \\ \omega = kx - \beta t + \omega_0. \end{cases} \tag{15}$$

Consequently, the system (15) is converted to a nonlinear ordinary differential system as follows:

$$\begin{cases} -\beta G' = k^5 G^{(5)} - 20k^3 G^{(3)} - 50k^3 G'G'' \\ \qquad + 80kG^2 G' + kH', \\ -\beta H' = -6k^3 HG^{(3)} - 2k^3 G''H' + 96kHGG' \\ \qquad + 16kH'G^2, \end{cases} \tag{16}$$

thus,

$$\beta H' - 6K^3 HG^{(3)} - 2K^3 G''H' + 96KHGG' + 16KH'G^2 = 0. \tag{17}$$

The first equation in (16) can be expressed as

$$H' = -\frac{\beta}{k} G' - k^4 G^{(5)} + 20k^2 GG^{(3)} + 50k^2 G'G'' - 80G^2 G'. \tag{18}$$

Integrating (18) once with respect to ω, while assuming the integration constant is zero, yields

$$H = -\frac{\beta}{k} G - k^4 G^{(4)} + 20k^2 GG'' + 15k^2 (G')^2 - \frac{80}{3} G^3. \tag{19}$$

By substituting (18) and (19) into the second equation of (16), the following ODE is obtained:

$$\begin{aligned} &-\frac{\beta^2}{k} G' - \beta k^4 G^{(5)} + 26\beta k^2 GG^{(3)} + 52\beta G'G'' \\ &+2k^7 G^{(5)} G^{(2)} - 160k^5 GG^{(2)} G^{(3)} - 90k^5 (G')^2 G^{(3)} \\ &-100k^5 G'(G'')^2 + 2880K^3 G'G''G^2 - 16k^5 G^{(5)} G^2 \\ &+480k^3 G^{(3)} G^3 - 3840kG^4 G' + 1440k^3 G(G')^3 \\ &-96k^5 GG'G^{(4)} + 6k^7 G^{(3)} G^{(4)} - 192\beta G^2 G' = 0. \end{aligned} \tag{20}$$

To determine the pole order, we substitute $G = y^\rho$ into (20), which results in $G''G^{(5)} \approx y^{2\rho-7}$ and $G^4 G' \approx y^{5\rho-1}$. By balancing the exponents, we find $\rho = -2$, indicating that the pole order is $N = 2$. Consequently, we have

$$G = b_0 + b_1 Q(\omega) + b_2 Q^2(\omega). \tag{21}$$

With the help of Mathematica, substituting (21) and its derivatives into (20), and then collecting terms with identical powers of Q^α while setting the coefficients of $Q^\alpha \left(\alpha = 0,1,2,\ldots,9\right)$ to zero, leads to a system of algebraic equations. This system is solved to obtain:

$$b_0 = \frac{k^2}{4},\ b_1 = 0, b_2 = -\frac{3}{4}k^2\lambda,\ \beta = -k^5. \tag{22}$$

By substituting these values into (21), the following exact solution is obtained:

$$\begin{cases} G(\omega) = \dfrac{k^2}{4} - \dfrac{3k^2\lambda}{4\left(pe^{\omega} + \dfrac{\lambda e^{-\omega}}{4p}\right)^2}, \\ H(\omega) = -\dfrac{k^6}{6}. \end{cases} \tag{23}$$

where $\omega = kx + k^5 t + \omega_0$.

We can randomly choose the parameters p and λ. Setting $\lambda = 4p^2$, we get the following solitary wave solution:

$$\begin{cases} G(\omega) = -\dfrac{k^2}{4}\left(3\operatorname{sech}^2(\omega) - 1\right), \\ H(\omega) = -\dfrac{k^6}{6}. \end{cases} \tag{24}$$

where $\omega = kx + k^5 t + \omega_0$.

Again, setting $\lambda = -4p^2$, we get the following solitary wave solution:

$$\begin{cases} G(\omega) = \dfrac{k^2}{4}\left(3\operatorname{csch}^2(\omega) + 1\right), \\ H(\omega) = -\dfrac{k^6}{6}. \end{cases} \tag{25}$$

where $\omega = kx + k^5 t + \omega_0$.

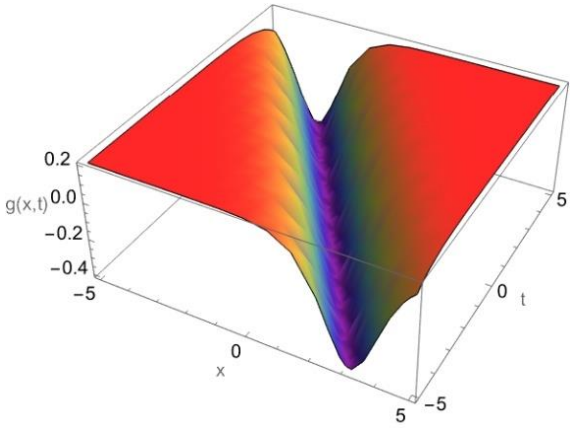


Figure 1: Solitary wave profile (24) at $k = 0.9,$ and $\omega_0 = 0.1$ in 3D plot.

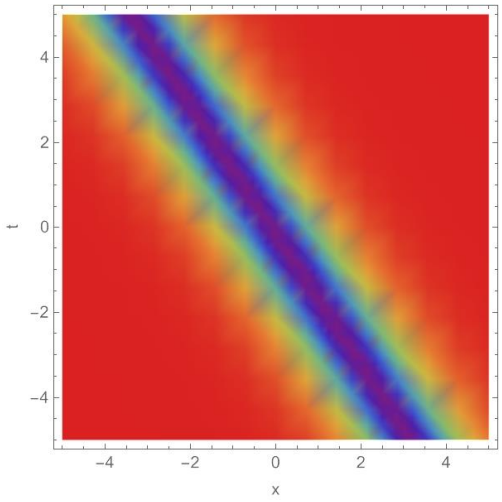


Figure 2: Solitary wave profile (24) at $k = 0.9,$ and $\omega_0 = 0.1$ in 2D density plot.

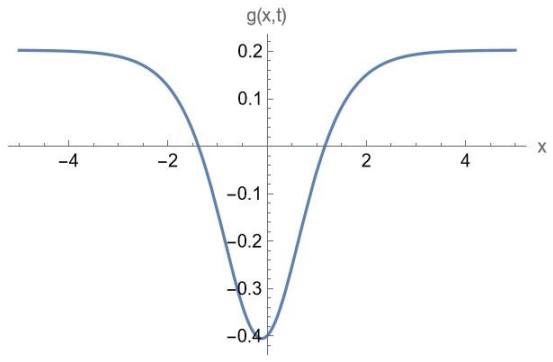


Figure 3: Solitary wave profile (24) at $t = 0, k = 0.9,$ and $\omega_0 = 0.1$ in 2D plot.

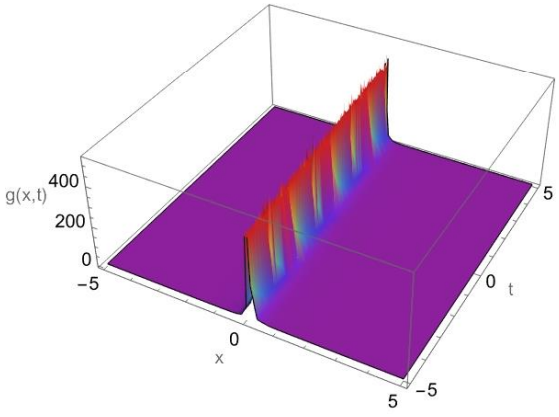


Figure 4: Solitary wave profile (25) at $k = 0.5,$ and $\omega_0 = 0.1$ in 3D plot.

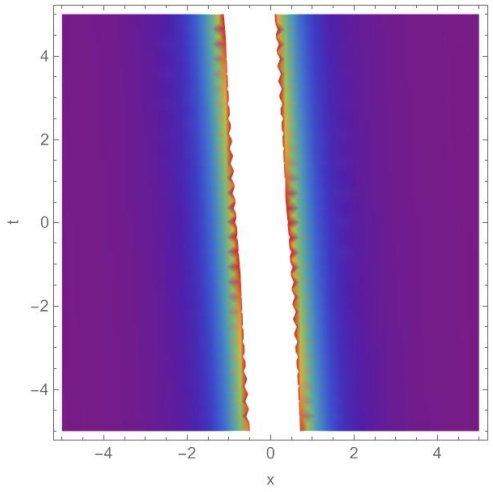


Figure 5: Solitary wave profile (25) at $k = 0.5,$ and $\omega_0 = 0.1$ in 2D density plot.

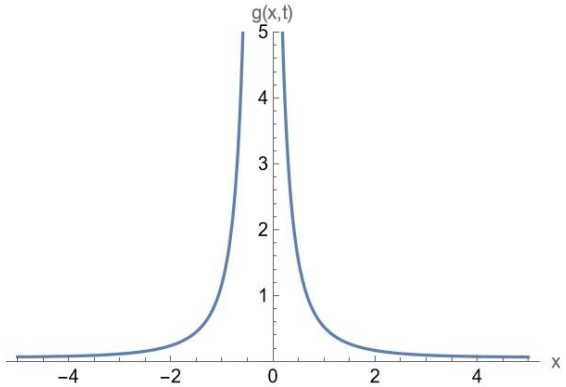


Figure 6: Solitary wave profile (25) at $t = 0, k = 0.5,$ and $\omega_0 = 0.1$ in 2D plot.

4 RESULTS AND DISCUSSION

We demonstrate the physical behavior of solutions by assigning particular values for the arbitrary parameters. These parameter choices play a crucial role in interpreting the physical features and properties of the derived solutions. Figures 1, 2 and 3 depict the 3D, 2D density and 2D profiles of the anti-bell-type soliton solution (24) respectively, for selected parameter values at $k = 0.9,$ and $\omega_0 = 0.1.$ Figures 4, 5 and 6 depict the 3D, 2D density and 2D graphs of the singular soliton solution (25) respectively for specific parameters at $k = 0.5,$ and $\omega_0 = 0.1.$

A comparison between the solitary wave solutions obtained in this study for the governing system and those reported in earlier literature [15] confirms that the solutions presented here are new and have not appeared before. The results will significantly contribute to investigate numerous phenomena that arise in nature and across various nonlinear medium.

In this research, we employed advanced symbolic computation techniques via Mathematica to conduct both symbolic mathematical analysis and numerical simulations. Mathematica has demonstrated its effectiveness and robustness as a tool for deriving solitary wave solutions.

5 CONCLUSIONS

This paper examined the use of the Kudryashov new function technique to analyze the (1+1) dimensional Mikhailov-Novikov-Wang system. By applying the wave transform, the original system is reduced to a fifth-order ODE. Consequently, this analysis has yielded new solitary wave solutions, including bell-shaped and singular soliton solutions. These solutions are considered completely new and have not been reported before. The obtained solutions offer important information about the wave dynamics of the model. They are especially useful for researchers working on nonlinear processes in areas like fluid mechanics and plasma physics. These findings show that the Kudryashov new function method provides an efficient and reliable approach for analyzing complex dynamical systems. This approach serves as an effective and robust tool for analyzing and interpreting the dynamic behavior of the system. It not only enhances the understanding of complex mathematical models, but also promising for broader future applications in various disciplines. In the future, one can further extend our results to solve other Boussinesq-type equations due to their significance in modeling nonlinear physical phenomena.

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A New Approach with Software Implementation to Extend the Pythagorean Theorem in Multi-Dimensions

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Keywords: Pythagoras Theorem, Extended Pythagorean Theorem, Programming the Expansion Intuition, Perfect Square Formula.

Abstract: The Pythagorean theorem, which states that "for any right triangle, the square of the hypotenuse is equal to the sum of the squares of the lengths of the two legs," applies only within a single plane (i.e., in two dimensions). This paper proposes an extension of the Pythagorean theorem to multiple planes and dimensions by introducing a novel concept called Abd's 1st Intuition or Abd's First Theory (AFT). In this extended version, a finite sequence of right triangles is constructed, starting from a specific initial right triangle with two legs and a hypotenuse. Each subsequent right triangle is formed with one leg as the hypotenuse of the previous triangle and the other leg as a new value. This process continues such that the n-th right triangle has legs corresponding to the previous hypotenuse and a new dimension's value, resulting in a new hypotenuse. Each transition to a new plane introduces a new dimension, culminating in an extended form of the theorem: the square of the hypotenuse of this final right triangle is equal to the sum of the squares of the legs from all the constructed triangles. Additionally, a sub-exponential growth pattern for the sequence of hypotenuses is derived, demonstrating that their growth rate depends on the acute angle adjacent to the legs. For the special case of isosceles right triangles, the hypotenuse of the n-th triangle can be explicitly calculated from the first leg of the base triangle. This generalized theorem is intended for applications in wireless and wired communication networks, cryptography, communications security, and other areas within applied programming and computer science.

1 INTRODUCTION

The tremendous development in all fields during the past four decades as a result of the widespread and rapid spread of information and communications technology and wireless communication networks required a reconsideration of mathematical theories and theorems in a way that serves the other applying sciences in our current era [1]. The importance of the Pythagorean theorem (PT) lies in its mathematical and engineering applications, such as calculating right triangles, trigonometric ratios, and finding the distances between points and determine angles and lengths in the engineering drawing [2] Also, this theorem is used in the design of buildings, where it is used by architects and construction engineers in the civil engineering, also in the electrical engineering to design the coils of the generators and motors [3],[4]. It is also used in the space engineering for calculating the distances among the celestial bodies [5], the movement of spacecraft, fighter jets, autopilot system, projectiles, missiles, submarines, and navigation at the sea [6], [7]. In addition, this theory appears in the scanning technology and wireless networks to determine the right and accurate distance between the source and the receiver with the necessary corrections for that [8], [9], while this theorem represents the basis for all Global Positioning System (GPS) measurements [10].

In this research, we present a new mathematical perspective on the PT by extending it to a finite number of planes and dimensions. We aim to make the theorem more comprehensive and then prove our new conjecture using the perfect square formula and

mathematical induction, supported by illustrative examples. Subsequently, we implement the theorem in Python to generalize its application and explore its potential uses in various fields Such as architecture, space engineering, navigation, rocket engineering, wireless communication networks, artificial intelligence, and others.

2 PYTHAGOREAN THEOREM AND ITS VARIOUS EXTANSION

The Pythagorean Theorem (PT) is one of the most important mathematical theorems developed in the history of mathematics. This theorem is named after Pythagoras, a Greek mathematician from the 6th century BC who developed it in the 5th century BC (Nanda 2016). The PT of any right triangle as given in Figure 1 (which is applied in one plane), states that the square of the hypotenuse, h_1 (the side opposite the right angle) is equal to the sum of squares of the other two sides, x_1 and x_2 (we call it legs) according to the mathematical expression [11, [12];

$$x_1^2 + x_2^2 = h_1^2 \quad (1)$$

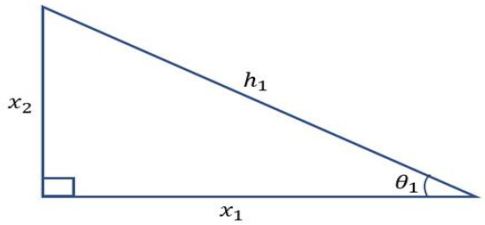


Figure 1: The right triangle.

It should be noted that the inverse of the PT is also true, meaning that a triangle is right-angled if the square of one of its sides (the longest side) is equal to the sum of the squares of the other two sides. While the square of the sum of two terms is equal to the sum of both its squares plus twice multiply them, as shown in the mathematical formula below [13];

$$(x_1 + x_2)^2 = x_1^2 + x_2^2 + 2x_1x_2 \quad (2)$$

This theorem has been extended and generalized in various ways over the centuries, leading to a rich field of mathematical research. The PT can be generalized to higher dimensions. In an n-dimensional Euclidean space, the distance between two points can be calculated using the generalized Pythagorean Theorem, also known as the Euclidean distance formula [14]. The Law of Cosines is a generalization of the Pythagorean Theorem to non-right triangles [15]. In non-Euclidean geometries, such as spherical and hyperbolic geometry, the PT does not hold in its classical form. However, there are analogous results in spherical geometry and in hyperbolic geometry that are critical in the study of curved spaces, such as in general relativity [16]. In number theory, there is Pythagorean triples which have been studied extensively and another extension of this concept is including the study of primitive Pythagorean triples and their properties in modular arithmetic and algebraic number theory [17].

In the concept of vector space and complex numbers, we can extend the PT in more than one level and in several dimensions via utilizing the idea of inner product associated with the Cauchy-Schwartz inequality and the parallelogram rule [18]. Also, we can extend the PT in the algebraic geometry to include various cases, like the Pythagorean technicals defined by equations similar to the Pythagorean conformity. Therefore, this scope shows the geometric characteristics relationship with algebraic texture [19]. Extensions of the PT are vastly utilized in physical fields, especially with vector amounts like the forces with their moments, the velocities with their accelerations, and electromagnetic fields with their directional effects. For example, Minkowski space (in special relativity) represents modulated version of the PT to calculate space-time periods [20]. Therefore, the PT represents the backbone of the math with the geometry as all, where its extensions into multi-dimensions via various planes, non-Euclidean spaces, algebraic geometry, number theory, and physical fields explain its deep effect and the wide scope of its applications. The continued studies of all these deepen our understanding of geometry and also join it to other mathematical corrections and feasible applications. In this work, we have expanded the PT into many planes via adding more right triangles that share sides with each other.

3 NEW EXTANDED VERSION OF THE PYTHAGOREAN THEOREM: ABD'S 1ST INTUITION

Our intuition gives attractive extension of the PT via the addition of finitely verity planes, each including a right triangle. This extension generates a multi-dimensional build that expands the traditional 2-dimension version of the PT.

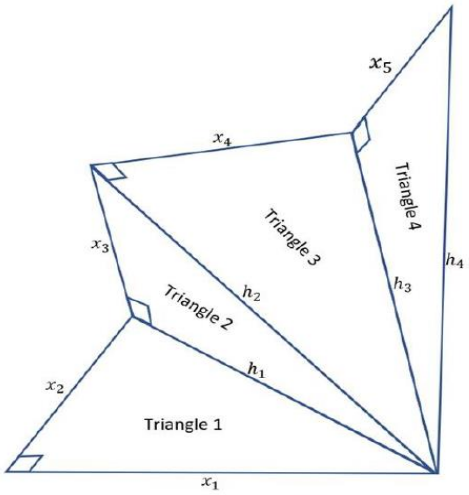


Figure 2: Creating four right triangles to illustrate Abd's 1st intuition.

Figure 2 illustrates our idea in the expanding mechanism, beginning with the foundation right triangle in the xy-plane (the first plane) with two right legs x_1 and x_2 and hypotenuse h_1, we build a second plane including a second right triangle with two right legs x_3 and h_1 and hypotenuse h_2. This second right triangle shares the hypotenuse h_1 with the base triangle, establishing a connection between the two. Subsequently, a third plane is introduced with a right triangle having legs x_4 and h_2, and hypotenuse h_3. This process continues, with each new triangle connecting to the previous one via a shared hypotenuse. Ultimately, this construction results in a geometric structure consisting of finitely many right triangles (say k triangles), each connected through shared hypotenuses. Refer to Figure 2 for a visual representation of this geometric construction of 4 right triangles.

In our institution, we conjecture that the sum of the squares of x_i (for $i = 1,2,\cdots,k$) is equal to the square of h_k, as given as follows

$$x_1^2 + x_2^2 + \cdots + x_{k+1}^2 = h_k^2 \quad (3)$$

This suggests that the extension not only creates a multi-dimensional structure but also preserves a relationship akin to the original Pythagorean Theorem, where the cumulative effect of the legs in each plane equates to the hypotenuse in the final plane.

3.1 Adopting the Perfect Square Formula

By using perfect squares law, we may leverage the familiar properties of squares to understand our institution, the extended PT in a higher-dimensional context. We may see that as we add more planes and triangles, the relationship $\Sigma_{i=1}^{k+1} x_i^2 = h_k^2$ remains consistent, showing that the geometric extension preserves the Pythagorean relationship in a more complex structure.

For the first right triangle, we have $x_1 = h_1 \cos\theta_1$ and $x_2 = h_1 \sin\theta_1$. From the perfect square law, we know that

$$x_1^2 + x_2^2 = (x_1 + x_2)^2 - 2x_1x_2 \quad (4)$$

Therefore we have

$$x_1^2 + x_2^2 = (h_1\cos\theta_1 + h_1\sin\theta_1)^2 - 2(h_1\cos\theta_1)(h_1\sin\theta_1)$$

This shows that

$$x_1^2 + x_2^2 = h_1^2 \quad (5)$$

For the second right triangle, we have $h_1 = h_2 \cos\theta_2$ and $x_3 = h_2 \sin\theta_2$. From the perfect square law, we know that

$$h_1^2 + x_3^2 = (h_1 + x_3)^2 - 2h_1x_3 \quad (6)$$

Therefore we have

$$h_1^2 + x_3^2 = (h_2\cos\theta_2 + h_2\sin\theta_2)^2 - 2(h_2\cos\theta_2)(h_2\sin\theta_2) \quad (7)$$

This shows that

$$h_1^2 + x_3^2 = h_2^2 \quad (8)$$

Then substitute (5) into the (8) yields

$$x_1^2 + x_2^2 + x_3^2 = h_2^2 \quad (9)$$

Moving to the next triangles, say to the k-th, we may substitute the finding in the $(k-1)$-th triangle to show that

$$x_1^2 + x_2^2 + x_3^2 + \cdots + x_{k+1}^2 = h_k^2 \quad (10)$$

This Equation represents our intuition, which is that "The square of the length of the hypotenuse in a right triangle resulting from several right triangles in space equals the sum of the squares of the lengths for all its legs (right sides)", hence k represents the number of right triangles or the number of levels in space and $k+1$ represents the number of dimensions, while x indicates the number of legs of the right triangles, and h represents the hypotenuse of the last triangle. Thus, the Intuition becomes as follows

$$x_1^2 + \sum_{i=1}^{i=k} x = h_k^2. \quad (11)$$

To illustrate the mechanism of this expansion and its stages, we present an example for that:

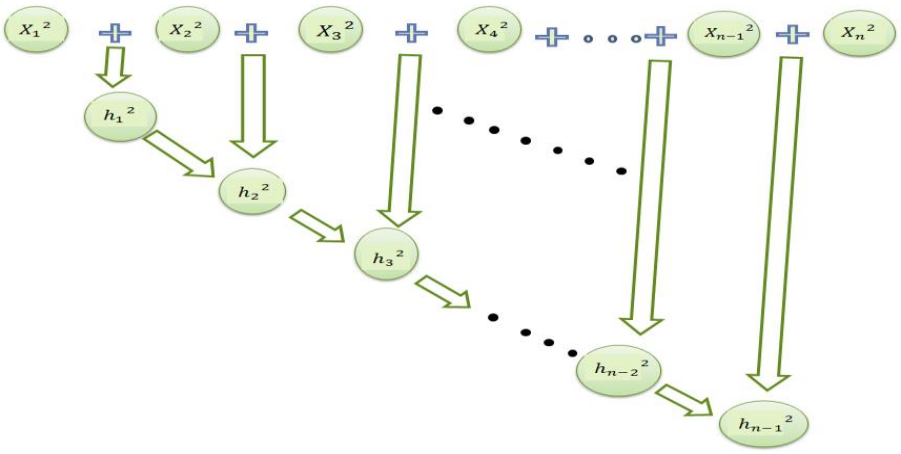


Figure 3: The action mechanism of Abd's 1st intuition.

Let, $k = 3$, $x_1 = 4$, and $x_2 = 3$.

$$h_1^2 = X_1^2 + X_2^2 \leftrightarrow 4^2 + 3^2 = 25 = 5^2 \quad (12)$$

And if $x_3 = 12$, then

$$5^2 + 12^2 = 169 = 13^2 \leftrightarrow 5^2 = 13^2 - 12^2 \quad (13)$$

Substitute (13) to (12) to obtain the (14):

$$4^2 + 3^2 = 13^2 - 12^2 \leftrightarrow 4^2 + 3^2 + 12^2 = 13^2 \quad (14)$$

And, if $x_4 = 84$, then

$$13^2 + 84^2 = 7225 = 85^2 \leftrightarrow 13^2 = 85^2 - 84^2 \quad (15)$$

Then, substitute (15) into (14) to obtain the (16) as shown below:

$$4^2 + 3^2 + 12^2 = 85^2 - 84^2 \leftrightarrow 4^2 + 3^2 + 12^2 + 84^2 = 85^2 \quad (16)$$

3.2 Programming the Expansion Intuition

To apply the expansion intuition, generalize it, and employ it in various fields, it will be programmed in the Python language [21], where Figure 3 shows the action mechanism for the new intuition, which facilitates the program construction to it as follows.

Figure 3 shows the programming for action mechanism of the new intuition as follows:

$$x_1^2 + x_2^2 = h_1^2, \text{then } h_1^2 + x_3^2 = h_2^2, \text{then } h_2^2 + x_4^2 h_3^2, \text{then } h_3^2 + x_5^2 = h_4^2, \text{ then}, \cdots, h_{k-2}^2 + x_k^2 = h_{k-1}^2 \quad (17)$$

This can be explained through the flow chart as shown in Figure 4.

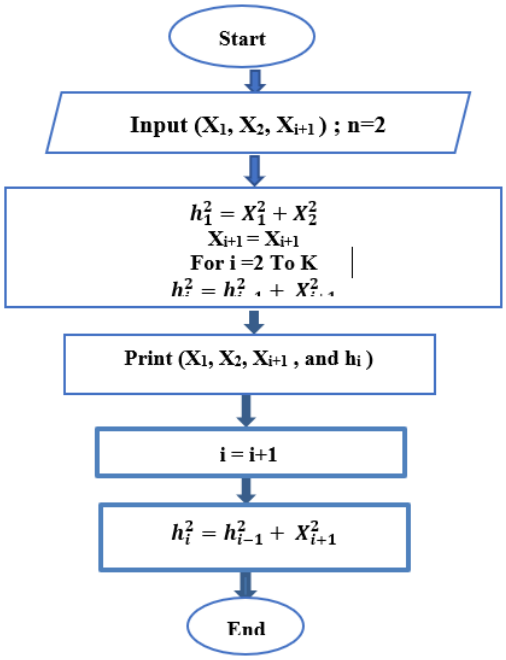


Figure 4: Flowchart for Abd's 1st intuition in k-dimension.

Therefore, we can express the programming according to the following steps:

Step 1: Sides of a right triangle (x_1, x_2) and the number of iteration to generate a right triangle (k)
Step 2: h_1 < ---- $x_1^2 + x_2^2$
Step 3: Loop of range (k)
Step 4: x_{i+2} < -----random number
Step 5: $h_{i+1}^2 = X_{i+1}^2 + h_i^2$
Step 6: Display h_{i+1} , X_1 , X_2 , X_{i+1}, h_i

3.3 Mathematical Induction to Prove the Intuition

Let us restate our finding in mathematical statement and prove it using the method of mathematical induction. Recall that we construct k right triangles beginning with the foundation based right triangle with legs x_1, x_2 and hypotenuse h_1. The next triangle is another right triangle where one leg is h_1 and the other is a new value, say x_3. The hypotenuse of this right triangle is denoted as h_2. We then construct another right triangle where one leg is h_2 and the other is another new value, x_4. We repeat this procedure to contruct k right triangles. Observe that $(k-1)$-th and k-th triangles share the side, h_{k-1}. We found that $x_1^2 + x_2^2 + \cdots + x_{k+1}^2 = h_k^2$, as stated by the following theorem and its proof.

Theorem 3.1. Consider a sequence of k right triangles constructed as follows:

The first right triangle has legs x_1, and x_2, with hypotenuse h_1. For each $k \in \mathbb{N}\backslash\{1\}$, the k-th triangle has legs x_{k+1} and h_{k-1}, with hypotenuse h_k.We found that $x_1^2 + x_2^2 + \cdots + x_{k+1}^2 = h_k^2$.

Proof: we are going to prove this result by induction method.

For the case $n = 1$, we consider the first right triangle, and Pythagoras Theorem gives that $x_1^2 + x_2^2 = h_1^2$. So the first case is proven.

We assume that, it is true for the $n = k$, i.e.

$$x_1^2 + x_2^2 + \cdots + x_{k+1}^2 = h_k^2 \qquad (18)$$

So now, we aim to prove the statement for $n = k + 1$.

By adding (x_{k+2}^2) to both sides for (13), we get

$$x_1^2 + x_2^2 + \cdots + x_{k+1}^2 + x_{k+2}^2 = h_k^2 + x_{k+2}^2 \qquad (19)$$

Proof: we firstly claim that $x_1 = h_k \cos^k \theta$ and prove it by induction method. For the case $k = 1$, it is obvious that $x_1 = h_1 \cos\theta$, since θ is the acute angle adjacent to x_2. We assume that it is also true for the case $k = n$, which is

$$x_1 = h_n \cos^n \theta \qquad (20)$$

Now let us consider the $(k+1)$-th right trianlge as Figure 5.

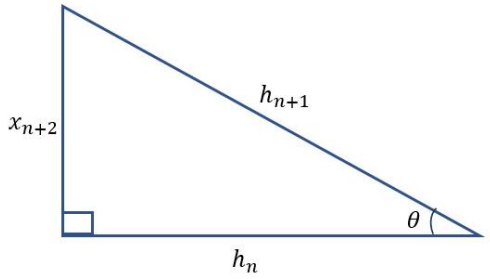


Figure 5: The $(k+1)$-th right trianlge.

Therefore

$$h_n = h_{n+1} \cos\theta \qquad (21)$$

Substitute (21) into (20), we then get on (22) as:

$$x_1 = (h_{n+1} \cos\theta)(\cos^n \theta) = h_{n+1} \cos^{n+1} \theta \qquad (22)$$

Therefore it is true that $x_1 = h_k \cos^k \theta$.

Now, for the sequence of $\{h_1, h_2, \cdots, h_k\}$ let us calculate it rate of growth $\frac{h_i}{h_{i+1}}$.

Since $h_i = \frac{x_1}{\cos^i \theta}$ and $h_{i+1} = \frac{x_1}{\cos^{i+1} \theta}$, then we found that

$$\frac{h_i}{h_{i+1}} = \cos\theta. \qquad (23)$$

Since $0 < \theta < \frac{\pi}{2}$, then $0 < \cos\theta < 1$ and this shows that the sequence $\{h_1, h_2, \cdots, h_k\}$ is increasing with sub-exponential growth, since the terms h_i's are increasing but at a progressively slower rate.

Now, we are going to consider another special case which is the right triangle with equal legs (isosceles right triangle). We then are able to determine the hyphotenus of each k-th isosceles right triangle.

Proposition 3.3: consider a sequence of k isosceles right triangles constructed as follows:

The first right triangle has two legs x_1 and hypotenuse h_1. For each $k \in \mathbb{N}\backslash\{1\}$, the k-th triangle has two legs h_{k-1}, and hypotenuse h_k.

Therefore,

$$h_k = \begin{cases} 2^n x_1 & \text{if } k = 2n \text{ for an integer } n \\ \frac{2^n x_1}{\sqrt{2}} & \text{if } k = 2n-1 \text{ for an integer } n \end{cases} \qquad (24)$$

Proof: let us consider the first and second isosceles right triangles and applying the basic trigonometry, we then have $h_1 = \frac{2x_1}{\sqrt{2}}$ and $h_2 = 2x_1$, then the finding is true for the case $k = 1$ and $k = 2$. By induction we assume that

$$h_k = \begin{cases} Kx_1 & \text{if } k = 2n \text{ for an integer } n \\ \frac{(K+1)x_1}{\sqrt{2}} & \text{if } k = 2n-1 \text{ for an integer } n \end{cases} \qquad (25)$$

for an integer k.

To prove for the case $k+1$, we consider two cases. The firsrt case is whenever $k+1 = 2n$ for an integer n and we consider the $(k+1)$-th triangle.

Therefore, $h_{k+1} = \frac{2h_k}{\sqrt{2}}$. Since $k + 1 = 2n$, then $k = 2n - 1$ and we have $h_k = \frac{2^n x_1}{\sqrt{2}}$. Therefore

$$h_{k+1} = \frac{2}{\sqrt{2}}\left(\frac{2^n x_1}{\sqrt{2}}\right) = 2^n x_1.$$

Now for the second case, we let $k + 1 = 2n - 1$ for an integer n. Therefore $k = 2n - 2 = 2m$ for some other integer $m = n - 1$. Hence $h_k = 2^m x_1 = 2^{n-1} x_1$. Then $h_{k+1} = \frac{2h_k}{\sqrt{2}} = \frac{2}{\sqrt{2}}(2^{n-1} x_1) = \frac{2^n x_1}{\sqrt{2}}$, and the proof is achieved.

4 CONCLUSIONS

Our study has led to a new creation of a modern expansion theorem derived from the Pythagorean Theorem (PT), viable in multi planes and dimensions. This extension confirms that the square of the hypotenuse of a right-angled triangle, created by integrating several different right triangles in space, equals the sum of the squares legs of all these triangles. Via projecting the Pythagorean rule to higher dimensions and adding a new dimension with each transition, this intuition was verified utilizing the rule of perfect squares and proved it via the mathematical induction process.

This new widened theorem shows a more overall process than the conventional PT applied on two dimensions only. Therefore, it can be utilized in varied fields including network security, coding, wireless communications networks, encryption, IoT (Internet of Things), and AI (Artificial Intelligence). This stratifies with the rapid progress in Information and Communication Technology (ICT) and gives valuable insights for applications via these scopes.

The improvement of PT via expanding to multiple dimensions has wide and influential applications. In communications, it improves signal processing and network design through advanced multidimensional analysis. Also, it can improve encryption algorithms and error detection with correcting it by using multidimensional spaces to protect data in the field of security and encryption. In artificial intelligence (AI), it supports and improves modern machine learning algorithms and techniques. It also helps in integrating sensors, analyzing data, and improving accuracy and functionality in the Internet of Things (IoT). In addition, this expansion helps in quantum computing and structural analysis, providing more accurate solutions and models in the fields of engineering and physics. These extensions are also invested in virtual reality experiences and improving 3D modeling of computer graphics and visualization. In general, this extension keeps pace with all modern and accelerating technological techniques across many and diverse fields. Finally, we recommend investing in this study and using it in the Network Security, Artificial Intelligence, Aerospace Engineering, and Mathematical and Physical applications.

ACKNOWLEDGMENTS

We sincerely thank Assistant Professor Ya'arub Mahmood Hamiedi, Professor of English Linguistics for helping us with this research.

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Stock Management Algorithms in a Passenger Wagon Depot

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Keywords: Stock Management, Algorithms, Passenger Wagon, Automation, Simulation Model.

Abstract: The process of delivering spare parts and components of different moving unit elements to the passenger wagon depot warehouse under the supervision of railway transport "Uztemiryolyolovchi" Joint Stock Company (JSC, also known as the Joint Stock Company) was examined in the article, along with the challenges of efficient stock management. The automated warehouse management system for railroad transportation is explained, along with its components, stages of development, concepts, and implementation. The efficient automation of warehouse accounting for prompt rolling stock (wagon) maintenance and repair is a special concern in the administration of passenger transportation, and simulation modeling is employed in this research. A multi-level research methodology is suggested in order to increase resource management efficiency: a) Conduct preliminary analysis of stock management systems; b) select the indicators required for the object's efficient operation and identify the factors influencing them; c) develop a simulation model of reserve management's block diagram and program, conduct an experiment based on it, and create a mathematical regression model with a set of equations; d) optimize the stock management system using the mathematical model that has been developed. The interconnectedness of the indices characterizing the reserves was examined using correlation-regression analysis. The correlation analysis's findings allowed for the determination of the regression equations, which in turn allowed for the evaluation of the elements' degree of influence on the final indicator of the stock management system's efficiency.

1 INTRODUCTION

Optimizing inventory management reduces costs and boosts business productivity, making it a crucial component of manufacturing company management. The improvement of inventory control models and algorithms has been the subject of numerous studies in recent years. Some of the most significant works in this field are listed below.

The study "Inventory Optimization Based on Simulation" by A. Bikov [2] concentrated on production planning techniques, inventory management strategies, mathematical models, and optimization techniques. The writers addressed a number of stock management-related topics, such as order quantity and manufacturing time optimization.

The unpredictability of the delivery procedures for spare parts and components of different moving unit elements (henceforth referred to as goods in the text) is a defining feature of the stock management systems of JSC "Uztemiryolyolovchi" passenger wagon depot warehouse. Both the delivery delay and the demand for the commodities during the completion of equipment and repair work (referred to as REW) are examples of supply uncertainty. Because demand varies over time and its average value (demand) varies throughout the year, it is vital to assess how items are used. As a result, activities involving the uncertainty of the order's delivery time and the variation of the demand value over time are extremely complicated, and using deterministic mathematical models in these circumstances is nearly impossible. It is suggested that in these situations, simulation techniques be used to ascertain the required stock of items.

2 MATERIALS AND METHODS

Improving the efficiency of the stock management system for carrying out REW in the passenger wagon depot is accomplished by lowering the costs of their (stock) storage, material transportation, and downtime due to a shortage of essential items in the

warehouse. It is required to identify the research objectives, which contain the primary eight stages of work accomplished to reach the research objective. The 8-step process outlined below is recommended to improve the effectiveness of passenger wagon depot warehouse stock management [6].

1) An first assessment of inventory management systems. Choosing the sort of system, establishing indicators for its effective functioning, and determining the elements that influence them.
2) The simulation model is used to create a stock management challenge.
3) Create the simulation model's block diagram and program.
4) Create a strategic plan for executing a simulation experiment.
5) Follow the strategic plan's simulation modeling (SM) guidelines.
6) Developing a mathematical regression model using a set of equations to determine the relationship between performance indicators and the factors that influence them.
7) Using the generated mathematical model, optimize the stock management system.
8) Calculating elasticity coefficients and factor specific weights.

Order size is an important element in an inventory management system with a set order size. This system is distinguished by the fact that it operates when the order level approaches the warehouse's existing stock limit. Note that this method is effective for the passenger wagon depot's manufacturing processes, and serial production is distinguished by the use of REW with, in most cases, the same operations, moving units.

The analysis of passenger wagon depot stocks and their management system was conducted using four effective indicators, two optimal factors impacting them, and five objective (fixed) elements. Table 1 outlines the variables used in the mathematical model.

Currently, there are two traditional approaches for evaluating and selecting suppliers: the point method and hierarchical analysis. Each approach has its own set of drawbacks, such as the level of subjectivity in the assessment, the availability of a sufficient number of criteria for assessing huge, uncertain data, and so on. The suggested simulation-optimization approach allows for the simulation of the passenger wagon depot's operation under various initial conditions, obtaining results for each scenario, and selecting the optimal supplier.

Table 1: Variables employed in the mathematical model for the investigation.

No	Code	The name of the variable
Performance indicators of the system of efficient management of reserves		
1	y_1	Total manufacturing expenses, soums.
2	y_2	average product storage, pcs
3	y_3	quantity of supplied items
4	y_4	number of days of REW suspension due to lack of goods
Factors to be optimized		
5	x_1	order delivery volume in product units
6	x_2	threshold level of stock in stock ordered in commodity units
Objective factors		
7	x_3	The average amount of goods required by the enterprise in a month
8	x_4	transportation costs of one batch of material, soums
9	x_5	cost of keeping a unit of product in one day, m
10	x_6	Losses incurred by REW during one-day interruptions of goods, sum
11	x_7	The average number of days it takes to deliver a product
12	x_8	product balance in the warehouse in commodity units

x_8 - taken modeling time is the same in all variants.

3 RESULTS

Taking into account the management system of reserves without deficit, it can be noted that it is characterized by constant demand, the uniformity of spending reserves and the absence of shortages. At the same time, the calculation of the optimal volume of delivered products can be done using the classic (1). Wilson formula [4, 5]:

$$Q = \sqrt{\frac{2\mu g}{s}} \quad (1)$$

where Q is the optimal volume of product delivery; μ - product consumption intensity; s-product unit storage fee; g-payment for delivery of one batch.

The dependence of the studied parameter on the influencing indicators is carried out by correlational analysis methods.

Using correlation-regression analysis, we study the interrelationship of indicators describing reserves, if the relationship between them is not strictly

functional or is broken by the influence of extraneous, random factors. After searching and assessing the narrowness of the relationship between two random features or factors based on correlation analysis, we establish a specific type of relationship between the studied parameters by applying regression analysis.

The automated "Backup" system based on the object-oriented approach is described in [3; 6; 15]. The "Reserve" program provides modeling of each block (object) using selected methods (Fig. 1).

By combining these models, it is possible to simulate different inventory management systems. We will build the models that make up the program.

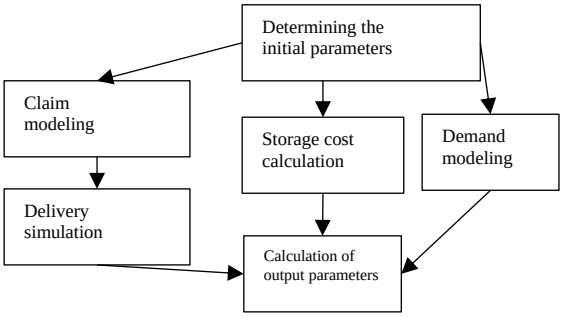


Figure 1: Reserve management system blocks.

The theory of resource management takes into account a vast variety of mathematical models. Two models are shared by them: EOQ (Q-model) (Economic Order Quantity) and periodic (P-model) [18, 19]. Many adjustments have been made to these models to cater for certain extra situations.

The generated models can be solved utilizing techniques such as linear programming, stochastic optimization, interval analysis apparatus, dynamic programming, queuing theory, and adaption theory. Because of the a priori uncertainty (demand, supply, delay time, etc.) of inventory jobs in passenger wagon depot warehouses, an effective solution (belonging to the given set of unknown demand) is conceivable utilizing concepts such as set theory and the concept of "unknown but bounded".

Imitation methods have grown popular as a result of advancements in computer technology, programming languages, and the use of object-oriented programming languages to solve resource management challenges.

Due to the development of computer technologies, programming languages, and the support of object-oriented programming languages in finding solutions to resource management problems, imitation methods have become widespread.

Below are some modeling algorithms of stock management systems developed on the basis of a modification of the classical algorithm.

The main tasks of the correlation-regression analysis in the logistics of supplying the warehouse with goods can be:

- to search and evaluate the correlation of the volume of consumption of goods with one or more of the above factors in order to formulate the right plans for ensuring the need for goods and materials;
- to determine the compatibility of inventory and the level of utilization of inventory by comparing the dynamics of receiving and spending warehouse resources;
- forecasting and budgeting of indirect costs related to stocks in the planning of goods purchases, etc.

Among the parametric criteria, the most common is the linear coefficient of Pearson's pairwise correlation:

$$r = \frac{\sum_{t=1}^{n}(x_t-\bar{x})*(y_t-\bar{y})}{n*\sigma_x*\sigma_y}, \quad (2)$$

the strength of the relationship between the selected variables can be determined by the formula that calculates the values of the linear correlation coefficients:

$$r_{xy} = \frac{m^*_{1xy} - m^*_{1x}*m^*_{1y}}{\sigma^*_x*\sigma^*_y}. \quad (3)$$

Using statistical tables, we determine the critical value of Student's criterion, and also calculate the critical value of the linear correlation coefficient according to the following (4):

$$r_{crit} = \pm\sqrt{\frac{t^2_{crit}}{t^2_{crit}+n-2}}. \quad (4)$$

In the next step, regression equations are determined based on the results of correlation analysis. Linear correlation coefficients make it possible to assess the degree of influence of factors on the resulting indicator of the efficiency of the stock management system.

Mathematical model of the system A set of controls that relate the resulting indicators of stock performance to the factors that influence them through regression equations.

Mathematical model presents simple regression equation, the relationship between the results of the effectiveness of the system management and the influencing factors.

$$y_j = f_j(x_1, x_2, x_3, x_4, x_5, x_6, x_7, x_8); j = \overline{1,4} \quad (5)$$

According to dependencies (5) the degree of influence of factors on the resulting indicators of the stock management system according to elasticity coefficients is evaluated and optimized.

Optimization is carried out separately for each variant of the strategic plan with specific values of the objective factors in these variants. The objective function is determined by the amount of total costs arising from the waiting, storage, transportation and downtime of the goods used in the execution of the REW to the mobile units in the workshop sections:

$$y_i = f_i(x_1, x_2, x_3, x_4, x_5, x_6, x_7, x_8); \rightarrow \min \quad (6)$$

Restrictions are placed on the effectiveness of the resulting indicators:

$$a_j \leq y_j \leq b_j; j = \overline{2,4}. \quad (7)$$

Constraints are placed on the factors to be optimized:

$$c_i \leq x_i \leq d_i; i = \overline{1,2}. \quad (8)$$

a j , b j ;j=(2,4)¯, c i , d i ; i=(1,2)¯the minimum and maximum values obtained during the simulation are taken as constraints.

Objective factors do not change during optimization.

$$x_3 - x_9 = \text{const}. \quad (9)$$

The values of the factors to be optimized are determined by the values of the objective factors according to the formulas obtained from the results of the regression analysis.

$$x_{iopt} = f_i(x_3, ..., x_8); i = \overline{1,2}. \quad (10)$$

4 DISCUSTION

It is necessary to highlight that research contains an interpretation and comparative analysis of the other researchers as Mitsel A.A., Gribanova YE.B. “Simulation modeling of economic objects”, Rijikov Y.I. “Queue Theory and Inventory Management” [11] and paper results obtained with link of these authors research respectively.

The simulation modeling method can be successfully used for human-machine evaluation of structural construction options of complex warehouse systems in order to achieve their optimal parameters and functional value characteristics within the existing constraints [1], [13]. This method takes into account the possible changes in the system caused by the influence of various factors, i.e. "what if...".

As mentioned above, the automated system of accounting for goods (ASAG) was implemented based on an object-oriented approach. ASAG includes simulation modeling of each computational process block (object) shown in Figure 1 based on a combination of models. Using selected methods, it allows to simulate different stock management systems by combining models. Note that among the classic simulation models there is the Q-model, whose algorithm has been discussed in detail in various works [7, 14]. According to the rules of this model, the next order for the delivery of goods is executed when the value (order point) falls to the level of Smin.

In addition, there is a P-model, according to which the order is made after a predetermined time. The algorithm of this model is built on the basis of making the following changes to the standard model (Figure 2).

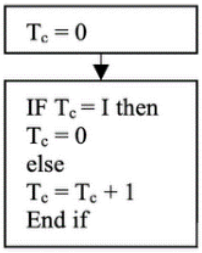


Figure 2: Algorithm for modeling systems with a periodic strategy.

Where T_c is a counter; i- order interval.

In this algorithm, the order placement symbol is no longer used, and there may be a situation where the order is placed before the next batch is delivered. Therefore, an array rather than a variable is used to store the value of the batch size.

In inventory management systems, product demand can be:

- deterministic or stochastic;
- continuously distributed or discrete.

Delivery time can also be random or deterministic (including zero). Modeling of random variables in the program is carried out according to the algorithms presented in. Input (demand, delivery time) they can have a normal.

Figure 3 presents a modification of the standard algorithm for modeling delayed demand. Based on the EBQ (Economic Batch Quantity) and EPQ (Economic Production Quantity) mathematical models presented in [9], [12], [16], [20], simulation models and algorithms were created.

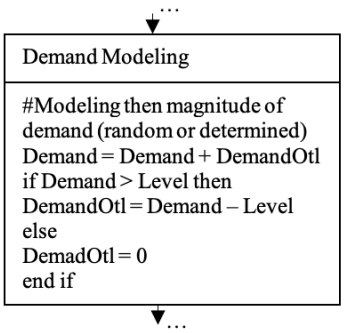


Figure 3: Delayed demand modeling algorithm.

Where demand - demand quantity; DemandOtl-deferred demand; Level - the current reserve level.
A distinctive feature of these models is that the delivery of batches is not carried out immediately, but over a period of time. The difference between the EBQ model and the EPQ model is that only material stocks are accumulated during the delivery period of goods without consuming them. Standard modeling to the algorithm we present the changes made (Fig. 4) [10], [17].

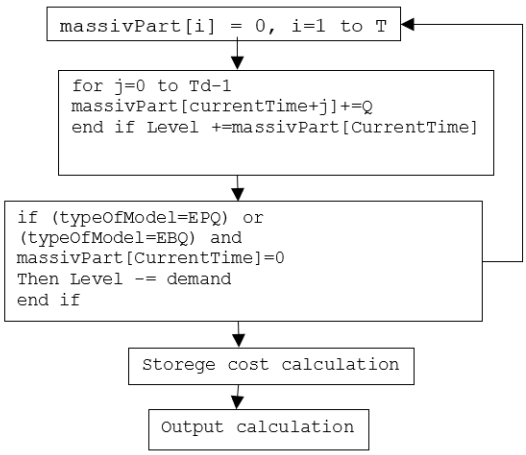


Figure 4: Algorithm for simulation of supply and demand.

In systems operating according to the EBQ and EPQ schemes: where CurrentTime is the time of the current model; T - modeling period; Td - goods delivery time to the warehouse; Q is a commodity of the party size; massivPart –one time for delivery to be given goods of the party value own_into received massive .

The manual mode of the ASUT program allows modeling of dynamic systems [8]. In this case, the input parameters of the model change over time. For example, demand may vary depending on the day of the week.

Let's look at the methods of calculating costs in inventory management systems. A model's cost function typically includes three types of costs: storage, order placement, and shortage penalties, and these may be volume-dependent and/or constant, and may not be considered in the modeling. In addition, when modeling systems in the ASUT program, discounts can be taken into account if the ordered product is ordered in a larger volume than specified [19].

We can conclude that since each enterprise has its own characteristics in defining the problem of cash flow management, production organization, and modeling, the cost function changes. For example, costs include the corresponding capital costs for holding stocks, which are calculated as the product of the cost of goods by the discount rate.

In the simulation model presented in primary financial statements are taken as input, and annual total costs include costs resulting from the loss of marginal profit and capital freeze. In addition, the following indicators can be used: the level of customer service, the implementation of the plan for the sale of goods, the evaluation of the work of suppliers (delivery on time), the reduction of excess stocks, the profitability of assets, etc.

5 CONCLUSIONS

Algorithms for determining and managing warehouse reserves at the "Uztemiryolyolovchi" JSC's passenger wagon depot have been developed by changing the classical algorithm. This method aims to optimize the order on the Reserve while also simulating the demand for reserves. The proposed algorithm allows you to replenish reserves and maximize their volume while taking into consideration the stochastic nature of demand and delivery time.

Key results demonstrate that the use of simulation-based optimization significantly reduces total inventory costs, minimizes REW (repair and equipment work) delays, and enhances resource allocation efficiency. The study also proved the effectiveness of a hybrid modeling strategy that includes deterministic formulas (e.g., Wilson’s formula) and stochastic simulation algorithms, thus addressing the limitations of using purely analytical or static methods in dynamic environments. The integration of the ASAG system and object-oriented simulation blocks has shown promising outcomes in real-world application scenarios. The simulation-optimization framework developed can be adapted to

other industrial depots facing similar logistical uncertainties.

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SECTION 3

APPLIED PHYSICS IN ENGINEERING AND TECHNOLOGY

Proceedings of the 13th International Conference on Applied Innovations in IT (ICAIIT)

JUNE 2025

A Comprehensive Review of Antireflection Coating Materials for Solar Cell Efficiency

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Keywords: Antireflection (AR) Coatings, Power Conversion Efficiency (PCE), Solar Photovoltaic (PV).

Abstract: The efficiency of solar photovoltaic systems is significantly impacted by reflection losses at the top of the solar cells. In order to lower these losses and increase the efficiency of solar cells in converting light into electricity, anti-reflection coatings are crucial. An extensive examination of the most recent advancements in anti-reflective (AR) coating technology designed specifically for solar cells is given in this research article. The paper covers the mechanical, optical, and chemical characteristics of a number of materials, including silicon dioxide (SiO_2), zinc oxide (ZnO), magnesium fluoride (MgF_2), and titanium dioxide (TiO_2). Also covers several nanocoating techniques in addition to single-layer and multi-layer coatings. Improved anti-reflective (AR) coatings are a result of developments in materials science and nanotechnology. Solar PV systems that have these coatings applied can function better and endure longer. This field also focuses on future research and development paths that could lead to more durable and efficient solar cells.

1 INTRODUCTION

Solar photovoltaic (PV), which harnesses energy from the sun, is more crucial in the modern day due to energy problems in many regions and the rapid depletion of fossil fuels. Currently, solar energy is harnessed using either photovoltaic (PV) or solar thermal technologies. Both approaches utilize protective glass to shield the solar cell from the harsh external climate, physical impacts, and other potential hazards [1]. The majority of solar PV equipment, whether it is first generating or second generation, employs borosilicate glasses as front glass. These glasses reflect approximately 9% of light because the discrepancy in refractive indices between the glass and air. The glasses utilized by lenses, lasers, and other optoelectronics equipment require exceptionally high transmission, as observed in various studies [2]. The number of silicon solar cells has been rising continuously since Bell Laboratories created the first silicon p-n junction solar cell ever [3]. Novel technologies were introduced in the 1990s and 2000s, including surface texturing, screen printing, passivated emitter and rear contact, and firing technology. These technologies were instrumental in lowering production costs and increasing cell efficiency, which in turn aided in the industrialization of silicon photovoltaics (PV). With a 90% global market share in photovoltaics, crystalline silicon solar cells, encompassing monocrystalline and polycrystalline technologies, are currently the most significant photovoltaic technology [4]. Thus far, silicon solar cells can be categorized as Si heterojunction solar cells or diffusion-based homojunction solar cells based on their respective device techniques. For PV production, homojunction c-Si solar cells are the most widely used variety at the moment. Passivated emitter and rear cells (PERC) and aluminium back surface field (Al-BSF) cells make up the majority of these cells; in 2019, they accounted for 40% and 50% of the market, respectively [5]. A solar cell's power conversion efficiency (PCE) can be increased, reflection loss can be decreased, and absorption can be increased by adding an anti-reflection (AR) coating. In order to lower the reflection loss, several researchers have applied single- and double-layer antireflection coatings on solar cells. AR coatings have been widely utilized to increase transmittance and decrease reflectance at the appropriate thickness. A decrease in reflectance results from an incongruity between the various layers caused by the AR

coating's differing refractive index. Researchers have developed AR coatings using a range of different materials, and there are both expensive and low-cost methods for applying AR coatings [6]. Classifying solar panels based on cell types is necessary for looking into anti-reflection coatings. The coatings' physical characteristics must not change, and they must be oxidation- and corrosion-resistant. The ability of anti-reflective coatings to hold their stability across extended fluctuations in temperatures and a range of seasonal weather conditions is another characteristic that they have in common. The surface adhesion is another anticipated with this seasonal shift. The coatings used on the glass surface prioritize protection against seasonal conditions [7]. Almost all of the technology of the present period is mostly dependent on nanotechnology. The digital world that seeks to become more efficient and compact has found its definitive solution in nanotechnology. Synthetic nanoparticles are materials that can be engineered. In comparison to their counterparts on a larger scale, they exhibit superior conductivity, strength, and chemical reactivity [8]. One of the most important elements in the advancement of solar cell efficiency and many optical devices functioning in particular electromagnetic spectrum ranges particularly in the visible and infrared spectra is anti-reflection coating. The fact that the materials used to make solar cells have nanomaterials as their particle size plays a significant role in increasing the efficiency of solar cells. When a material's dimension falls inside the nanoscale dimension, its optical and electronic properties alter, allowing it to utilize more spectrum than just the electromagnetic one [9]. The superior surface-to-area ratio and physicochemical properties of nanoparticles and their associated materials offer various advantages for their application. Because of these characteristics, they are perfect for usage in industrial settings and can be coated as nanostructured layers on thin-film solar cells. The optical pathway for light absorption is the primary justification for the use of nanoparticles in appliances. This is significantly greater than the common materials with subpar reflections. However, because of their numerous reflections, photovoltaic cells are far more accessible to a wider range of applications [10].

In this paper, the latest applications of anti-reflective optical films in different types of solar cells are reviewed, and the experimental data are summarized. Important developments in ARC technology are examined, with a focus on a range of materials, including magnesium fluoride (MgF_2), zinc oxide (ZnO), titanium dioxide (TiO_2), and silicon dioxide (SiO_2). The study looks at single-layer and multi-layer coatings, focusing how nanotechnology can enhance the mechanical, optical, and chemical characteristics of these coatings. By greatly reducing reflection losses, nano-coatings showed promising results in improving solar cell performance. The study discusses the importance of ideal coating thickness and material stability in various environmental conditions. It emphasizes the need for self-cleaning coatings and stable ARCs for solar technology. Future research directions aim to improve solar PV systems' efficiency and shift towards sustainable energy sources. In summary, anti-reflective coating (ARC) suppresses surface light loss, thereby improving the power conversion efficiency (PCE) of solar cells, which is its basic function

2 ANTIREFLECTION COATING FOR SOLAR CELLS

In the manufacture of silicon photovoltaic cells, the reduction of reflection losses must be considered. These losses are estimated to be more than 30% on the surface of the silicon [11]. Appreciable improvements in the performance of the photovoltaic cell can be achieved by reducing these losses. The quality of anti-reflective coatings (ARC) is therefore an essential parameter for achieving high-efficiency solar cells. The basic optical theory of anti-reflective coating design commonly uses anti-reflective materials, and their classic combinations are introduced [12]. Therefore, the focus of current research is mainly on anti-reflection coatings with multi-material composition or the gradient refractive index (GRIN) structure that performs, resulting in a significant increase in adhesion and osmosis efficiency [13]. Where the operation of anti-reflective layers is based on the adaptation of the refractive index of the layer in such a way as to produce destructive interference at a certain wavelength, taking into account the thickness of the layer. The material used as an anti-reflective layer must be non-absorbent in the solar spectrum range [14].

In this context we review in detail the structures of AR coatings and their techniques in addition to the most common transparent conductive nanoparticle materials used recently for anti-reflective coating of solar cells, such as zinc oxide

(ZnO), Magnesium fluoride (MgF_2), titanium dioxide (TiO_2), etc.

2.1 Single-Layer Coating (SLARC)

At an interface when the two mediums have different refractive indices (n), light is reflected; the greater the difference, the greater the reflection. According to this rule, putting a layer of intermediate refractive index between the two media can help reduce reflection by minimizing the difference between them. This is the basic principle of single-layer anti-reflection coatings, which initially noticed by Lord Rayleigh and which can be proven by the following equation:

$$ni = \sqrt{n1n2} \quad (1)$$

$$di = \frac{\lambda}{4 \times ni} \quad (2)$$

where ni is the refractive index of ARC (the ideal refractive index RI), $n1$ and $n2$ are the refractive indices of the existing layers [15], and di is the ARC thickness. It should be mentioned that these equations only work with nonadsorbing, homogeneous media; otherwise, the loss of each medium will complicate the computation. The primary obstacle for single-layer quarter-wavelength ARCs is their disappearance at glazing incidence angles due to a loss in reflectivity for partial wavelengths and incidence angles. The different incident light optical path-lengths of the glazing incidence are the reason of this. And since the glazing incidence depends on the norm, the phase difference between the incident and reflected waves cancels each other out. Additionally, since low RI single-layer ARCs often have fewer RI substrates, it is challenging to locate any material in their clear surface as shown in Figure 1 (a) [13]. Khalaf A. M. and Obaid A. S. (2018) [9], the study focused on the impact of a single layer of anti-reflective coating (Ge) on the quantitative efficiency of a silicon solar cell. MATLAB programs were used to analyze the cell's efficiency within the 400-700 nm visible wavelength region. The results showed that applying an anti-reflective coating with adjusted particle size significantly at the specified design wavelength of 550 nm for vertical fall the maximum quantitative efficiency of 96.9004% was attained of silicon solar cells and the quantitative efficiency for horizontal polarization (P) was 96.3131%. However, the study had limitations, such as the observed increase in reflectivity and decrease in efficiency as the design wave length increased.

2.2 Multi-Layer Coating (MAR)

Multilayer anti-reflection (MAR) coatings solve the restrictions of single - layer AR coatings. The apply of multiple layers with high and low refractive index creates a coating that reduce the reflection across a wide wavelength range [15]. throughout suitable selection to the thickness of the layer and the materials type, and this could control the light interference by decreasing the losses of reflection [16].

The theory of the multi-layered antireflection coatings is the same as for the single-layer antireflection coatings, but the mathematical model employed in this case includes a vector analysis of the reflected individual rays. As shown in Figure 1 (b), the light reflected from the junction of the two layers, i and j (suppose there are no losses), is provided in (3) as follows:

$$Rij = |Rij| e^{[-2(\delta i + \delta ij)]} \quad (3)$$

where:

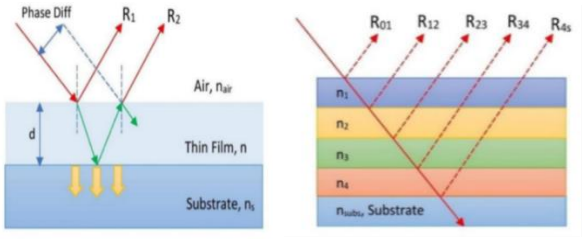
$$|Rmn| = \frac{(ni - nj)}{(ni + nj)}$$

and

$$\delta i = \frac{2\pi nidi \cos\theta i}{\lambda}$$

$di, \theta i$ and λ are the optical film thickness, the angle of refraction and wavelength of the light respectively. After that, reflectance at each layer's interface is integrated to determine the overall reflectivity ($Rsum$), which is written as:

$$Rsum = R01 + R12 + R23 + \cdots + Rns. \quad (4)$$



(a) (b)

Figure 1: Schematic illustration of diffusion of light in (a) single layer film (ns > n) and (b) multiple layer film [17].

In order to provide an antireflection effect, the index of refraction and optical film thickness can be suitably optimized to lower the *Rsum* [18]. In 2023, Koysuren O. et al. [19], the study discussed the development of solar cell coatings that can self-clean themselves, reducing efficiency losses caused by contaminants. The SiO_2/WO_3 and $SiO_2/WO_3/ZnO$ composite coating was used to coat glass substrates, showing over 90% photocatalytic dye removal efficiency after 240 minutes of UVA light irradiation. The composite coating maintained the cell's efficiency nearly identical to untreated cells, with minimal effect on efficiency.

2.3 Gradient Refractive Index (GRIN) Coating

The GRIN coating is a unique non-uniform ARC that has a refractive index that gradually changes from the incident medium to the substrate in a vertical direction. It could be considered as a structure consist of a series of very thin films, which lead to broadening the antireflection bandwidth and angle. If each film thickness is much smaller than the reference wavelength, then the GRIN film could be considered as a continuously system without interfaces. For this cause, the light reflection that entering to the substrate from the incident medium is very small [20]. Lord Rayleigh has really theoretically verified the gradual transition problem, which states that the beam bends and the reflection reduces as the density in a variable medium change [18]. Various profiles of gradient refractive index layers have been proposed for omnidirectional and broadband anti-reflection coatings, which consist of linear, gaussian, parabolic, exponential, exponential-sine, quintic, cubic, and Klopfenstein [17].

The following lists typical expressions for the continuously changing RI with linear, cubic, and quintic profiles.

- Linear index profile:

$$n = ni + (ns - ni)t,\ 0 \le t \le 1; \quad (5)$$

- Cubic index profile:

$$n = ni + (ns - ni)(3t2 - 2t3); \quad (6)$$

- Quintic index profile:

$$n = ni + (ns - ni)t(10t3 - 15t4 - 6t5). \quad (7)$$

Where ni and ns are the refractive indices of the incident and substrate media, t is the thickness of gradient interface region, respectively. [18]. Adwan Y. M. et al (2023) [21], the study focused on the optical characteristics of a three-layer anti-reflective coating (TLAR) structure using graded refractive index material (GIM). The structure, which consists of materials with varying indices, can increase transmittance while decreasing reflectance, improving solar cell performance. The study found that the highest reflectance occurs when the ARC thickness is $x = 0$, and it gradually declines before increasing again. The study also found that TE-polarized light is reflected more when the contact angle rises, lowering transmittance.

3 EFFECTS OF MAJOR COATING MATERIALS ON PV EFFICIENCY

3.1 SiO_2 as Antireflection Coating

SiO_2, also known as silicon dioxide, is the second most prevalent element on Earth, after oxygen, and makes up around 25.7% of the Earth's crust [22]. It is cost-effective and has excellent optical, mechanical, and electrical features, although it is seldom encountered in its pure crystal form in nature. Each of the elements undergo a chemical reaction in order to create Si-O-Si connections, and it is the arrangement of the atoms in the system which determines the precise shape of the SiO2 particles as shown in Figure 2 [23]. There are several works that has been achieved to synthesis a nanometer SiO_2 antireflection coating for solar modules. Wei W. et al (2015) [24], the paper focused on the development of a nanometer SiO_2 antireflection coating for solar modules, aiming to reduce reflection losses and improve efficiency. The coating, created using the sol-gel process, achieved a 5.6% increase in transmittance and reduced reflection losses to 3.44%. It also demonstrated uniform antireflection and high scratch resistance, demonstrating its potential for PV system efficiency.

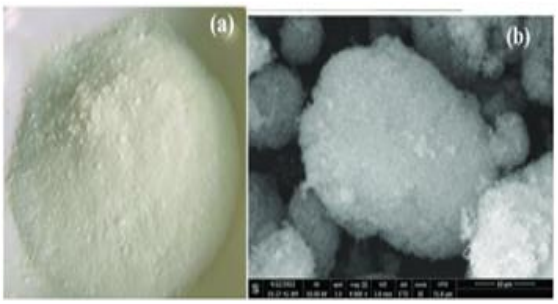


Figure 2: SiO_2 material (a) raw material (b) SEM image [25].

Sharma R. et al. (2017) [26], researchers studied the impact of anti-reflection coatings on silicon solar cells using PV Education and the PC1D simulator. They found that DLARC, specifically SiO_2/TiO_2 DLARC, improved photovoltaic performance and short current, resulting in a 14.34 solar efficiency. This was achieved by lowering the weighted reflectance of the solar cells, resulting in better efficiency and shorter current. Liao K. et al (2020) [27], the study was focused on the open-circuit voltage, conversion efficiency, and fill factor of solar panels made with different antireflection coating (ARC) stacks and compared them. Where the coating had a thickness of approximately 70 nm with a refractive index of about 2.01. And thus Mc-Si solar cells covered with TiO_2-$SiO_2/SiO_2/SiNx$ ARC stacks under encapsulation circumstances had an average conversion efficiency of 16.27%. Comparing this value to commercial Mc-Si solar cells (16.09%) made with SiNx ARCs, the difference was 0.18% as shown in Figure 3.

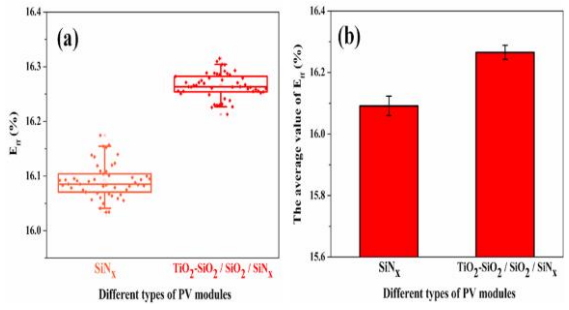


Figure 3: (a) Displays the distribution of conversion efficiency in comparison, and (b) displays the average conversion efficiency of solar modules assembled using various ARC stacks.

Table 1 shows the thickness and limitations with the efficiency of the solar cell using the material SiO_2.

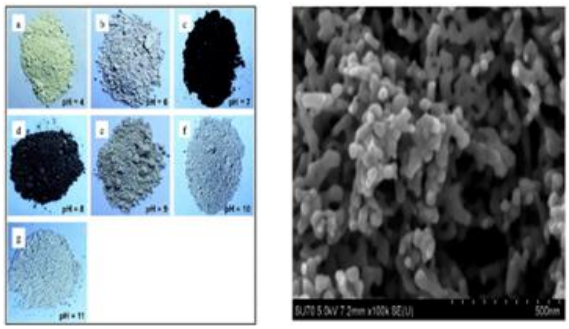
3.2 ZnO as Antireflection Coating Material

Zinc oxide (ZnO) is an atypical semiconductor with a direct band gap of 3.4 electron volts (eV) and an incredibly high exciton binding energy of 60 millielectron volts (meV). It is considered to be the most promising semiconductor for a wide range of applications. These features are really intriguing for enhancing several optoelectronic devices. The unique properties of ZnO can be attributed to the fact that among all the elements in the sixth group of the periodic table, oxygen offers the greatest potential for ionization. As a result, the Zn (3d) and O (2p) orbitals interact significantly with one another [28]. Antibacterial treatments, sunscreen lotions, photocatalysis, catalysts, UV absorption, and biological applications are just a few of the many uses for zinc oxide (ZnO) [29]. This is because it is inexpensive, has little toxicity, and is very biocompatible. A wide variety of industrial products, such as paint, rubber, cosmetics, and coating, incorporate zinc oxide (ZnO) as an additive.

Useful in mechanical actuators with enhanced piezoelectric and pyroelectric capabilities, as well as piezoelectric sensors, ZnO's wurtzite structure, which lacks a center of symmetry, produces strong electromechanical coupling effects [30]. Figure 4 shows the raw material and SEM image.

Table 1: Previous research results of SiO_2.

Ref	Material used	Thickness	Limitation	Results
Wei W. et al (2015) [24]	SiO_2	----------	Over the 400–800 nm wavelength range, the transmittance grows at short wavelengths and drops at long wavelengths as lifting speed increases.	A 5.6% improve-ment in transmit-tance and a reduction in reflection losses to just 3.44 percent
Sharma R. et al (2017) [26]	SiO_2 /TiO_2 SLARC and DLARC	110, 105, 85, 75, 65, and 60 nm	The front surface of the solar cell with SLARC enhances its performance to a certain extent.	Efficiency 14.34 %
Liao K. et al (2020) [27]	TiO_2–SiO_2/ SiO_2/ SiNx	70 nm for SiNx 25 nm for SiO_2 55 nm for TiO_2–SiO_2	It appears that there is need for improvement in the sintering and welding processes, as the average fill factor of PV modules covered with TiO_2-SiO2/SiO_2/SiNx ARC layers did not show consider-able improve-ment.	Efefficiency 16.27%



(a) (b)

Figure 4: The ZnO material. (a) photographs of ZnO nanoparticle powders with different pH values [31] (b) SEM microscopy of zinc oxide ZnO nanoparticles at x 100 magnifications [32].

Several studies have been achieved for using ZnO nanoparticles as anti-reflective coting layer. Jalali A. et al (2019) [33] study in deep the effects of sol-gel solution quantity on the performance of silicon solar cells that use ZnO thin films as anti-reflective layers. The sol-gel method was used to make thin films of ZnO, which were then spin-coated onto a P-N silicon base. Where Nano-structured ZnO films with hexagonal crystallites and particle sizes ranging from 30 to 50 nm were successfully made on the silicon base. As anti-reflection sheets, coated ZnO films made silicon solar cells work better. It was found that the quantity of the sol-gel solution was significantly related to the efficiency of the silicon solar cell.

Sagar R. and Rao A. (2020) [34], focused on using transition metal oxide thin films (zinc oxide, magnesium oxide, and aluminum oxide) as anti-reflection coatings on silicon-based solar cells to improve energy conversion efficiency. Quantum efficiency measurement showed increased external quantum efficiency (EEQ) in the 450-680 nm wavelength range, with aluminum oxide exhibiting the highest EEQ. And the transition metal oxide films showed compact structures and good substrate adhesion. However, spectral response declined near the silicon gap, emphasizing the need for optimized anti-reflection layer design. Jamaluddin N. I. M. et al. (2024) [35], the study investigated the effectiveness of the performance of six different ARC materials on silicon solar cells using PC1D simulation software. The optimal wavelength for ARC development was found to be between 500 and 800 nm. AND found silicon nitride (Si_3N_4) and zinc oxide (ZnO) were found to be the most effective ARC materials for single-layer design in terms of reflectance reduction and efficiency (Eff) of 21.69% and 21.67%, respectively. Table 2 shows the comparison between several studies that utilized ZnO nanoparticles as anti-reflective coting layer.

3.3 MgF_2 as Antireflection Coating Material

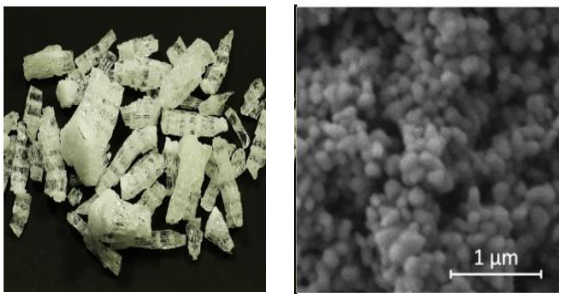
Magnesium fluoride is a colorless crystal (MgF_2) has a tetragonal crystal system and a rutile structure. It is frequently used as a coating material due to its superior optical properties.

Table 2: Previous research results of ZnO.

Ref.	Material used	Thickness	Limitations	Results
Jalali A. et al (2019) [33]	ZnO thin film	230 nm	Although ZnO thin films have many desirable properties, there is a restriction on their use.	Efficiency 9.19%
Sagar R. and Rao A. (2020) [34]	ZnO, MgO, and Al_2O_3 thin film	77 nm, 90 nm and 79 nm	The non-optimal thickness of the light absorber layer placed in the manufactured devices could be the cause of the quantum efficiency reduction at longer wavelengths.	Efficiency 9.633%, 9.864%,and 9.125%
Jamaluddin N. I. M. et al (2024) [35]	SiC, SiO_2, Si_3N_4, TiO_2, ZnO, and ZnS.	36.159nm, 101.351nm, 74.257nm, 62.396nm, 78.411nm, 63.479nm	SiC has the highest reflectance hence, causing the coating has to display the lowest efficiency	Efficiency 17.1%, 20.23%, 21.69%, 21.05%, 21.67%, and 21.16%

Also, Magnesium fluoride (MgF_2) coatings have several outstanding characteristics, such as a low refractive index (n = 1.38), a broad transparent range spanning from 120 nm to 8000 nm, and a significant energy gap of 11 eV. MgF_2 is a compound composed of several ions with varying charges that are attracted to each other through electrostatic forces [36].

Consequently, compressing it at normal temperature and pressure conditions is exceedingly challenging. Furthermore, MgF_2 possesses the benefits of exceptional mechanical resilience, outstanding thermal endurance, and a remarkable resistance to laser damage. It mitigates external material wear and extends the lifespan and efficiency. Hence, MgF_2 coatings has a multitude of uses in the production of optical coatings as shown in Figure 5 [36].



(a) (b)

Figure 5: MgF_2 material. (a) raw material [37] (b) SEM image [38].

Several studies have been achieved for using MgF_2 nanoparticles as anti-reflective coting layer. Nayak J. et al (2024) [39], with the transfer matrix method, the magnetic and electric fields of the light hitting the coats' surface were changed to make the AR coatings. A MATLAB program based on the transfer matrix model was used to figure out the reflectance. Materials with a high refractive index, like ZnS, Si_3N_4, and MgF_2, were used to model single, double, and three-layer ARCs. It was found that the reflected loss for single-layer ARCs was less than 34.66%, for double-layer ARCs it was 8.47%, and for three-layer ARCs it was 5.71%. Single-layer ARCs had an external quantum efficiency (EQE) of 65.34% on a silicon base, while double-layer ARCs had an EQE of 81.81%. Khadir Al. et al (2020) [40], the study used computer models to look into how an anti-reflective coating (ARC) affects the performance of CuInGaSe2 (CIGS) solar cells. It employed the Atlas module from SILVACO T-CAD for the numerical models. And it taken into consideration how different ARC and CIGS layer thicknesses affected a single magnesium fluoride (MgF_2) ARC layer in order to get the best electrical qualities from the solar cell. When a single 0.11µm MgF_2 ARC monolayer was used, the modelling showed that the CIGS solar cell's conversion rate went up by 8.10%. Future solar cell designs and implementations can benefit greatly from the findings as shown in Figure 6. Table 3 shows the thickness and limitations with the efficiency of the solar cell using the material MgF_2.

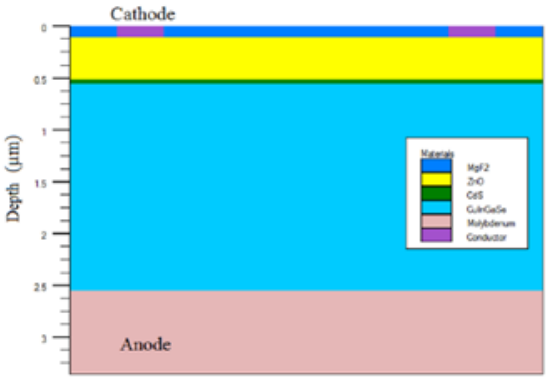


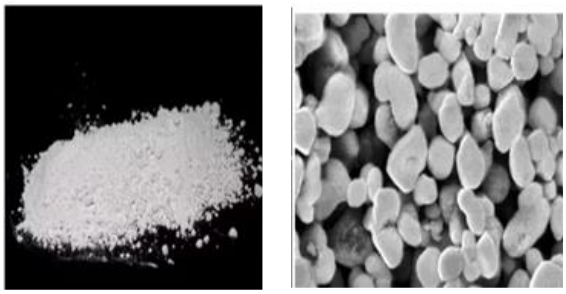
Figure 6: The simulated CIGS solar cell's schematic structure.

3.4 TiO_2 as Antireflection Material

TiO_2, a photo semiconductor, has gained significant attention as the most extensively studied material among metal oxides. This is due to its versatile uses in areas such as photocatalysis and photovoltaics, making it valuable for both fundamental study and practical use [41]. Owing to its lack of toxicity, excellent efficiency, photochemical stability, and afford ability. The material has been classified as a broad band gap semiconductor with an energy gap of 3.00 eV in the rutile phase and 3.20 eV in the anatase phase [42]. The rutile phase is the predominant natural form of TiO_2 among its various polymorphs, including anatase and brookite. Thermodynamically, rutile is the most stable phase of TiO_2 at all temperatures as shown in Figure 7 [43].

Table 3: Previous research results of MgF_2.

Ref.	Material was used	Thickness	Limitation	Results
Nayak J. et al (2024) [39]	ZnS, Si_3N_4, and MgF_2 double layer.	200 nm, 400 nm	Possible explanation for low EQE at higher wavelengths (>700 nm): ARCs and silicon surface have different impedances miss -matching.	The ARCs with a single layer on a silicon substrate achieved an external quantum efficiency (EQE) of 65.34%, whereas the ARCs with two layers achieved 81.8%.
Khadir Al. et al (2020) [40]	MgF_2	0.11μm	when ZnO and CdS layer thickness increases, conversion efficiency falls.	Growth in conversion efficiency from 18.99 to 20.52%



(a) (b)

Figure 7: TiO_2 material. (a) raw material [44] (b) SEM Image [45].

Several studies have been achieved for using TiO2 nanoparticles as anti-reflective coting layer. Rad A. S. et al (2020) [46], the research investigates the effect of porosity and roughness on the performance of anti-reflection and self-cleaning meso-porous TiO_2 coatings on glass substrates for solar systems protection. Using the sol-gel method and varying quantities of Pluronic F127, mesoporous TiO_2 coatings were produced. Etching the TiO_2 coatings with 5% HF enhanced their anti-reflective capabilities. Also, coating surface roughness was enhanced, and holes smaller than 30 nm formed as a result of F127 usage. Glass coated with TiO_2 and treated with F127 has a greater transmittance of the visible spectrum waves than uncoated glass. Therefore, coatings of higher porosity and roughness on mesoporous TiO_2 demonstrated better anti-reflective and self-cleaning capabilities.

Sagar R. and Rao A. (2021) [47], focused on improving the photon-to-electron conversion efficiency (PEC) of commercial monocrystalline silicon solar cells by using nanoscale TiO_2 and Ta_2O_5 as antireflection coatings (ARCs). Nanoscale TiO_2 and Ta_2O_5 ARCs were deposited on commercial silicon solar cells, with thickness determined using sputtering techniques. Where the efficiency (Eff) improved from 17.18% to 17.87% with TiO_2 ARCs and 18.8% with Ta_2O_5 ARCs. This improvement was attributed to increased fill factor and reduced reflectance. Table 4 shows the thickness and limitations with the efficiency of the solar cell using the material TiO_2.

3.5 Different Other Materials as Antireflection Coatings

Huanga Z. et al (2018) [48], researchers had developed a method to create antireflective and self-cleaning coatings on silicon-based solar cells, aiming to improve light harvesting efficiency and protect cells from dust and environmental damage. The transparent photopolymer with leaf surface morphologies was cured onto Si slabs, resulting in a 10.9% increase in photovoltaic cell power. The coatings were resistant to ambient liquid pH levels and hydrophobic, and can be printed onto glass for large-scale production.

Li W. et al. (2019) [49], the study aimed to create high-performance, broadband antireflective (AR) and superhydrophobic coatings on glass surfaces for solar cells. The coating was created using spin coating and hexadecyltrimethoxysilane (HDTMS), resulting in a hydrophobic surface and porous structure. The coating increased the short-circuit current density and conversion efficiency of dye-sensitized solar cells by 10.12%. However, the research had drawbacks, such as increased transparency and root means square roughness over time, and the formation of scratches that could damage the AR layer.

Jalaly S. et al. (2019) [50], the study discussed the use of a polymer-based anti-reflection coating (ARC) for solar panels, specifically designed for building-integrated and building-applied photovoltaic systems.

Table 4: Previous research results of TiO_2.

Ref.	Material was used	Thickness	Limitations	Results
Rad A. S. et al (2020) [46]	TiO_2	80–90 nm	T0, T1, T2, and T3 samples showed had less porosity that's lead to less light transmittance than uncoated glass	T4 and T5 samples, which had TiO_2 coatings with 1.6 X 10-3 M and 2 X 10- 3 M of f127 in the coating solution, respectively, demonstrated higher visible light transmit-ttance compared to the non-coated glass.
Sagara R. and Rao A. 2021 [47]	TiO_2 and Ta_2O_5	55.2 nm and 70.8 nm	Both layers exhibit a sharp rise in reflec-tance between 370 and 440 nm.	Efficiency 17.87% and 18.8%

The ARC can be adjusted to fit specific light angles, and tests show a 5.6% increase in outdoor conversion efficiency compared to plain polymer-based sheet ARCs.

Kaliyannan G. V. et al. (2019) [51], a study on nanostructured gahnite spinel material for anti-reflection coating (ARC) on polycrystalline silicon solar cells was conducted. Transparent gahnite nano-microfilms were formed using radio frequency magnetron sputtering, resulting in a high optical transmittance of 97% and a maximum power conversion efficiency of 21.27% in open air and 23.83% in controlled air. The material's physical properties decreased with deposition time.

Huang X. et al. (2021) [52], carbon nanotube/silicon (CNT/Si) solar cells have been enhanced by solution-processable MoOx, which acted as a chemical dopant and anti-reflection coating. This coating reduced series resistance and increased short-circuit current density, resulting in a 39% increase in power conversion efficiencies (PCE) and 80% stability for two months without a protective layer. MoOx also improved CNT film properties, reduced reflection, and increased the built-in potential at the CNT/Si junction, resulting in a 10.0% increase in PCE.

El-Khozondar H. J. et al. (2021) [6], the research focused on the design and efficiency of solar cells with antireflection coatings, aiming to improve performance with low cost and high efficiency. Four suggested designs that utilize Borofloat glass, SiNx, and sol-gel-based materials were present. The transfer matrix method determined total transmission and reflection, considering film thicknesses and incidence angles. The sol-gel layer was promising for high-performance cells, while the second and third structures were suitable for low-efficiency cells.

Abed R. N. et al. (2021) [53], a unique nanocoating was created using CuO:NiO and carbon (fly ash) on copper and glass surfaces. The coating's optical characteristics were examined using UV-vis and reflectance spectroscopy. The study found that the high electron absorption energy varied according to the composition's absorption wavelength, potentially enhancing solar energy absorption. As CuO:NiO concentration increased, the nano thin films darkened and became less transparent.

Shah D. K. et al. (2022) [54], the study aimed to determine the optimal thickness of a niobium pentoxide (Nb_2O_5) anti-reflective coating (ARC) layer for c-Si solar cells. A low-cost sol-gel spin coating technique was used to achieve the maximum thickness. Simulation studies revealed the 75-nm thick ARC layer had the lowest average reflectance and achieved an external quantum efficiency of over 95% and a maximum power conversion efficiency of 17.92%.

4 ANALYSES OF ANTIREFLECTION COATINGS AND TECHNIQUES

According to these studies, by decreasing reflection and increasing absorption, all antireflective materials have been shown to increase solar cell efficiency. For instance, because of its special qualities and great effectiveness in optical and electrical applications, zinc oxide (ZnO) is considered to be one of the most promising materials. However, available industrial methods make it impossible to ensure its quality and stability, requiring additives to increase stability. Differently composed materials, including transition metal oxides (like magnesium

and aluminum oxide), have been shown to increase power conversion efficiency when used as thin films on silicon solar cells. Their ability is found in their specific optical characteristics, which can be modified for certain uses.

A thin film of niobium pentoxide (Nb_2O_5) has also been employed because of its promise as an antireflective coating. Using sol-gel techniques, it may be additionally enhanced to reduce light reflection and increase power-generating capacity, exhibiting its practical use. The efficiency of solar cells could be greatly increased by hybrid coatings like CuO: NiO and composite coatings like SiO_2/WO_3/ZnO. However, because they depend on more special components, these materials may be less accessible than ordinary materials. Additionally, one of the best materials to lower the performance of solar cells is nano-coatings, which contain materials that enhance the advantages of nanotechnology and have the potential to improve anti-reflective and self-cleaning properties, so preserving efficiency in outdoor conditions. But its cost is relatively high due to advanced manufacturing processes.

Finally, several technologies are in use that are important to enhancing the performance of solar cells, such as SLARC, MAR, and GRIN. Based on research reviews, the gradient refractive index (GRIN) technology is considered an interesting selection for high-performance solar cells since it may improve transmittance over a broad wavelength range while decreasing reflectance. Its layered structure allows for more improvements in enhancing solar energy efficiency.

5 DISCUSSIONS

The review highlights the crucial role of anti-reflective coatings (ARCs) in improving the efficiency of solar cells. These coatings work by minimizing the reflection losses on the surface of the solar cells, thereby increasing the amount of light absorbed and converted into electrical energy. This is a key factor in enhancing the overall performance of photovoltaic cells, which is critical as the world seeks more efficient and sustainable energy solutions. Nanotechnology is revolutionizing the manufacturing and functionality of ARC materials, enhancing solar cell efficiency and resistance to external influences. Nanocoating's, with their unique surface characteristics, are being developed for various applications. Thus, this review focus in analysis the efficiency of solar photovoltaic systems with anti-reflection coatings based on recent related works, focusing on titanium dioxide, silicon dioxide, zinc oxide, magnesium fluoride and other different materials. Results show the material performs better, especially when nanostructured or in multilayer forms, increasing power conversion efficiency. This article discusses challenges in applying AR coatings in the real world, including accuracy, consistency, and thickness. It calls for further research on self-cleaning AR coatings, long-term performance, and solar cell efficiency. In the following we can summarize the aspects of:

5.1 Material Properties and Their Impact

In follows the main advantage and challenges which has been concluded by analysis of this review for each type of anti-reflective coatings materials.

A) SiO2 (Silicon Dioxide):
   1) SiO2 is favored for its excellent optical, mechanical, and electrical properties.
   2) The Sol-gel process to create nanometer SiO2 coatings shows promising results, with significant improvements in transmittance and reduction in reflection losses.

However, maintaining uniformity and mechanical durability of these coatings can be challenging, and stress build-up can lead to rapid failure.

B) ZnO (Zinc Oxide):
   1) ZnO's high exciton binding energy and direct band gap make it ideal for optoelectronic applications.
   2) Studies have demonstrated that ZnO thin films, particularly when optimized for sol-gel solution concentrations, can greatly enhance solar cell performance.

Challenges include managing the formation and stability of ZnO nanoparticles and ensuring consistent coating quality.

C) MgF2 (Magnesium Fluoride):
   1) MgF2's low refractive index and broad transparent range make it a valuable ARC material.
   2) Research indicates that single-layer MgF2 coatings can significantly improve the conversion efficiency of CIGS solar cells.

However, the difficulty in compressing MgF_2 and ensuring uniform application can pose manufacturing challenges.

D) TiO2 (Titanium Dioxide):
   1) TiO2 is a highly versatile material with applications in both photocatalysis and photovoltaics.
   2) Its various polymorphs, particularly rutile and anatase, offer different advantages for ARCs.

The challenge lies in optimizing the porosity and roughness of TiO_2 coatings to maximize their anti-reflective and self-cleaning properties.

5.2 Innovations and Future Directions

A) Multilayer Coatings:
   1) As single-layer and double-layer ARCs may not suffice, researchers are focusing on multilayer ARCs to achieve better refractive index matching and broader bandwidths;
   2) The gradient refractive index (GRIN) structure is a promising innovation, virtually eliminating interfaces and enhancing adhesion and osmosis efficiency.

B) Self-Cleaning Coatings:
   1) The incorporation of photocatalytic materials like WO3 into ARCs can improve self-cleaning capabilities, reducing efficiency losses due to contamination;
   2) Composite coatings like SiO2/WO3/ZnO show significant potential in maintaining high efficiency while offering self-cleaning properties.

C) Bionic and Nano-Structured Coatings:
   1) Replicating natural structures, such as leaf surfaces, onto solar cells can enhance light harvesting and provide self-cleaning benefits;
   2) Nanostructured materials like gahnite spinel and carbon nanotube-based coatings offer innovative ways to improve solar cell performance.

In summary, the comprehensive review underscores the importance of selecting the right materials and design strategies for ARCs to achieve high-efficiency solar cells. Each material brings unique properties and challenges, and ongoing research is crucial in overcoming these obstacles and exploring new possibilities. The future of solar technology lies in the continuous innovation and optimization of these anti-reflective coatings, paving the way for more sustainable and efficient energy solutions.

6 CONCLUSIONS

In summary, research on anti-reflective coatings (ARCs) for solar cells demonstrates their critical role in the development of photovoltaic technology, particularly in terms of extending their lifespan and improving their energy conversion efficiency. Because anti-reflective coatings drastically reduce reflection losses—which are serious issues when attempting to extract the maximum amount of energy from light—they are not just an add-on; they are critical to getting the most out of solar cells. It has been discovered that materials like MgF_2, TiO_2, ZnO, SiO_2 and hybrid composites can have their optical, mechanical, and chemical properties precisely adjusted to significantly alter their performance. The application of nanotechnology to anti-reflective coatings is a significant advancement since it improves light absorption and coating durability, two factors critical to the long-term viability of industrial solar cells. It's crucial to remember that AR coatings do have certain drawbacks. A number of questions have been raised regarding their potential longevity in various conditions, the potential impact of surface pollution on performance, and the necessity of using cautious application techniques.

The main conclusions of this study are that additional research on AR coatings is necessary in order to enhance coating techniques, discover novel material combinations, and develop long-lasting self-cleaning technologies such as Smart ARCs can adapt to environmental changes, enhance light absorption, and improve solar cell energy capture. and Sustainable materials, such as low-impact or biodegradable materials, can solve environmental issues without losing functionality. Also, Research into natural polymers and hybrid coatings can result in exceptional performance.

Future studies and projects can improve the stability and efficiency of solar cells by employing these innovative suggestions, which will help to create a more sustainable energy environment. The advancement of the solar field will depend on ongoing research into new materials, methods, and cooperative initiatives.

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Mathematical Model for Aerodynamic Interaction of High-Speed Passenger and Freight Trains on Adjacent Tracks. Part I: Preliminary Conclusions on Problem Formulation and Solution Approach

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Keywords: High Speed, Aerodynamics, Safe Crossing, Freight Train, Standard, Air Flow, Anemometer, Kinematic Viscosity.

Abstract: The article highlights the theoretical formulation of the problem of studying the issues of mutual aerodynamic influence that affects the organization of safe crossing of high-speed passenger and freight trains when they move along adjacent railway tracks. The issue of operating freight trains without unnecessary stops in railroad sections with mixed traffic of different types of trains acquires urgent importance. With the help of the Navier-Stokes equation, which is primary in hydrogasdynamics for the study of aerodynamic effects, the nature of air flows that occur during the movement of trains of different categories has been studied. The problem of modeling the dynamics of changes in aerodynamic pressure impulses with respect to time and coordinate is described in detail. Preliminary conclusions on the determination of aerodynamic pressure showed that all speeds of freight and high-speed passenger trains used on the correct sections of Uzbekistan railways shouldn't exceed the calculated total pressure, to ensure the safety of train movement.

1 INTRODUCTION

When evaluating the aerodynamic forces, pressure, and safe distance experienced by passengers standing on adjacent tracks within a moving train or on a platform beside the tracks, the pressure (Pa) specified in safety standards, the distance of the object from the centerline of the tracks S (in meters), and the train speed V (in km/h) are expressed as a function. Solving this problem will enhance the capacity of the train section while ensuring traffic safety, thereby addressing issues related to the passage of freight trains in high-speed passenger train sections [1], [2].

The determination of the aforementioned parameters requires consideration of multiple factors. Specifically, the air surrounding the train is treated as an incompressible turbulent flow, and the Navier-Stokes equation is solved. The general form of the Navier-Stokes equation is as follows (1) in Appendix [1]-[2], [12].

Here u_x , u_y , u_z - projections of the velocity vector on the x, y, z axes; p - pressure; ρ - density; T - temperature; μ - is the coefficient of dynamic viscosity of air.

The continuity (2), derived from the principles of state and mass conservation, is obtained as follows:

$$\frac{\partial \rho}{\partial t}+\frac{\partial(\rho u_x)}{\partial x}+\frac{\partial(\rho u_y)}{\partial y}+\frac{\partial(\rho u_z)}{\partial z}=0 \quad (2)$$

If we consider this process in the $\{x, y\}$ plane and for an ideal gas, ($u_x >> u_z, u_y$, $\rho = const.$) The Euler equation in a particular case has the following (3) in Appendix [1]-[2], [12].

The relationship between the boundary layer width and the ratio of velocities in the incompressible turbulent airflow, as a function of the x coordinate (distance to the head of the train), was determined using the power law [1], [2]:

$$\begin{cases} \frac{u_x}{U} = \sqrt[7]{\frac{y}{\delta}} \\ \delta(x)_{turbulent} = 0{,}37 \cdot \sqrt[5]{\frac{\nu \cdot x^4}{U}} \\ \delta(x)_{laminar} \approx 5 \cdot \sqrt{\frac{\nu \cdot x}{U}} \end{cases} \quad (4)$$

Here u_x - the projection of the velocity vector in the direction of the *ox* axis is expressed in meters per second (m/s); U - train speed, m/s; y - The distance from the side surface of the train in the direction of the *oy* axis is expressed in meters (m); δ - boundary layer width of incompressible turbulent air flow, m; x - distance to the head of the train, m; ν - coefficient of kinematic viscosity of air at a given temperature, m^2/s;

Figures 1-2 illustrates the relationship between the width of the boundary layer of the air flow $\delta(x)$) and the speed of the air flow on the coordinate axis ($U \leq 250\ km/coam$).

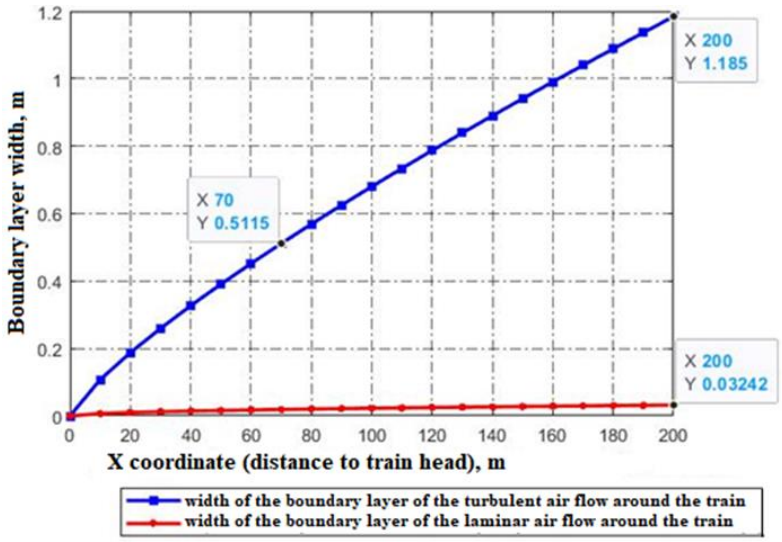


Figure 1: A plot of airflow boundary layer width versus the x coordinate (distance to the head of the train).

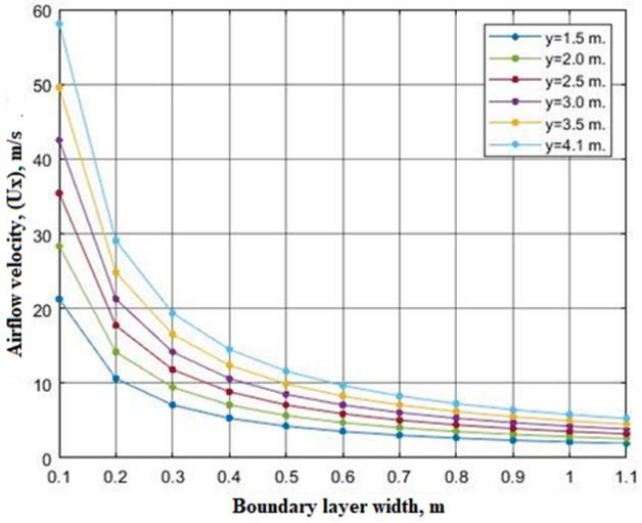


Figure 2: A graph of the relation of air flow speed to the width of the boundary layer.

2 MATERIALS AND METHODOLOGY

From the presented graphs, it is evident that both the width of the boundary layer and the velocity of the airflow exhibit a continuous and smooth variation. When observing the train's movement in the XOZ plane, the simplified vector Navier-Stokes equation can be employed, utilizing the "Reynolds-averaged Navier-Stokes (RANS) method." The specific form of this equation is as follows (5) in Appendix [1]-[2].

It is possible to simplify the above expressions depending on the characteristic indicators, in particular (6) in Appendix.

In a similar manner, the characteristic parameters of the second part will take on the following (7) in Appendix.

Based on (6) and (7), expression (5) can be simplified as follows:

$$\begin{cases} \bar{u}_x \frac{\partial \bar{u}_x}{\partial x} + \bar{u}_z \frac{\partial \bar{u}_x}{\partial z} + \frac{1}{\rho}\frac{\partial \bar{p}}{\partial x} = \nu \cdot \frac{\partial^2 \bar{u}_x}{\partial x^2} - \frac{\partial \bar{u}_x' \bar{u}_z'}{\partial z} \\ \frac{1}{\rho}\frac{\partial \bar{p}}{\partial z} = 0 \\ \frac{\partial \bar{u}_x}{\partial x} + \frac{\partial \bar{u}_z}{\partial z} = 0 \end{cases} \quad (8)$$

Based on (8), it is possible to determine the function describing the dependence of the velocity components on the width of the boundary layer and the Reynolds number, specifically for the conditions where $u_x >> u_z, u_y$.

$$\frac{u_x}{U} = \frac{1}{0{,}41} \cdot \ln(\frac{z}{z_o}) \quad \text{or}$$

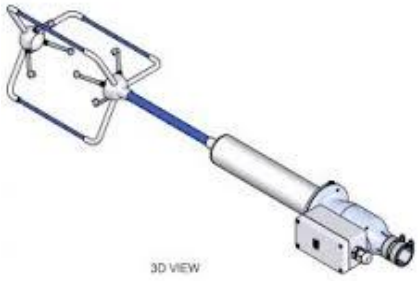
$$u^+ = \frac{1}{0{,}41} \cdot \ln(\frac{z \cdot U}{\nu}) + K = \frac{1}{0{,}41} \cdot \ln(z^+) + K$$

The obtained results in both planes demonstrate a systematic variation in the velocity components.

To ensure the reliability of the theoretical calculations, it is crucial to compare them with the results of experimental tests conducted by various researchers [3]-[12]. These experiments employed specialized anemometric instruments equipped with a Pitot tube (as shown in Figure 3) to measure residual differential pressures, dynamic pressures, and flow velocity.

The aerodynamic characteristics and mutual interaction of high-speed passenger and freight trains differ significantly from the interaction between two high-speed passenger trains. Extensive scientific research conducted by English, French, and Polish scientists [1], [3], 5], [7] has revealed a pronounced aerodynamic effect when high-speed trains move in opposite directions. The aerodynamic effects of high-speed passenger trains at intersections were compared in three different scenarios [13]:

- The observing train is moving at a speed lower than the passing train;
- The velocities of the observer and the passing train are equal;
- The following train is stationary.



(a)



(b)

Figure 3: Equipment used in experimental work. a) 3D-Axis Ultrasonic Anemometr, b) PCE-HVAC Model 2 Pitot Tube Anemometer.

Experimental results indicated that the highest aerodynamic pressure was observed in case 3, as shown in Table 1. Furthermore, it was observed that the magnitude of the residual pressure difference on the windows of high-speed trains moving in opposite directions at different speeds (160/140 km/h) was lower than the standard value (of 1800 Pa).

Experimental results [5] showed that the maximum pressure created when two trains cross has the following values.

Figure 4 quantifies this relationship, showing how aerodynamic pressure (Pa) escalates with increasing train speeds at varying horizontal distances between track centers (3.8–4.5 m). Figure 4 visualizes the pressure trends from Table 1, highlighting the exponential growth with speed.

Table 2 complements these findings by quantifying the maximum aerodynamic pressure exerted on the sidewall of the ET2M train during crossings with the Sapsan high-speed train under varying speed conditions

It is crucial to investigate the aerodynamic characteristics of airflow around trains to ensure safe crossings when encountering oncoming trains. Drawing upon scientific studies [6], [7], [9], [14] the model representing the airflow nature at train intersections can be expressed as follows in Figure 5.

Table 1: The aerodynamic pressure values obtained from the experimental test results.

Train speed, km/h	Aerodynamic pressure, Pa			
	Horizontal distance between road axes, m			
	3,8 (m)	4,0 (m)	4,2 (m)	4,5 (m)
160	328,3	282,0	245,2	202,8
180	415,5	356,8	310,3	256,6
200	512,9	440,6	383,1	316,8
220	-	533,1	463,5	383,3
240	-	634,4	551,6	456,2
250	-	688,4	598,6	495,0
260	-	-	647,4	535,4
280	-	-	750,8	620,9
300	-	-	861,9	712,8
320	-	-	-	811,0
340	-	-	-	915,6
350	-	-	-	970,2

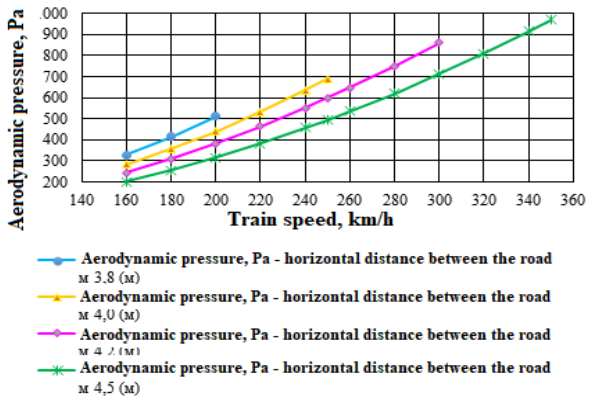


Figure 4: Value of aerodynamic pressure based on experimental results

Table 2: The maximum pressure exerted on the side wall of the car when trains are crossing.

Speed of trains at intersection, km/h		The maximum aerodynamic pressure on the sidewall of the ET2M train, Pa
Sapsan	ET2M	
160	0 (stayed on the neighboring road)	230
200	0	325
250	0	525
250	60	580
250	100	610
250	120	650
275	0	600
275	120	780

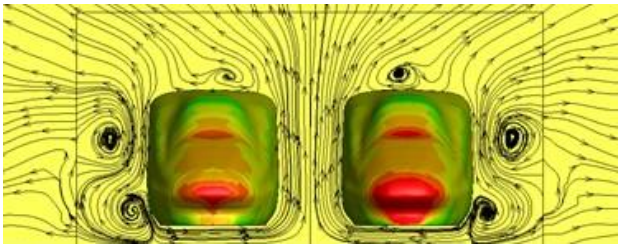


Figure 5: The nature of the air flow around oncoming trains.

In the context of the current study, the analysis focuses on a specific scenario involving a freight train. The freight train under consideration has a total length of 815 meters and a gross weight of 1392 tons. This freight train is composed of an electric locomotive called "Afrosiyob" with a gross weight of 310 tons, which is accompanied by 11 cars with a combined length of 183 meters. Additionally, two locomotives are also present in this configuration. The objective is to examine the safety conditions at the intersection, as depicted in Figure 6.

Based on extensive scientific research conducted by various scholars [3]-[5], [9], the characteristics of airflow and air pressure during the crossing of two trains can be summarized as depicted in Figure 7. The safe crossing of trains and their stability [15], along with the study of the impact of airflow on loads in open rolling stock resulting from their interaction with high-speed trains, is a critical and pressing issue. In this regard, the airflow between high-speed passenger and freight trains is considered as an incompressible turbulent air flow [1].

To ensure train stability, it is crucial to take into account the merging and reinforcement of the air

currents formed around the two trains. In this scenario, the primary transverse aerodynamic force is generated at the head of the rolling stock, while the force between the cars is relatively lower [1], [3], [11]. The dominant aerodynamic force, which significantly affects train stability, acts in the transverse direction along the axis of the train. This force is attributed to the organization of turbulent air flows in that particular direction. Experimental tests [4], [7], [9] confirm that the flow speed around trains decreases gradually and follows a certain pattern as it moves away from the train.

Considering the above observations, the overall air flow velocity can be expressed in the following manner, taking into account the principle that the speed of the flow around trains decreases gradually in relation to the speed of the ambient air flow at points distanced from the train.

$$\vartheta = V_n\left[1-\left(\frac{y}{\delta}\right)^{1/n}\right] \qquad \delta = a\cdot X^{m}. \quad (9)$$

Here, ϑ - air flow speed around the train, m/s; V_n - speed of a high-speed passenger train, m/s; y - transverse distance from the vehicle body to the observation point, m; n, m, a - fixed coefficients depending on the composition and type of train; X - horizontal distance to the front of the train, m

According to the European standard "EN 14067 Railway application - Aerodynamics - Chapter 4" [3]-[4], the calculation of the speed components of a turbulent air stream formed around a circular train is determined as follows (10):

$$C_{vi} = \vartheta_i / V_п;\ C_{vx} = \vartheta / V_п;\ C_{uy} = u / V_п;\ C_{wz} = w / V_п \quad (10)$$

Here ϑ, u, w - velocity components of the air flow formed around the train, m/s; V_n - speed of a high-speed passenger train, m/s; C_{vx}, C_{uy}, C_{wz} - constant speed coefficients depending on the composition and type of train $0{,}17 \le C_{vx} \le 0{,}4$; $C_{uy} \le 0{,}1$; $C_{wz} \le 0{,}1$; $C_{vx}(freighttrain) \le 0{,}57$.(freight train).

Based on Figure 7 and scientific research, it can be observed that pressure pulses exhibit varying patterns at different points, dependent on time and coordinates. Therefore, a model was developed to simulate the aerodynamic pressure impulses, as depicted in Figure 8.

The graph illustrates that the pressure exhibits a linear trend in zones 0, 4, 8, n-1, and a sinusoidal pattern in zones 1, 2, 3-5, 6, 7-9, 10, 11-n-4, n-3, n-2, but in reverse order. Consequently, the variation in coordinates and pressure impulses is expressed in the following manner:

$$\begin{cases} x_1 - x_0 = (\vartheta_1 + \vartheta_2)\cdot(t_1 - t_0) \\ x_2 - x_1 = (\vartheta_1 + \vartheta_2)\cdot(t_2 - t_1) \\ x_3 - x_2 = (\vartheta_1 + \vartheta_2)\cdot(t_3 - t_2) \\ \vdots \\ x_n - x_{n-1} = (\vartheta_1 + \vartheta_2)\cdot(t_n - t_{n-1}) \end{cases} \quad (11)$$

In a linear order, the pressure values in the change zones were determined by expression (12) in Appendix.

The pressure value was determined using expression (13) in Appendix by changing the zones in a sinusoidal order.

The magnitude of the aerodynamic force acting on each of them is determined by integration. From expressions (12), (13) it can be seen that the peak aerodynamic pressure corresponds to zones 1, 2, 3. Also, based on the results of the above scientific research and European standards, assuming the maximum value of aerodynamic pressure corresponding to zones 1, 2, 3, affecting the stability of trains, proportional to the square of the speed of the train, the pressure of a high-speed passenger train on a freight train in the transverse direction along the axis of *oy* and *oz* (14) in Appendix was determined by the expression [12].

Here k_1 – coefficient of influence depending on the geometric structure of the train ($k_1^{freight} \approx 1$; $k_1^{passenger} \approx 0{,}53$; $k_1^{high-speed} \approx 0{,}43$); y – distance between axes of adjacent roads ($y = 4{,}1\ м$), m; A_1 – width of the investigated object or rolling stock (width of a locomotive or carriage of a high-speed passenger train, $A_1 = 2{,}96\ m$), m; A_2 – width of the object or rolling stock under study (width of a locomotive or wagon of a freight train, $A_2 = 3{,}1\ m$), m; ρ – air density ($\rho \cong 1{,}2041\ kg/m^3$), kg/m^3; ϑ_1, ϑ_2 – speed of high-speed passenger and freight trains ($\vartheta_1^{\max} = 250\ km/h \approx 69{,}44\ m/s$, $\vartheta_2^{\max} = 90\ km/h \approx 25\ m/s$), m/s.

C_{p1} – Coefficient of aerodynamic pressure in the direction of the axes *oy* and *oz*.

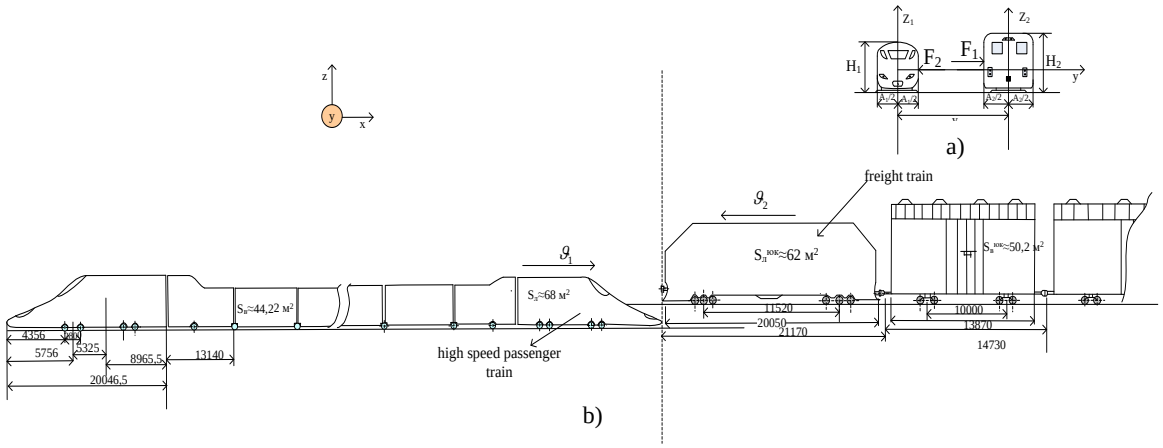


Figure 6: Design diagram of the intersection of the Afrosiab high-speed electric train and a freight train on neighboring tracks: a) section, b) side view.

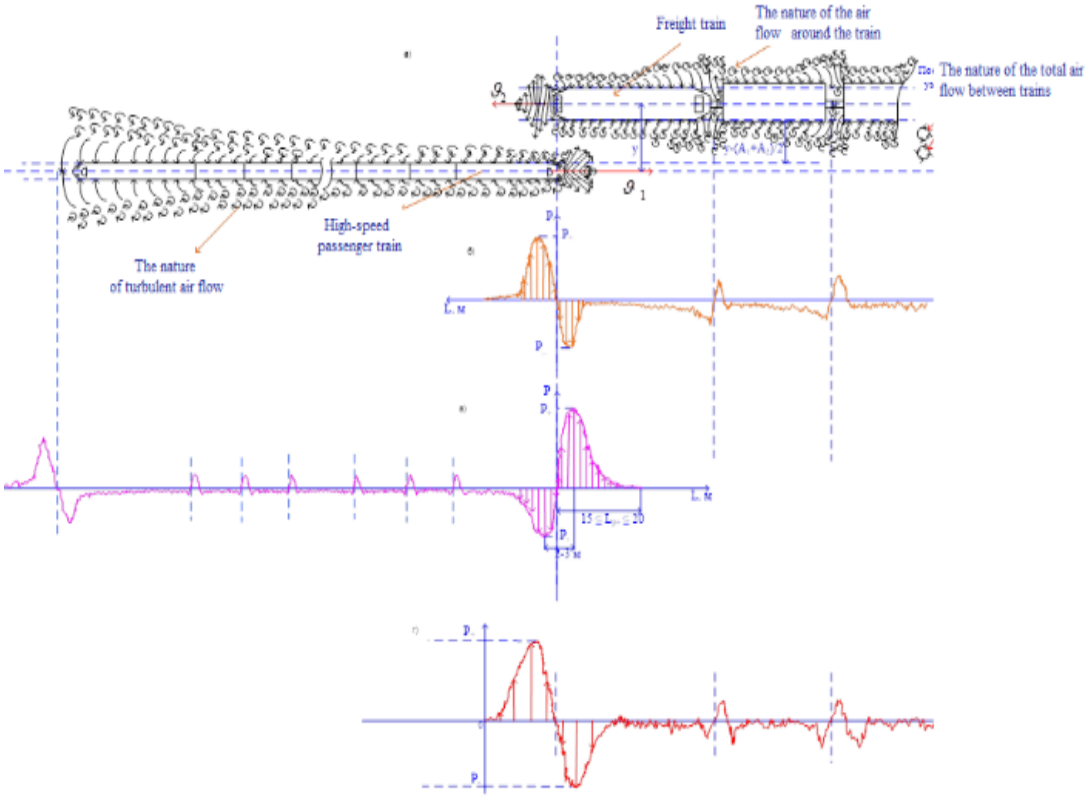


Figure 7: Calculation diagram of the intersection of the Afrosiab high-speed electric train and a freight train on adjacent tracks: a) - top view, b), c), d) - freight, high-speed and the nature of the dynamics of changes in aerodynamic pressure at their intersection.

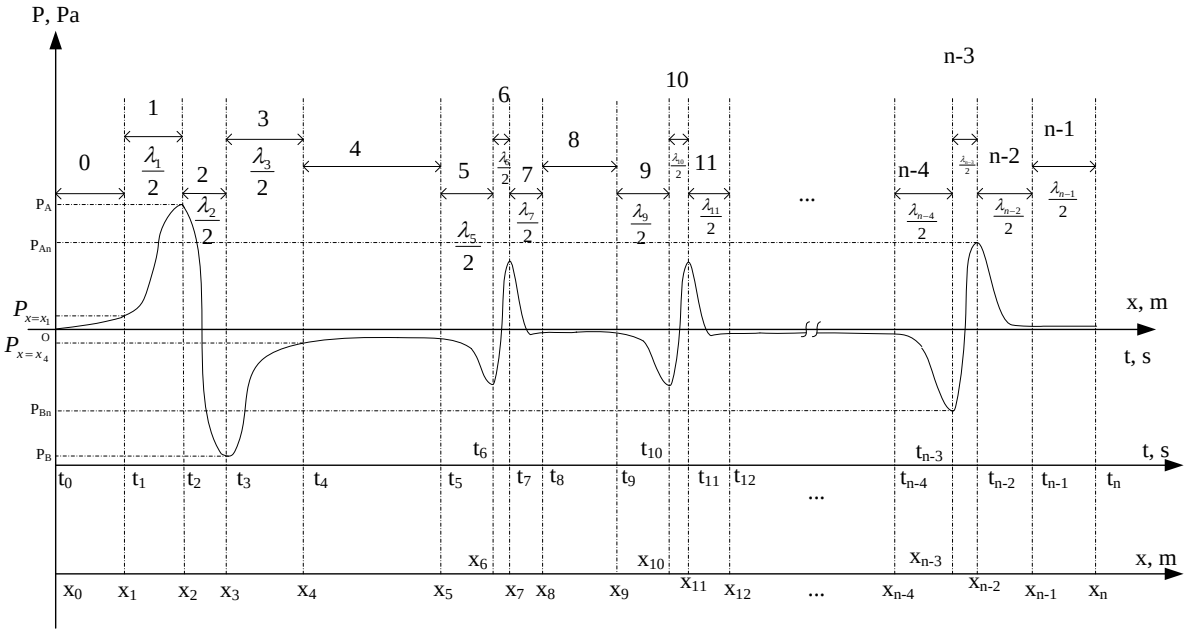


Figure 8: The law of change of the impulse of aerodynamic pressure depending on time and coordinates.

Since in our case $\vartheta_{max} = \vartheta_1 = 250\ {}^{km}/_{h} \approx 69{,}44\ {}^{m}/_{s}$, the maximum number $M = {}^{\vartheta_{max}}/_{c} \approx 0{,}21$ and the Reynolds number $4{,}5\cdot10^{8} \le \mathrm{Re} = {}^{\vartheta_1 \cdot L_п}/_{\nu} \le 7{,}1\cdot10^{8}$ (+20 ^{0}C $\nu = 1{,}81\cdot10^{-5}\ {}^{m^2}/_{s}$) were taken, and the process under consideration was studied as incompressible air in a stationary state. Similarly, the aerodynamic effect of a freight train on a passenger train was expressed as follows:

$$\begin{cases} \Delta P_{2y} = k_1^{freight} \cdot C_{p2y} \cdot \rho \cdot \dfrac{\vartheta_2{}^2}{2 \cdot 3,6^2} \\ C_{p2y} = \dfrac{8}{\left(y - \dfrac{A_1}{2} + 2{,}10\right)^2} \\ \Delta P_{2z} = k_1^{freight} \cdot C_{p2z} \cdot \rho \cdot \dfrac{\vartheta_2{}^2}{2 \cdot 3,6^2} \\ C_{p2z} = \dfrac{8{,}5}{(z-1{,}9)^2} + 0{,}1 \end{cases}$$

In a comprehensive analysis, the peak pressure occurring in the transverse direction as a consequence of train intersection and negatively impacting their stability was determined by applying the principle of superposition, considering the combined effect of pressure values and the compressive force resulting from the addition of their air flow velocities in the most unfavorable scenario. Furthermore, in accordance with the technical specifications for load fastening and enhancement, an average external wind pressure of 50 kgs/m^2 ≅ 500 Pa was assumed.

$$\Delta P_y^{\text{total}} = \sum_{i=1}^{n} \Delta P_{iy} = \Delta P_{1y} + \Delta P_{2y} + \cdots + \Delta P_{ny}$$

$$\begin{cases} F_{y1} = \Delta P_y^{\text{total}} \cdot S_2 \\ F_{y2} = \Delta P_y^{\text{total}} \cdot S_1 \end{cases}$$

3 RESULT AND DISCUSSION

As a result of determining the resulting aerodynamic and total pressure using the MATLAB program, the following graphs were constructed (Figure 9 and Figure 10).

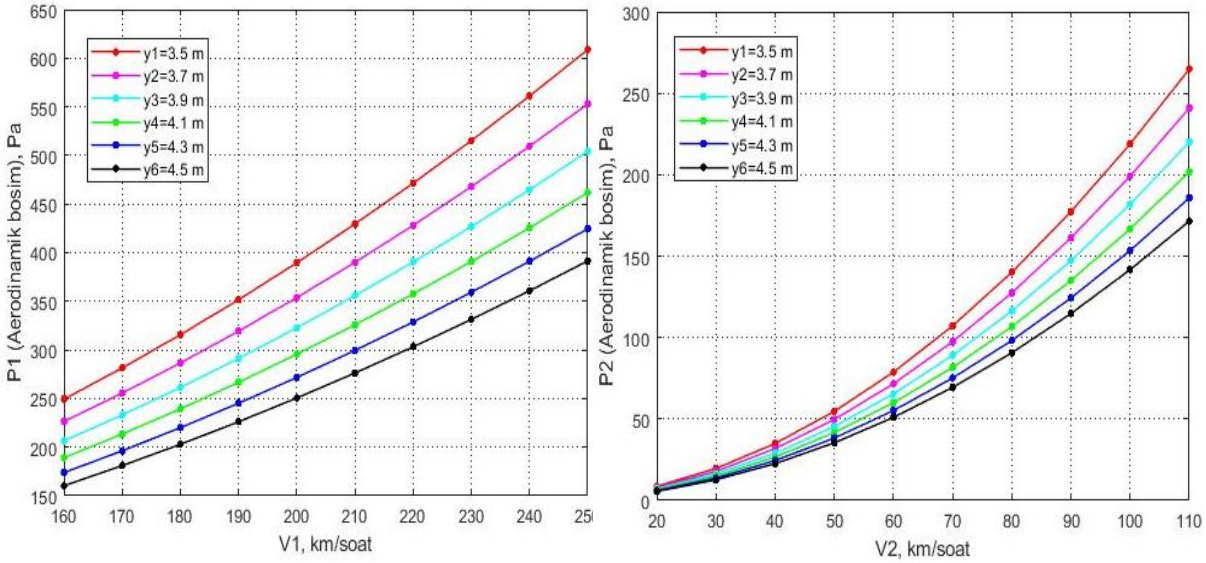


Figure 9: The value of the aerodynamic pressure of high-speed (left) and freight trains (right).

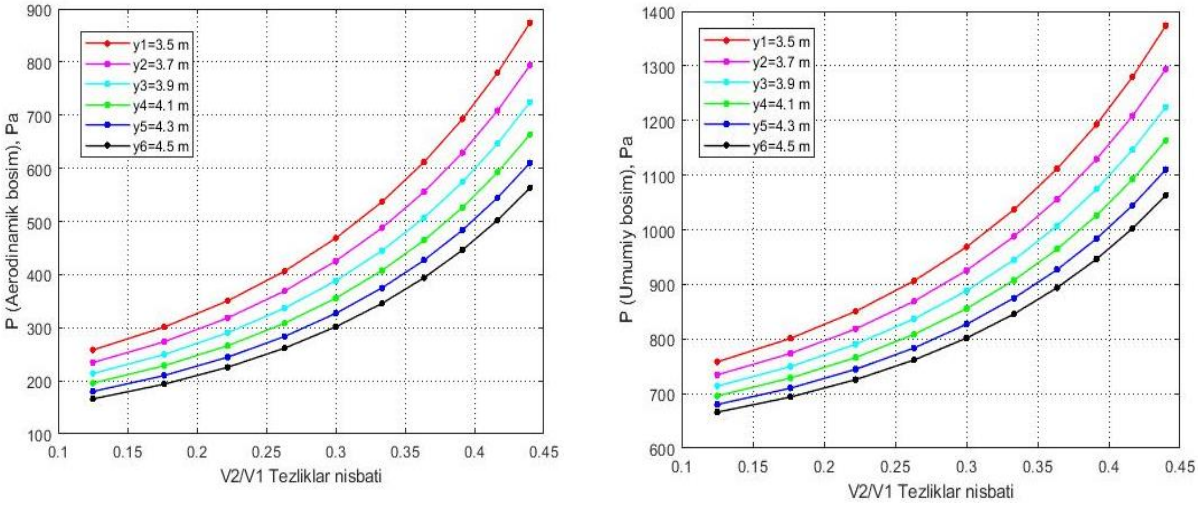


Figure 10: The value of the aerodynamic pressure created between trains (left) and the total pressure (right) taking into account the influence of external wind.

4 CONCLUSIONS

The issue of operating freight trains without unnecessary stops in railroad sections with mixed traffic of different types of trains acquires urgent importance. Based on the analysis of scientific research conducted from this perspective, it was determined that the nature of the air flow around trains during intersection at speeds of up to 250 km/h for passenger trains and up to 100 km/h for freight trains is compressible (M <0,3). The peculiar solutions of the Navier-Stokes equations were also considered in determining the width of the boundary layer of air flow velocity. In order to ensure safe movement during the intersection of trains, the law of change of the aerodynamic pressure impulse occurring between them in a time- and coordinate-dependent manner was modeled; the general aerodynamic pressure that could affect the moving units was determined based on the principle of

superposition and the achieved results were compared with regulatory values.

Based on the proposed mathematical model, the calculated value of the aerodynamic pressure is 628,7 Pa (250/100), and when compared with the results of scientific research [4], the relative error is about, indicating that the accuracy of the engineering calculations is within the standard value of approximately ( ).

The results of the calculations showed that when the distance between the rails is 4,1 meters and the relative speed of movement of freight and high-speed passenger trains is 0,44 (for example, the maximum speed of the freight train is 110 km/h, and the speed of the high-speed passenger train is 250 km/h), the total pressure is 1164 Pa. This amount of total pressure constitutes 64,7% of the value of the calculated pressure (1800 Pa) specified in the "Safety Standards for Railway Transport" NB JT ST 03-98. Therefore, it can be concluded that all speeds of freight and high-speed passenger trains used on the correct sections of Uzbekistan railways do not exceed the calculated total pressure, which ensures the safety of train movement. In the future, detailed studies will be conducted on the aerodynamic forces that arise during the crossing of trains in complex conditions (on curved sections of the railway, in areas with strong wind influence, etc.) based on the mathematical model developed.

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APPENDIX

The Appendix contains the detailed mathematical expressions used in the study, specifically formulas (1), (3), (5), (6), (7), (12), (13), and (14), which support the aerodynamic modeling and calculations presented in the main text.

$$\begin{cases} \rho \cdot (\frac{\partial u_x}{\partial t} + u_x \frac{\partial u_x}{\partial x} + u_y \frac{\partial u_x}{\partial y} + u_z \frac{\partial u_x}{\partial z}) + \frac{\partial p}{\partial x} = X + \mu(\frac{\partial^2 u_x}{\partial x^2} + \frac{\partial^2 u_x}{\partial y^2} + \frac{\partial^2 u_x}{\partial z^2}) + \rho \cdot g_x \\ \rho \cdot (\frac{\partial u_y}{\partial t} + u_x \frac{\partial u_y}{\partial x} + u_y \frac{\partial u_y}{\partial y} + u_z \frac{\partial u_y}{\partial z}) + \frac{\partial p}{\partial y} = Y + \mu(\frac{\partial^2 u_y}{\partial x^2} + \frac{\partial^2 u_y}{\partial y^2} + \frac{\partial^2 u_y}{\partial z^2}) + \rho \cdot g_y \\ \rho \cdot (\frac{\partial u_z}{\partial t} + u_x \frac{\partial u_z}{\partial x} + u_y \frac{\partial u_z}{\partial y} + u_z \frac{\partial u_z}{\partial z}) + \frac{\partial p}{\partial z} = Z + \mu(\frac{\partial^2 u_z}{\partial x^2} + \frac{\partial^2 u_z}{\partial y^2} + \frac{\partial^2 u_z}{\partial z^2}) + \rho \cdot g_z \end{cases} \quad (1)$$

$$\begin{cases} \frac{\partial \vec{u}_i}{\partial t} + u_x \frac{\partial \vec{u}_i}{\partial x} + u_y \frac{\partial \vec{u}_i}{\partial y} + u_z \frac{\partial \vec{u}_i}{\partial z} = -\frac{1}{\rho} \nabla_i P \ , \ \varepsilon = \frac{\delta}{L} << 1 \ , \ Re = \frac{L \cdot U}{\nu} = \frac{L \cdot U \cdot \rho}{\mu} >> 1 \\ \rho \cdot (\frac{\partial u_x}{\partial t} + u_x \frac{\partial u_x}{\partial x} + u_y \frac{\partial u_x}{\partial y}) = -\frac{\partial p}{\partial x} \\ \rho \cdot (\frac{\partial u_y}{\partial t} + u_x \frac{\partial u_y}{\partial x} + u_y \frac{\partial u_y}{\partial y}) = -\frac{\partial p}{\partial y} \\ \frac{\partial u_x}{\partial x} + \frac{\partial u_y}{\partial y} = 0, \ u_x = \frac{\partial \psi}{\partial y}, u_y = -\frac{\partial \psi}{\partial x} \Rightarrow \frac{\partial^2 \psi}{\partial x^2} + \frac{\partial^2 \psi}{\partial y^2} = 0 \end{cases} \quad (3)$$

$$\begin{cases} \bar{u}_x \frac{\partial \bar{u}_x}{\partial x} + \bar{u}_z \frac{\partial \bar{u}_x}{\partial z} + \frac{1}{\rho} \frac{\partial \bar{p}}{\partial x} = \nu \cdot (\frac{\partial^2 \bar{u}_x}{\partial x^2} + \frac{\partial^2 \bar{u}_x}{\partial z^2}) - (\frac{\partial \bar{u}_{xI}{}^2}{\partial x} + \frac{\partial \bar{u}_{xI} \bar{u}_{zI}}{\partial z}) \\ \bar{u}_x \frac{\partial \bar{u}_z}{\partial x} + \bar{u}_z \frac{\partial \bar{u}_z}{\partial z} + \frac{1}{\rho} \frac{\partial \bar{p}}{\partial z} = \nu \cdot (\frac{\partial^2 \bar{u}_z}{\partial x^2} + \frac{\partial^2 \bar{u}_z}{\partial z^2}) - (\frac{\partial \bar{u}_{zI}{}^2}{\partial z} + \frac{\partial \bar{u}_{xI} \bar{u}_{zI}}{\partial x}) \\ \frac{\partial \bar{u}_x}{\partial x} + \frac{\partial \bar{u}_z}{\partial z} = 0 \end{cases} \quad (5)$$

$$\begin{cases} \frac{\partial u_x}{\partial x} = O\left(\frac{U}{L}\right) \ ; \ \frac{\partial u_z}{\partial z} = O\left(\frac{W}{\delta}\right) = O\left(\frac{U\xi}{L}\right) \ ; \ u_x \frac{\partial u_x}{\partial x} = O\left(\frac{U^2}{L}\right) \ ; \ \frac{\partial u_x}{\partial t} = O\left(\frac{U}{T}\right) = O\left(\frac{U^2}{L}\right) \ ; \\ u_z \frac{\partial u_x}{\partial z} = O\left(W \frac{U}{\delta}\right) = O\left(\frac{U\delta}{L} \cdot \frac{U}{\delta}\right) = O\left(\frac{U^2}{L}\right) \ ; \ \nu \frac{\partial^2 u_x}{\partial x^2} = O\left(\frac{\nu \cdot U}{L^2}\right) = O\left(\frac{1}{\frac{UL}{\nu}} \cdot \frac{U^2}{L}\right) = O\left(\frac{1}{Re \frac{U^2}{L}}();\right) \\ \nu \frac{\partial^2 u_x}{\partial z^2} = O\left(\frac{1}{\xi^2 \cdot Re \frac{U^2}{L}}() \frac{\delta}{L} \frac{1}{\sqrt{Re}}\right) \end{cases} \quad (6)$$

$$\begin{cases} \frac{\partial u_z}{\partial t} = O\left(\frac{W}{T}\right) = O\left(\frac{1}{\sqrt{\mathrm{Re}}} \cdot \frac{U^2}{L}\right); u_x \frac{\partial u_z}{\partial x} = O\left(W \frac{U}{L}\right) = O\left(\frac{1}{\sqrt{\mathrm{Re}}} \cdot \frac{U^2}{L}\right); \\ u_z \frac{\partial u_z}{\partial z} = O\left(\frac{W^2}{\delta}\right) = O\left(\frac{1}{\sqrt{\mathrm{Re}}} \cdot \frac{U^2}{L}\right); \nu \frac{\partial^2 u_z}{\partial x^2} = O\left(\frac{\nu \cdot W}{L^2}\right) = O\left(\frac{1}{\mathrm{Re}\sqrt{\mathrm{Re}}} \cdot \frac{U^2}{L}\right); \\ \nu \frac{\partial^2 u_z}{\partial z^2} = O\left(\frac{\nu \cdot W}{\delta^2}\right) = O\left(\frac{1}{\xi^2 \cdot \mathrm{Re}\sqrt{\mathrm{Re}}} \cdot \frac{U^2}{L}\right); \end{cases} \quad (7)$$

$$\begin{cases} P_0 = P_{x=x_0} + (\frac{P_{x=x_1} - P_{x=x_0}}{x_1 - x_0}) \cdot (x - x_0) = P_{x=x_0} + (\frac{P_{x=x_1} - P_{x=x_0}}{x_1 - x_0}) \cdot (\vartheta_1 + \vartheta_2) \cdot (t - t_0) \\ P_4 = P_{x=x_4} + (\frac{P_{x=x_5} - P_{x=x_4}}{x_5 - x_4}) \cdot (x - x_4) = P_{x=x_4} + (\frac{P_{x=x_5} - P_{x=x_4}}{x_5 - x_4}) \cdot (\vartheta_1 + \vartheta_2) \cdot (t - t_4) \\ P_8 = P_{x=x_8} + (\frac{P_{x=x_9} - P_{x=x_8}}{x_9 - x_8}) \cdot (x - x_8) = P_{x=x_8} + (\frac{P_{x=x_9} - P_{x=x_8}}{x_9 - x_8}) \cdot (\vartheta_1 + \vartheta_2) \cdot (t - t_8) \\ \vdots \\ P_{n-1} = P_{x=x_{n-1}} + (\frac{P_{x=x_n} - P_{x=x_{n-1}}}{x_n - x_{n-1}}) \cdot (x - x_{n-1}) = P_{x=x_{n-1}} + (\frac{P_{x=x_n} - P_{x=x_{n-1}}}{x_n - x_{n-1}}) \cdot (\vartheta_1 + \vartheta_2) \cdot (t - t_{n-1}) \end{cases} \quad (12)$$

$$\begin{cases} P_1 = P_{x=x_1} + \frac{1}{2}\cdot(P_A - P_{x=x_1})\cdot(1 - cos(\frac{2\pi}{\lambda_1}\cdot(x - x_1))) = P_{x=x_1} + (P_A - P_{x=x_1})\cdot sin^2(\frac{\pi}{\lambda_1}\cdot(x - x_1)) = \\ = P_{x=x_1} + (P_A - P_{x=x_1})\cdot sin^2(\frac{\pi}{\lambda_1}\cdot(\vartheta_1 + \vartheta_2)\cdot(t - t_1)) \\ P_2 = \frac{1}{2}\cdot(P_A + P_B) + \frac{1}{2}\cdot(P_A - P_B)\cdot cos(\frac{2\pi}{\lambda_2}\cdot(x - x_2)) = \\ = \frac{1}{2}\cdot(P_A + P_B) + \frac{1}{2}\cdot(P_A - P_B)\cdot cos(\frac{2\pi}{\lambda_2}\cdot(\vartheta_1 + \vartheta_2)\cdot(t - t_2)) \\ P_3 = P_{x=x_4} - \frac{1}{2}\cdot(P_{x=x_4} - P_B)\cdot(1 - cos(\frac{2\pi}{\lambda_3}\cdot(x - x_3 + \frac{\lambda_3}{2}))) = \\ = P_{x=x_4} - \frac{1}{2}\cdot(P_{x=x_4} - P_B)\cdot(1 - cos(\frac{2\pi}{\lambda_3}\cdot((\vartheta_1 + \vartheta_2)\cdot(t - t_3) + \frac{\lambda_3}{2}))) \\ \vdots \\ P_{n-4} = P_{x=x_{n-4}} - \frac{1}{2}\cdot(P_{x=x_{n-4}} - P_{Bn})\cdot(1 - cos(\frac{2\pi}{\lambda_{n-4}}\cdot(x - x_{n-4}))) = \\ = P_{x=x_{n-4}} - \frac{1}{2}\cdot(P_{x=x_{n-4}} - P_{Bn})\cdot(1 - cos(\frac{2\pi}{\lambda_{n-4}}\cdot(\vartheta_1 + \vartheta_2)\cdot(t - t_{n-4}))) \\ P_{n-3} = \frac{1}{2}\cdot(P_{An} + P_{Bn}) - \frac{1}{2}\cdot(P_{An} - P_{Bn})\cdot cos(\frac{2\pi}{\lambda_{n-3}}\cdot(x - x_{n-3})) = \\ = \frac{1}{2}\cdot(P_{An} + P_{Bn}) - \frac{1}{2}\cdot(P_{An} - P_{Bn})\cdot cos(\frac{2\pi}{\lambda_{n-3}}\cdot(\vartheta_1 + \vartheta_2)\cdot(t - t_{n-3})) \\ P_{n-2} = P_{x=x_{n-1}} - \frac{1}{2}\cdot(P_{x=x_{n-1}} - P_{An})\cdot(1 - cos(\frac{2\pi}{\lambda_{n-2}}\cdot(x - x_{n-2} + \frac{\lambda_{n-2}}{2}))) = \\ = P_{x=x_{n-1}} - \frac{1}{2}\cdot(P_{x=x_{n-1}} - P_{An})\cdot(1 - cos(\frac{2\pi}{\lambda_{n-2}}\cdot((\vartheta_1 + \vartheta_2)\cdot(t - t_{n-2}) + \frac{\lambda_{n-2}}{2}))) \end{cases} \quad (13)$$

$$\begin{cases} \Delta P_{1y} = k_1^{high-speed}\cdot C_{p1y}\cdot\rho\cdot\frac{{\vartheta_1}^2}{2\cdot 3,6^2} \\ C_{p1y} = \frac{8}{\left(y - \frac{A_2}{2} + 2,10\right)^2} \\ \Delta P_{1z} = k_1^{high-speed}\cdot C_{p1z}\cdot\rho\cdot\frac{{\vartheta_1}^2}{2\cdot 3,6^2} \\ C_{p1z} = \frac{8,5}{(z - 1,9)^2} + 0,1 \end{cases} \quad (14)$$

Mathematical Model for Aerodynamic Interaction of High-Speed Passenger and Freight Trains on Adjacent Tracks. Part II: Theoretical and Practical Basis for Solving the Problem in Complex Conditions

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Keywords: High-Speed, Aerodynamics, Safe Crossing, Curvature of Railway, Stability Coefficient, Empty Wagon, Freight Train, Standard, Air Current.

Abstract: The article studies the issues of mutual aerodynamic influence that affects the organization of safe crossing of high-speed passenger and freight trains when they move along adjacent railway tracks in difficult conditions (on curved sections of railways, under the influence of a strong side wind). At the same time, special attention is paid to the stability of the rolling stock. The reserve of the rollover stability coefficient for each unit of rolling stock was calculated depending on the radius of the curve of the railway section and the pressure of the side wind, and the limits of train speeds were set for their safe crossing in difficult conditions. The article also defines the limits of the safe crossing speeds of high-speed passenger and freight trains of different categories under these complex conditions under the curved conditions of the railways and the "Wind rose" study, which in turn is achieved to ensure the safety of train traffic. As a result, the effective use of train permeability and carrying capacity of railway sections is achieved.

1 INTRODUCTION

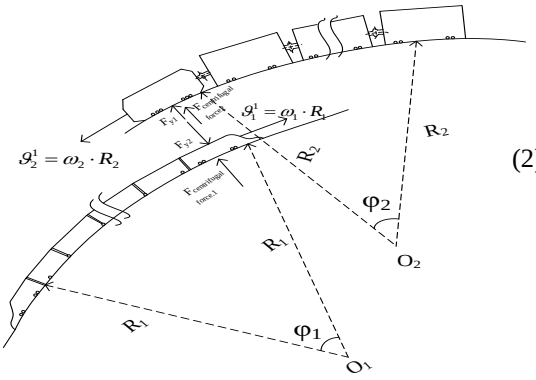
This scientific article serves as a logical continuation of the previous scientific study entitled "Mathematical model for aerodynamic interaction of high-speed passenger and freight trains on adjacent tracks, Part I: Preliminary conclusions on problem formulation and solution approach". The previous study focused on analyzing wind speeds using a 'wind rose' method and specifically examined double-track sections with mixed traffic and various types of trains operated by Uzbekistan Railways JSC, taking into account their geographical locations. By analyzing the areas where strong wind pressure may occur, an estimation was made regarding the margin of the stability coefficient for rolling stock units to prevent transverse overturning when trains traverse curved railway sections.

2 MATERIALS AND METHODS

When freight and high-speed passenger trains cross curved sections of the railway, the stability of the car is negatively affected due to the combination of centrifugal force and aerodynamic pressure forces caused by the resonance phenomenon. Figure 1 illustrates the forces acting on a unit of rolling stock moving along track 1. Considering that a high-speed passenger train experiences a greater force when moving along the same track compared to track 2, the maximum impact forces can be expressed as follows [1]-[3], [5]-[12].

$$\begin{cases} F_1^{total} = F_{y1} + F_2^{\text{centrifugal force}} \\ F_2^{total} = F_{y2} + F_1^{\text{centrifugal force}} \end{cases}. \quad (1)$$

Considering the reduction in the influence of aerodynamic force on movement along the 1st track, an increase in impact on movement along the 2nd track was observed. In this case, the calculation considered the centrifugal force, which is directly proportional to the square of the velocity and inversely proportional to the radius of curvature (refer to Figure 2).



(2)

Figure 1: Influence of forces on a rolling stock unit on a railway track curve.

Furthermore, the calculations used standard values for undamped accelerations resulting from train movement on railway curves, which were determined for each traffic structure [4]-[6], [16]-[19].

$$F_2^{\text{centrifugal force}} = M_2 \cdot a_y^{freight}$$

$$F_1^{\text{centrifugal force}} = M_1 \cdot a_y^{high-speed}$$

$$a_y^{freight} = \frac{(\vartheta_2^1)^2}{3,6^2 \cdot R_{1,2}} \cdot cos(\gamma) - g \cdot sin(\gamma)$$

$$a_y^{high-speed} = \frac{(\vartheta_1^1)^2}{3,6^2 \cdot R_{1,2}} \cdot cos(\gamma) - g \cdot sin(\gamma) - g \cdot \alpha$$

Based on Figure 2 and considering that $tg(\gamma) \approx \sin(\gamma) \approx \frac{h}{2l}$ and $\cos(\gamma) \approx 1$ for small angles γ, (1) and (2) can be simplified to the following form:

$$\begin{cases} F_1^{total} \approx F_{y1} + M_2 \cdot \left(\frac{(\vartheta_2^1)^2}{3,6^2 \cdot R_{1,2}} - g \cdot \frac{h}{2l} \right) \\ F_2^{total} \approx F_{y2} + M_1 \cdot \left(\frac{(\vartheta_1^1)^2}{3,6^2 \cdot R_{1,2}} - g \cdot \frac{h}{2l} - g \cdot \alpha \right) \end{cases} \quad (3)$$

Here:

- S_1, S_2 - side surfaces of sections of high-speed passenger and freight trains, respectively, m^2.
- M_1, M_2 - Masses of high-speed passenger and freight trains, respectively, kg.
- α - inclination angle of the body of a high-speed train ($\alpha = 3° \approx 0{,}0523\, radian$).
- R_1, R_2 - radii of curvature respectively ($R_{1,2} \geq 1200\ m$), m.
- $\vartheta_1^1, \vartheta_2^1$ - maximum speeds of high-speed passenger and freight trains on curves, respectively ($160 \leq \vartheta_1^1 \geq 250\ km/h$, $25 \leq \vartheta_1^1 \geq 110\ km/h$), km/hour.
- h - height of the outer rail relative to the inner rail at railway bends ($h \leq 0{,}150\ м$), m.
- $2l$ - the width of the distance between the rims in the wheelset ($2l \cong 1{,}6\ m$), м.
- a_y - undamped lateral accelerations in curves ($-0{,}3 \leq a_y^{freight} \leq +0{,}3\ m/s^2$, $-0{,}7 \leq a_y^{\text{passenger train.}} \leq +0{,}7\ m/s^2$, $-1{,}2 \leq a_y^{\text{high-speed.}} \leq +1{,}2\ m/s^2$), m/s^2;
- g - acceleration of gravity ($g \approx 9{,}81\, m/s^2$), m/s^2.

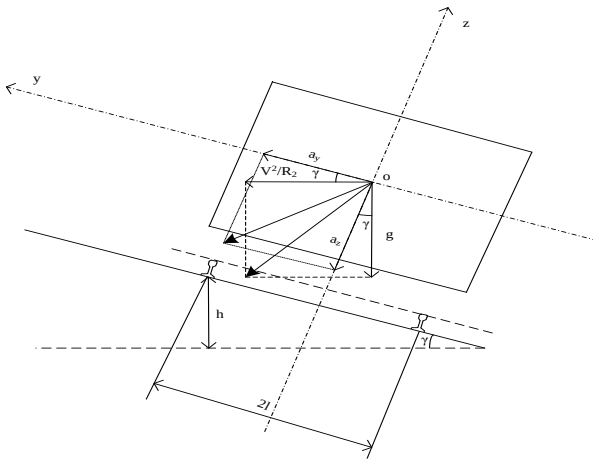


Figure 2: Components of centrifugal force and acceleration at railway turns.

In order to assess the mutual aerodynamic influence when combining high-speed passenger and freight trains on curved sections of the railway, it is crucial to consider the most challenging scenarios. The calculations were conducted using data obtained from sections capable of accommodating high-speed traffic within the Tashkent Railway Distance (PC-2) of UTY JSC [11]. Detailed results of these calculations are presented in Figures 3 and 4.

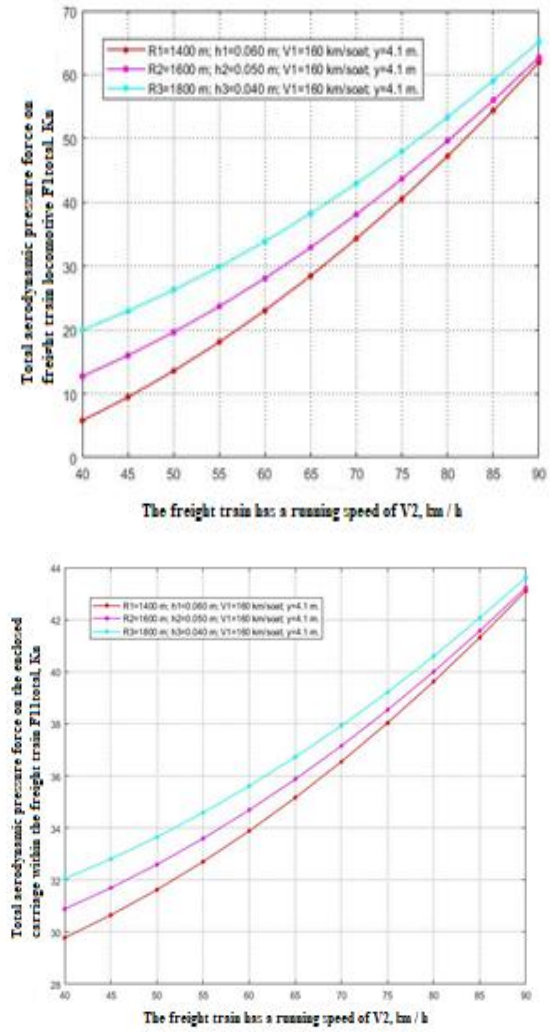


Figure 3: Sum of total aerodynamic forces on a freight train locomotive (left) and an empty four-axle universal closed car (right).

It is necessary to evaluate the margin of the stability coefficient to prevent transverse overturning caused by the total aerodynamic force acting on the components of the motion structure. To do this, the stability coefficient margin of an empty covered wagon, which represents a unit of rolling stock, is calculated in the transverse direction. The minimum acceptable values for the stability coefficient margin to prevent rollover in the transverse direction are determined as $K_{\text{stab.}}^{passenger} \geq 1{,}4$; $K_{\text{stab.}}^{empty\ car} \geq 1{,}3$; $K_{\text{stab.}}^{high-speed} \geq 1{,}6$ as stated in [12-15]. In the general case, the stability coefficient margin is determined by the following expression:

$$K_{\text{stab.}} = F_{st} \Big/ F_{dy} = F_{st} \Big/ (F_{\text{centrifugal force}} + F_{wind}^{aerody.}), \quad (4)$$

here, F_{st} , F_{dy} - static and dynamic forces from the wheel to the rail, kN, respectively.

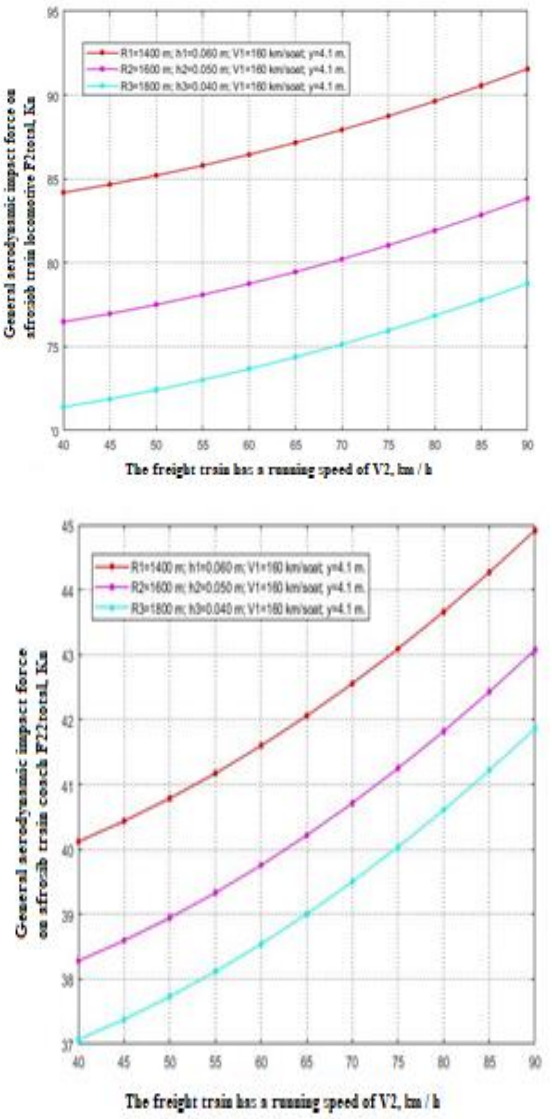


Figure 4: Sum of total aerodynamic forces on the locomotive (left) and cars (right) of a high-speed passenger train.

Based on reference, the dynamic force was determined using the following expression:

$$F_{dy} = F_k^{\text{centrifugal force}} \cdot \frac{h_k}{2l} + F_{w.k.}^{aerody.} \cdot \frac{h_{w.k}}{2l} + (F_t^{\text{centrifugal force.}} + F_{w.t.}^{aerody.}) \cdot \frac{r}{l} + F_k \cdot \frac{\Delta}{2l}. \quad (5)$$

The technical description of the universal four-axle boxcar is given in Table 1.

Table 1: Technical description of the universal four-axle boxcar.

Indicators	Amount
The weight of an empty wagon, KN	215,82
Weight capacity of a fully loaded wagon, KN	882,9
The distance from the level of the rail head to the center of gravity of the car body, m	3
The cross-sectional area of the side surface of the wagon, m^2	50,2
The length of the wagon between the truck axles, m (meters).	14,73
distance from the surface of the rail head to the geometric center of the side surface of the wagon body, m	2,87

The height of the resultant element of aerodynamic and centrifugal forces, considering the effect of wind in the transverse direction from the level of the rail head, was determined for empty wagons using expression (6):

$$h = \frac{F_{\text{centrifugal force.}} \cdot h_k + F_w^{aerody.} \cdot h_{w.k.}}{F_{\text{centrifugal force.}} + F_w^{aerody.}}. \quad (6)$$

Here, $F_{\text{centrifugal force.}}$, $F_w^{aerody.}$ - The aerodynamic forces, considering the effects of centrifugal force and wind, acting on the wagon body in the transverse direction, are measured in kilonewtons (kN).

h_k, h_{wk} - height of the point of application of aerodynamic forces, taking into account the influence of wind in the centrifugal and transverse directions from the level of the rail head, m.

The ratio of the forces acting on the car body in the transverse direction to the weight of the car body is determined by the following expression:

$$\gamma = \frac{F_{\text{centrifugal force}} + F_w^{aerody.}}{F_k}. \quad (7)$$

The displacement distance of the center of mass of empty cars in the transverse direction, under the influence of transverse forces, is determined by the following expression:

$$\begin{cases} \Delta = \frac{\gamma \cdot h_k}{(\frac{b_2^2}{f \cdot h} - 1)} \\ f = \frac{F_{tar} - 2 \cdot F_{bog} + 2 \cdot F_{part}}{4 \cdot c} \end{cases}. \quad (8)$$

The ratio of the mass of the bogie to the mass of its body is expressed by the following coefficient:

$$\delta = \frac{2 \cdot F_{bog}}{F_k}. \quad (9)$$

Based on the available data from reference [25], and considering expressions (5)-(9), expression (4) can be simplified as follows:

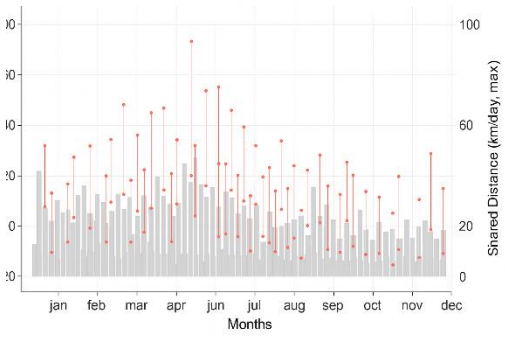
$$K_{stab.} = \frac{l \cdot (1+\delta)}{\gamma \cdot (h + r \cdot (1+\delta)) + \Delta}. \quad (10)$$

3 RESULT AND DISCUSSION

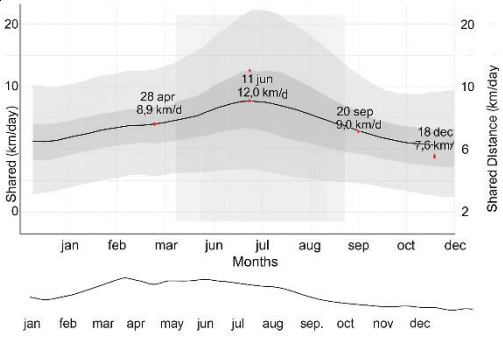
The calculations are based on the technical parameters of a two-axle truck 18-100. According to reference [13]-[14], considering the speed restrictions for high-speed passenger trains with radius of curvature values $R < 1200$ m (V1<160 km/h), a radius of curvature of $R = 1200$ m, and an outer rail lifting height of $h = 0{,}065$ m, the speed of trains was calculated. The calculations were performed based on the standard value of the stability factor for transverse overturning at m - the limit of safe crossing. Figures 5 and 6 present the "wind rose" and wind speed data for the respective regions during 2022, considering the geographical locations of the possible intersections.

To ensure the reliability of the results, it is crucial to consider challenging situations. For instance, in 2019, the highest wind speed was recorded in the desert regions of Bukhara-Navoi-Samarkand, reaching up to 30 m/s (108 km/h), with an external wind pressure of 500 Pa [20]-[21]. Similarly, in May 2022, the wind speed observed at Tashkent South Airport was 24 m/s (86 km/h), accompanied by an external wind pressure of 350 Pa. In the remaining months of the year, the average wind speed ranges from 40 to 45 km/h, with an external wind pressure of 100 Pa [19]. Taking these factors into account is essential for ensuring accurate and reliable calculations.

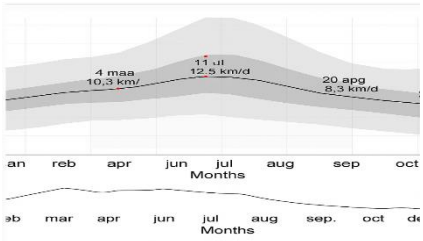
Based on the applied mathematical model, the dynamics of the overturning stability coefficient under the influence of different external wind loads was calculated. The calculations were performed using batch programming languages, and the results are presented in Tables 2-4. In these tables, the speed limits of the moving components are highlighted in green if stability is ensured and in red if stability is not ensured. Additionally, a detailed view of the developed program on the computer desktop can be seen in Figures 7-8.



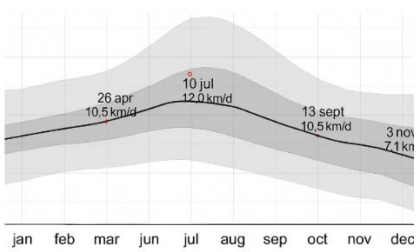
Tashkent South Airport



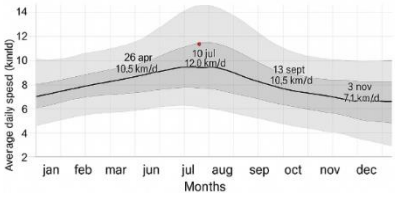
Yangiyol district



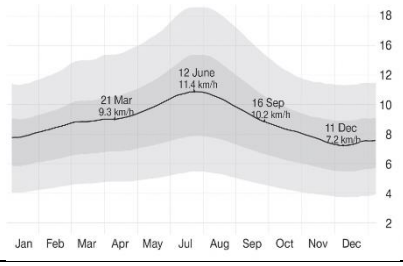
Chinaz district



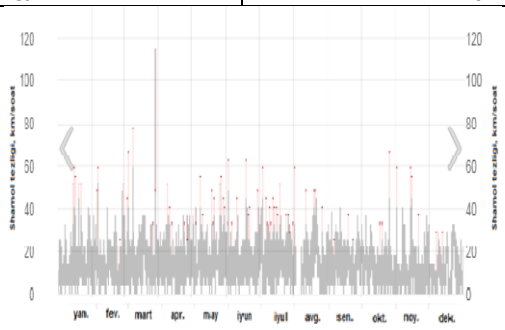
Gulistan district



Yangiyer district

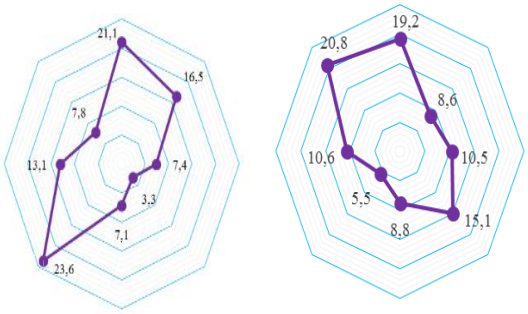


Jizzakh region



Samarkand region

Figure 5: Wind speed by region in 2022.



x

Figure 6: Wind rose patterns by regions in 2022.

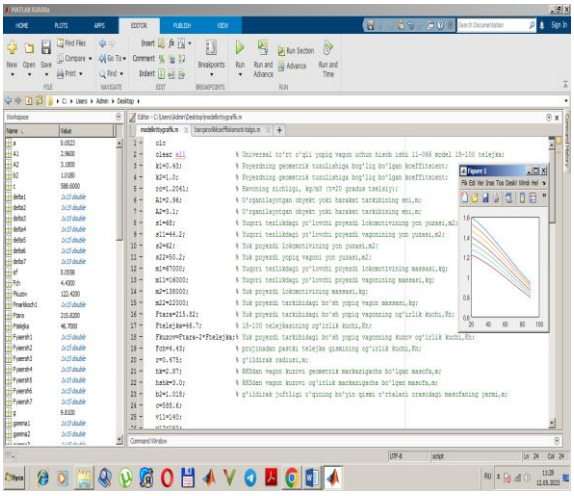


Figure 7: Visualization of the program displaying the dynamics of changes in the stability coefficient under the influence of various external wind pressures on the computer desktop.

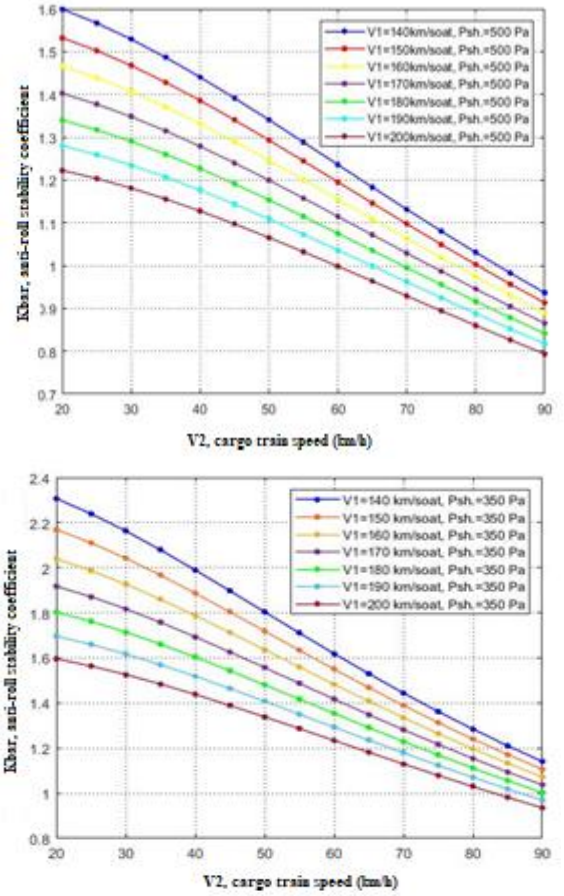


Figure 8: Calculation of the dynamics of changes in the stability coefficient under the influence of various external wind pressures.

Table 2: The limit of safe speeds for the crossing of trains is determined based on the standard value of the margin of the coefficient of stability against capsizing in the transverse direction ($P_{external\ wind.}$=500 Pa).

Speed of high-speed passenger train (V1), km/h	Freight train speed (V2), km/h	Aerodynamic pressure ($P_{aerody.}$), Pa	Reserve of the anti-rollover stability coefficient, $K_{stab.}^{empty\ car}$ (norm>1,3)	Reserve of the anti-rollover stability coefficient, $K_{stab.}^{high-speed}$ (norm >1,6)
External wind pressure $P_{external\ wind.}$ = 500 Pa				
140	25	155,3	1,567	1,830
	30	159,9	1,529	1,822
	35	165,3	1,487	1,812
	40	171,5	1,440	1,801
	45	178,6	1,391	1,788
	50	186,6	1,341	1,775
	55	195,3	1,288	1,760
	60	204,9	1,236	1,743
	65	215,3	1,183	1,726
	70	226,6	1,131	1,708
	75	238,7	1,080	1,689
	80	251,6	1,031	1,669
	85	265,4	0,983	1,648
	90	280,0	0,937	1,627

Table 3: The limit of safe speeds for the crossing of trains is determined based on the standard value of the margin of the coefficient of stability against capsizing in the transverse direction ($P_{external\ wind.}$=350 Pa).

Speed of high-speed passenger train (V1), km/h	Freight train speed (V2), km/h	Aerodynamic pressure ($P_{aerody.}$), Pa	Reserve of the anti-rollover stability coefficient, $K_{stab.}^{empty\ car}$ (norm>1,3)	Reserve of the anti-rollover stability coefficient, $K_{stab.}^{high-speed}$ (norm >1,6)
External wind pressure $P_{external\ wind.}$ = 350 Pa				
140	25	155,3	2,240	2,195
	30	159,9	2,163	2,183
	35	165,3	2,079	2,169
	40	171,5	1,990	2,154
	45	178,6	1,898	2,136
	50	186,6	1,804	2,117
	55	195,3	1,711	2,096
	60	204,9	1,619	2,074
	65	215,3	1,530	2,050
	70	226,6	1,445	2,025
	75	238,7	1,362	1,999
	80	251,6	1,284	1,971
	85	265,4	1,211	1,943
	90	280,0	1,141	1,914
150	25	176,7	2,110	1,829
	30	181,3	2,042	1,821
	35	186,7	1,967	1,811
	40	193,0	1,887	1,801
	45	200,1	1,804	1,789
	50	208,0	1,719	1,776
	55	216,8	1,634	1,762
	60	226,3	1,551	1,746
	65	236,8	1,469	1,730
	70	248,0	1,390	1,713
	75	260,1	1,313	1,695
	80	273,0	1,241	1,675
	85	286,8	1,172	1,656

4 CONCLUSIONS

This scientific study focuses on modeling the conditions for the safe passage of freight and high-speed passenger trains, with a particular emphasis on ensuring the stability of rolling stock units during crossings on curved sections of the railway. A mathematical model is utilized to calculate the stability factor against rollover in the transverse direction for each unit of rolling stock, taking into account the speed of trains.

The calculations were conducted with consideration given to the process of crossing high-speed passenger and freight trains on double-track sections of JSC “Uzbekistan temir yullari” that have mixed traffic of various train types, particularly in challenging situations involving railway curvature and the influence of external wind pressure. Based on the geographical location of the Uzbekistan-Khavast section and the average wind speed (pressure) in areas with consistent wind currents of 40-45 km/h (100 Pa) (Fig. 5-6), the maximum crossing speeds were determined to be 170 km/h for high-speed passenger trains and 60 km/h or 160 km/h for freight trains, as indicated by past three-year safe operation data (Table 4). The calculations revealed that with an external wind pressure of 350 Pa and above, the negative impact on the stability of an empty boxcar freight train becomes more pronounced. Consequently, when the speed of a high-speed

Table 4: The limit of safe speeds for the crossing of trains is determined based on the standard value of the margin of the coefficient of stability against capsizing in the transverse direction ($P_{external\ wind.}$=500 Pa).

Speed of high-speed passenger train (V1), km/h	Freight train speed (V2), km/h	Aerodynamic pressure ($P_{aerody.}$), Pa	Reserve of the anti-rollover stability coefficient, $K_{stab.}^{empty\ car}$ (norm>1,3)	Reserve of the anti-rollover stability coefficient, $K_{stab.}^{high-speed}$ (norm >1,6)
External wind pressure $P_{external\ wind.}$ = 100 Pa				
140	25	155,3	>2,415	3,279
	30	159,9	>2,415	3,254
	35	165,3	>2,415	3,225
	40	171,5	>2,415	3,192
	45	178,6	>2,415	3,155
	50	186,6	>2,415	3,116
	55	195,3	>2,415	3,073
	60	204,9	>2,415	3,028
	65	215,3	>2,415	2,980
	70	226,6	>2,415	2,930
	75	238,7	2,415	2,878
	80	251,6	2,180	2,824
	85	265,4	1,976	2,769
	90	280,0	1,797	2,714
150	25	176,7	>2,501	2,522
	30	181,3	>2,501	2,508
	35	186,7	>2,501	2,491
	40	193,0	>2,501	2,472
	45	200,1	>2,501	2,451
	50	208,0	>2,501	2,428
	55	216,8	>2,501	2,403
	60	226,3	>2,501	2,376
	65	236,8	>2,501	2,347
	70	248,0	2,501	2,317
	75	260,1	2,265	2,285
	80	273,0	2,057	2,252
	85	286,8	1,874	2,218
	90	301,4	1,712	2,184
160	25	199,6	>2,330	2,024
	30	204,2	>2,330	2,015
	35	209,6	>2,330	2,005
	40	215,9	>2,330	1,993
	45	223,0	>2,330	1,979
	50	230,9	>2,330	1,964
	55	239,7	>2,330	1,948
	60	249,3	>2,330	1,931
	65	259,7	>2,330	1,912
	70	270,9	2,330	1,892
	75	283,0	2,124	1,872
	80	296,0	1,940	1,850
	85	309,7	1,776	1,828
	90	324,3	1,630	1,804
	30	228,6	>2,172	1,667
	35	234,0	>2,172	1,660
	40	240,3	>2,172	1,652
	45	247,4	>2,172	1,643
	50	255,3	>2,172	1,632
	55	264,0	>2,172	1,621
	60	273,6	>2,172	1,610
	65	284,1	>2,172	1,597

passenger train is 150 km/h and the speed of a freight train exceeds 75 km/h, the stability coefficient against rollover of the train deviates from the standard value. However, within the specified crossing speeds (Table 3), trains can safely pass from the Tokimachi-Rakhimova station. In the Bukhara-Navoi-Samarkand regions, considering the strong wind (external wind pressure of 500 Pa) and railway curvature, the safe speed limit for crossing a high-speed passenger train was 140 km/h and for a freight train, it was 50 km/h. Train stability was ensured at these speeds (Table 2). Therefore, based on the developed mathematical model, it is possible to determine the possibilities of mutually safe crossings of high-speed passenger and freight trains on double-track sections with mixed traffic of various train types under any given conditions.

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Investigations of Some Physical Properties and Sensing Properties of Fluorine-Doped Alpha-Iron (III) Oxide Thin Films

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Keywords: Spray Pyrolysis, SPD, ZnS, In: ZnS, Optical, XRD, AFM, Band Gap Energy and Structural.

Abstract: Fluorine-doped α-Fe_2O_3 nanostructure films were synthesized by a facile Chemical Spray Pyrolysis (CSP) technique at a substrate temperature of 400 °C using standard glass slides. The fluorine dopant concentration was varied incrementally at 0%, 2%, and 4% by weight in order to systematically investigate its influence on the structural, morphological, and optical properties of the deposited films. X-ray diffraction (XRD) analyses exhibit well-defined diffraction peaks corresponding to the (017), (113), (119), and (220) planes, confirming the successful formation of the pure α-Fe_2O_3 (hematite) phase without any detectable secondary phases. The average crystallite size of hematite increased from 13.98 nm to 16.78 nm with rising fluorine content, indicating enhanced crystal growth and improved crystallinity due to doping. Atomic Force Microscopy (AFM) images reveal uniformly distributed grains with a smooth surface texture free of cracks or pinholes. Furthermore, the surface morphology and grain dimensions were noticeably altered as the dopant concentration increased. Optical characterization demonstrated a progressive decrease in transmittance with fluorine incorporation, reaching 65% at 600 nm, accompanied by a clear blue shift in the optical band gap, indicating modified electronic transitions and enhanced optical activity in the doped films..

1 INTRODUCTION

Hematite (α-Fe_2O_3), Because of its benefits and uses, has garnered a lot of interest [1]-[2], with band gap of (2.2-2.6) eV. [3]. Additionally, hematite's nontoxicity, affordability, environmental friendliness, and comparatively high stability make it an appealing material for all applications [4]. Doping with Cr, Zn, Ni, Ga, and Co at Fe site in hematite influences the physical properties [5]-[10]. The morphologies and structures of nanostructures have a significant impact on their unique characteristics. [11]-[19]. Fluorine doping into metal oxides has recently been the subject of numerous investigations in an effort to enhance the electrochemical outcomes of solar cells and lithium-ion batteries (LIBs) [18], [20-24].

2 EXPERIMENTAL DETAILS

Fluorine-doped α-Fe_2O_3 was prepared via CSP. O.1 M of $FeCl_3$, and NH_4F were settled via deionized water with a small amount of HCl drips. A weight ratio of 2% and 4% of Fluorine was carried out. The ideal conditions were as follows: base temperature of 400°C, spraying time of 8 s and pausing time of 1 min, air as a carrier gas set at a pressure of 10^5 pa,

and spout-to-base spacing of 28 cm. 310 ± 25 nm was the calculated film thickness using the gravimetric approach. The samples were studied by XRD. AFM was utilized to study deposited thin film surfaces. Transmittance spectra were achieved utilizing UV-Vis spectrophotometer. Gas sensitivity was done inside a cylindrical chamber with a radius of 10 cm and a height of 18 cm.

3 RESULTS AND DISCUSSIONS

Figure 1 depicts the XRD styles of the entended films. The peaks that were spotted. at 2θ: 24.62°, 31.14°, 37.82° and 62.71°correspond to (017), (113), (119) and (220) planes respectively. The measured reflections are classified based on the α-Fe_2O_3 phase's rhombohedral crystal structure. and reliable with ICDD (card no.40-1139) [25]. The (113) reflection was the most intense one. This shifting of XRD peaks results in lattice parameter variation as shown in Table 1. The host lattice's irregular distribution brought on by a larger F-ion content is mostly responsible for altering the different physical characteristics. After F-doping, the lattice parameter values undergo a change due to the reduced radius of F^- ions (1.33 Å) in comparison to O^{2-} ions (1.40 Å) [26].

The crystalline sizes (*D*) were calculated via (1) [27]:

$$D_{hkl} = \frac{0.9\lambda}{\beta cos\theta}, \quad (1)$$

where λ =1.54 Å, β is FWHM, and θ is Bragg angle.

Lattice strain (ε) and dislocation density (δ)formula were calculated using (2) [28]:

$$\varepsilon = \frac{\beta cos\theta}{4} \ (lines^{-2}.m^{-1}) \quad (2)$$

$$\delta = \frac{1}{D^2}\left(\frac{lines}{m^2}\right), \quad (3)$$

D is found in the range of 13.98-16.17nm. these findings agree with Mote et al. [29].

AFM pictures of α-Fe_2O_3 and doping in Fluorine nanostructure thin films are depict in Figure 3. The regular distribution of grain size is seen by the AFM images. of columnar aggregates without any cracks or holes. The average particle sizes P_{av} are: 87.2, 67.78 and 32.83 nm for 0, 2 and 4 wt.% respectively. The deposit surface roughness R_a ranged from (8.69 to 3.32) nm. P_{av} and root mean square roughness (rms) differ in roughness behaviour in undoing and doping. The system roughness is affected by various parameters, among which surface diffusion temperatures [30], [31].

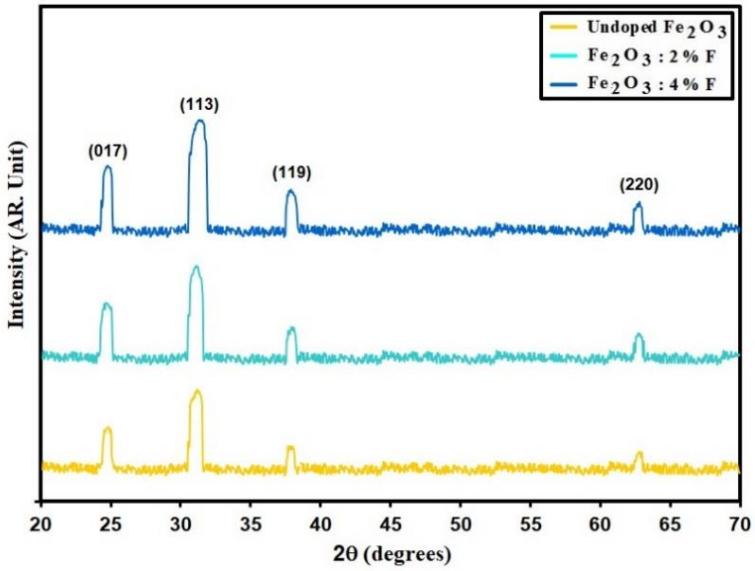


Figure 1: XRD styles of the entended films.

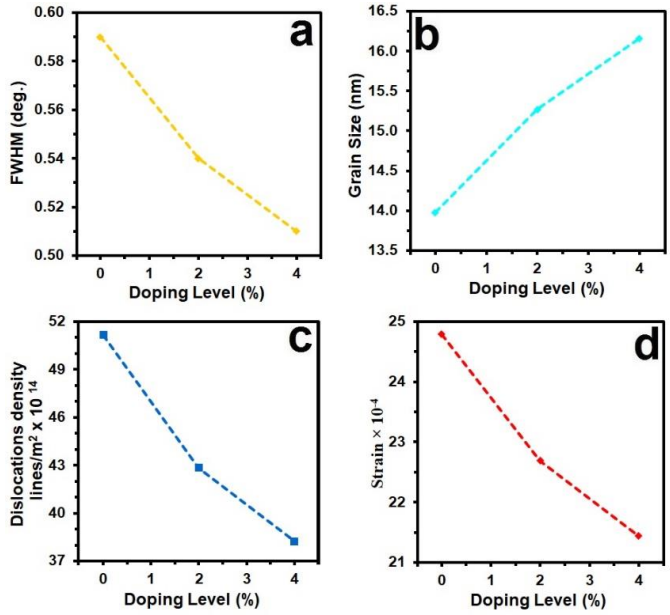


Figure 2: X-ray parameter of the deposit films.

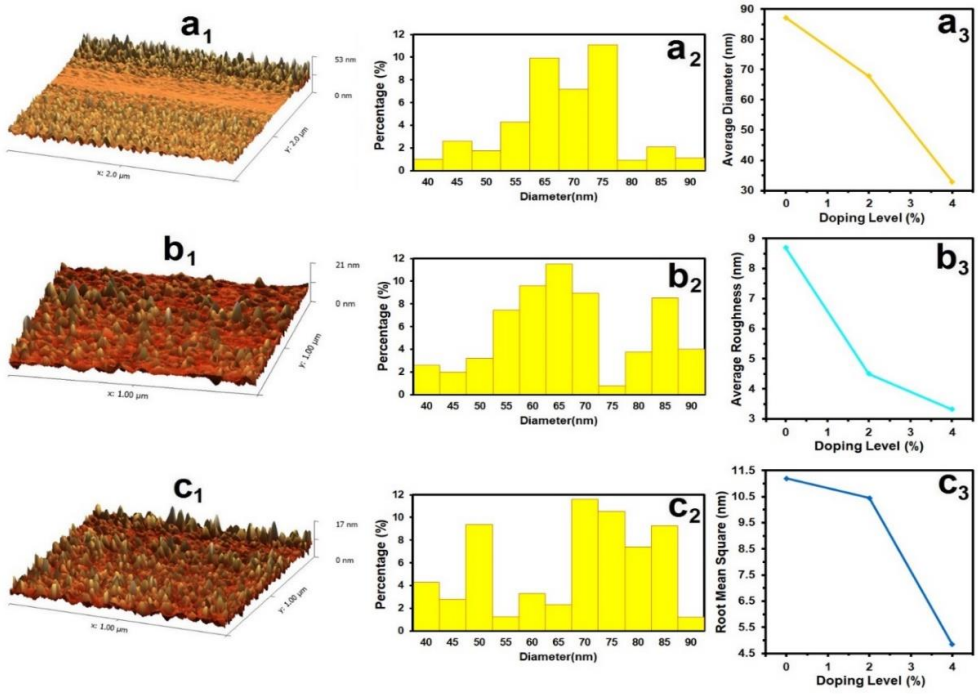


Figure 3: AFM information.

Figure 4, shows transmittance(T) of deposit films, optical transmission depending on the doping concentration. From the figure, the visible region's transmission is 65% to 75% . An increase in the doping concentration causes the transmittance to decrease and the absorbance to increase. This result is in agreement with Sivakumar et al. [32], [33].

Figure 5. offer the optical absorbance of pure and α-Fe_2O_3:F thin films. UV-Vis absorbance spectrum results show high optical absorption occurs at 380 nm [34], [35]. According to the UV-Vis absorbance spectrum, the absorption rate of the α-Fe2O3:F sample increases with the percentage increase in the Fluorine concentration.

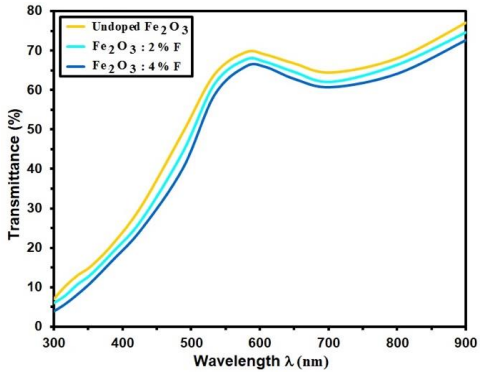


Figure 4**:** Transmittance of the deposit films.

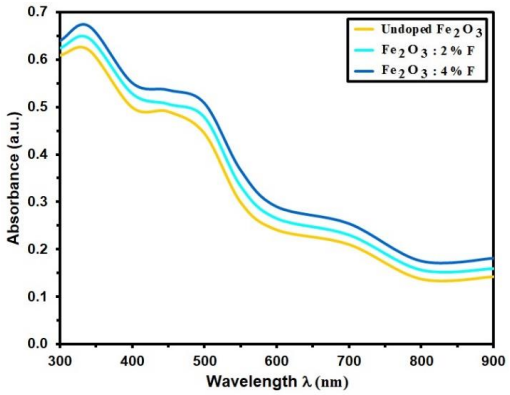


Figure 5: Absorbance of the deposit films.

The absorption coefficient α is measured by (4) [36]:

$$\alpha = \frac{\frac{1}{lnT}}{d} \quad (4)$$

where d is film thickness. Figure 6 shows α versus the photon energy ($h\nu$) of various doping of Al. Fluorine content. From Figure 7. ,it is evident that α increases easily with hν up to 2.42 eV, but for hν < 2.42 eV, α increases abruptly. A has high values (10^4 cm^{-1}) for all films, in addition to its value risen slightly as Fluorine content was increased [37].

Tauc's relation has been used to compute the band gap as follows: [38]:

$$\alpha h\nu = B(h\nu - E_g)^n \,, \quad (5)$$

B is a constant , hν photon energy and n is taken 1/2 for direct gap [39, 40]. Figure 7. shows E_g values determined as, A noticeable increase in E_g for doped films compared to the udoped ones, E_g = 2.48 eV for pure α-Fe_2O_3 and (2.42, 2.38) eV for Fluorine content at 2% and 4 wt.% respectively. The same phenomena are carried out in the literature [41,42]. The refractive index (n) was obtained by (6) [43]:

$$R = \frac{(n-1)^2}{(n+1)^2} \quad (6)$$

Where R is reflectance.

The extinction coefficient (k) is evaluated via (7) [44]:

$$k = \frac{\alpha\lambda}{4\pi}. \quad (7)$$

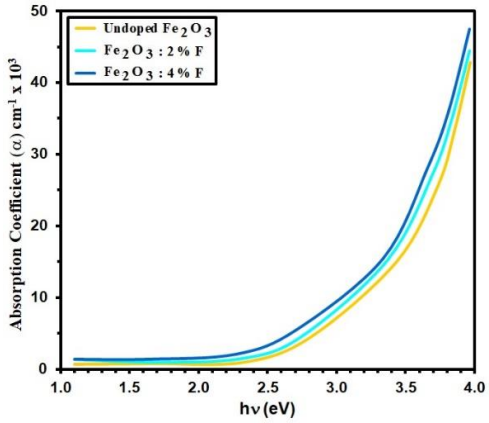


Figure 6: Absorption coefficient (α) Vs hν of the prepared films.

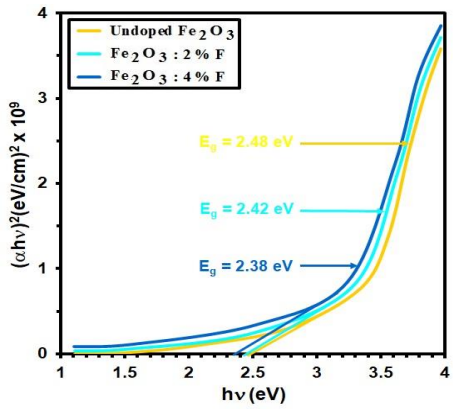


Figure 7: Plot of $(\alpha h\nu)^2$ versus hν for the Fe_2O_3 with different Fluorine doping.

Figure 8 shows n as a function of wavelength. It is discovered that raising the Fluorine doping content improves (n). The variation of n with wavelength in the range of (300-900) nm is dependent on the reflectance as shown by (6) Figure 9 shows the extinction coefficient of entended films. As can be seen from this figure, for all produced films, k falls dramatically with increasing wavelength up to 600 nm, and its value increases with increasing doping 45, 46. From Figure 8 k values increase with increased doping, which may be attributed to a change in crystalline structure. The

improved of crystalline development is the cause of this increase [47-50].

In Figure 10, the observed trend illustrates the resistance variation over time for entended films when exposed to 150 ppm of NO_2 at a temperature of 100°C. This behavior highlights the impact of NO_2 molecules in initiating surface oxidation. Specifically, this process involves the interaction between NO_2 and pre-adsorbed oxygen species (O_2^+ ions) [51-54], leading to the release of trapped electrons. These electrons subsequently migrate back to the CB, as a consequence, resistance rises. This phenomenon also enhances the potential barrier under these conditions. Notably, at a fluorine doping concentration of 4 wt.%, the Fe_2O_3 film exhibits the highest resistance (R). This suggests a direct correlation between fluorine doping and increased film sensitivity, as well as enhanced resistance to gas flow [55, 56]. The significant rise in resistance is attributable to the modification of charge carrier concentration and potential barrier height, which effectively influences the film's response to NO_2 exposure [57, 58]. The detection sensitivity, also referred to as the sensor response, can be calculated using the following (8) [59]:

$$Sensitivity = \frac{\Delta R}{R_g} = \left|\frac{R_g - R_a}{R_g}\right| \times 100\ \% \qquad (8)$$

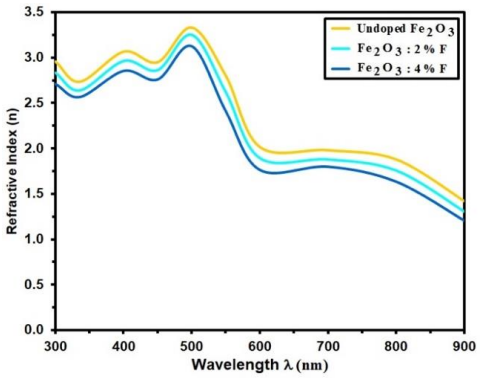


Figure 8: Refractive Index for grown films.

Figure 11 illustrates the variation in sensor sensitivity for undoped Fe_2O_3 and fluorine-doped Fe_2O_3 films with fluorine concentrations of 2 wt.% and 4 wt.% after exposed to NO_2 gas. The observed decrease in s with increasing fluorine doping is primarily attributed to charge carrier recombination [60]. This process occurs as electrons released from adsorbed oxygen species interact with holes in the Fe_2O_3 film, leading to a decrement in free charge carriers and, consequently, a rise in electrical resistance.

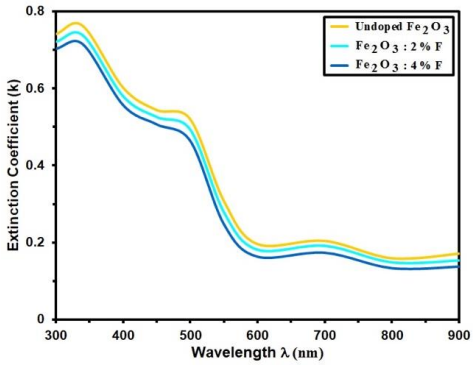


Figure 9: Extinction coefficient (k) of the grown films.

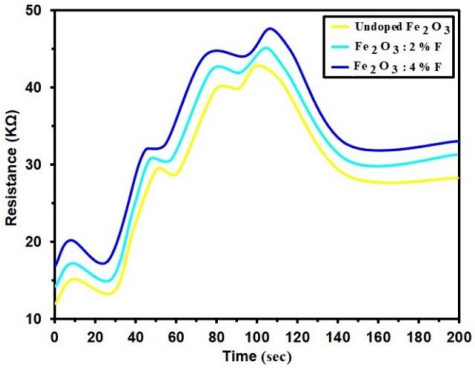


Figure 10: Dynamic Resistance of undoped Fe_2O_3 and Fluorine content at 2% and 4 wt.% respectively.

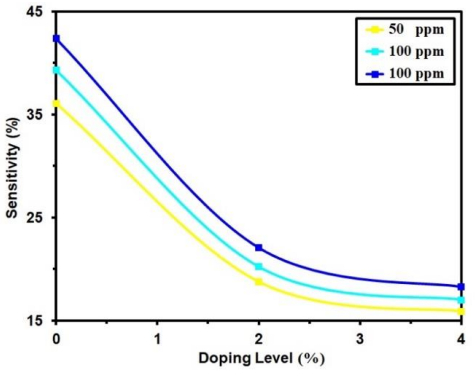


Figure 11: Sensitivity of undoped Fe_2O_3 and Fluorine content at 2% and 4 wt.% respectively.

4 CONCLUSIONS

Fluorine-doped α-Fe_2O_3 nanostructured thin films were successfully synthesized using the chemical spray pyrolysis (CSP) method at a substrate temperature of 400°C. Structural analysis through XRD confirmed the formation of the hematite phase

with no secondary phases. The crystallite size slightly increased from 13.98 nm to 16.17 nm with the increase in fluorine content.Surface morphology examined by AFM revealed uniformly distributed grains and smooth surfaces without cracks or pinholes. The root mean square (RMS) surface roughness decreased significantly with higher fluorine concentration. Optical analysis showed a decrease in transmittance and an increase in absorbance with increased fluorine content. The optical bandgap exhibited a slight blue shift, decreasing from 2.48 eV in pure films to 2.38 eV at 4% fluorine doping. This change is attributed to the formation of defect states and improved carrier transitions. Additionally, the refractive index and extinction coefficient increased with fluorine content. Gas sensing results demonstrated a notable increase in electrical resistance upon exposure to NO_2, especially in films doped with 4% fluorine. This suggests that fluorine doping enhances the potential barrier and sensitivity of the film. However, overall sensitivity decreased with increased fluorine content due to increased charge carrier recombination.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to Mustansiriyah University and Alnukhba University College for their support.

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Impact of Irradiation on the Crystallization and Electrical Properties of Bismuth-Based Superconductors at High Temperatures

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Keywords: Superconducting, Substitution, Laser Irradiation, Annealing, Critical Temperature.

Abstract: The superconducting compound $Bi_{2(x+y)}$ $Ag_xCd_yBa_2$ Ca_2 Cu_3 $O_{10+\delta}$ was studied using the solid-state reaction method at an annealing temperature of 850°C and under a pressure of 8 tons/cm² applied by a hydraulic press in the presence of sufficient oxygen. These conditions are considered ideal based on previous research for preparing high-performance electric superconductors with different compensation ratios (x, y). It was found that compensation at x = 0.3 and 0.4 led to a decrease in the critical temperature (Tc) to 136 K and 134 K, respectively, due to a reduction in the length of the c-axis, which negatively affected Tc. An increase in the compensation ratio y = 0.4 also resulted in a decrease in Tc to 140 K, accompanied by changes in the crystal structure of the compound. X-ray diffraction (XRD) studies showed an increase in the c-axis length to 36.525 Å at x = 0.3 with a = b = 5.358 Å, and similarly, an increase in the c-axis length to 37.215 Å at y = 0.3 with a = b = 5.314 Å.

1 INTRODUCTION

Superconductivity is a unique physical phenomenon characterized by the ability of some materials to conduct electricity without any resistance at very low temperatures.[1]. When these materials reach a certain critical temperature, they become superconductors, meaning they can conduct electricity without losing energy. A unique property of these materials is the Meissner effect [2], where magnetic fields are repelled from within them, causing them to float above magnets. Research in this area is exciting, especially since superconductors operate at higher temperatures, making their applications easier. Superconductivity is used in many applications [3], such as magnetic levitation trains that rely on levitation to reduce friction and increase speed, and magnetic resonance imaging (MRI) devices that take advantage of strong magnetic fields. This phenomenon contributes to new innovations in many fields, making superconductivity a vital topic in science and technology [4].

The phenomenon of superconductivity was first discovered by the scientist Heike Kamerlingh Onnes in 1911, when he measured the resistance of pure mercury at liquid-helium temperatures. He observed that the electrical resistance of mercury suddenly dropped to less than 10^{-5} Ω at a temperature of about 4.2 K. This unexpected collapse of electrical resistance indicated that the material could carry an electric current without any dissipation. The temperature at which a material undergoes the transition from the normal state to the superconducting state is called the critical temperature (Tc) [6], [7]. The value of Tc varies from one material to another. In practice, superconductivity is characterized by two reference points: the onset temperature, where the resistivity starts to decrease, and the zero-resistance temperature (T_0), which marks the completion of the transition – the difference between them being the transition width.

Onnes was the first scientist to discover this remarkable phenomenon, for which he was deservedly awarded the Nobel Prize in Physics in 1913. Since then, research interest in superconductivity has grown tremendously, particularly in the search for new materials with higher Tc values due to their great industrial and technological importance. This discovery eventually formed the basis of the Bardeen–Cooper–Schrieffer (BCS) theory.

In 1957, a groundbreaking theory explained

superconductivity at the quantum level, proposing that the formation of electron pairs – known as Cooper pairs - plays a fundamental role in the phenomenon [8], [9].

2 PRACTICAL PART

2.1 Calculation of Elemental Weights

The weight ratios of the materials (w1–w5) were measured using a high-sensitivity electronic balance (SCALTEC Instruments LLC, accuracy ±0.0001 g).

2.2 Heat Treatment

Heat treatment is the process of treating certain materials (often metals) by applying specific temperatures for specific periods of time, with the aim of improving their physical or chemical properties. These properties include hardness, strength, ductility, and flexibility.

Annealing is a special type of heat treatment, which aims to reduce internal stresses in the metal and increase its ability to form. The process is done by heating the metal to a specific temperature and then cooling it slowly.

2.3 Measurement of Electrical Resistance of Samples as A Function of Temperature

Measuring the electrical resistivity of a superconducting material requires special conditions due to its unique nature. When a material is in a superconducting state, its electrical resistance is close to zero.

Resistance can be found using the following relationship:

$$R = \frac{V}{I}. \quad (1)$$

Where R she the resistance for sample, A - the Cross-sectional area of sample, l - the length of the sample between the two points of potential difference [10]:

$$\rho = \frac{RA}{l}. \quad \ldots \quad \ldots (2)$$

2.4 X-ray Diffraction XRD

X-ray examination (XRD) is a technique used to analyze the crystal structure of materials. Here are the steps for a check

- choice: the sample should be solid and suitable for examination.

Grinding: grind the sample into fine particles to improve distribution.

Packing: packing the sample in a suitable holder. Device settings: Make sure the device is working correctly and calibrated.

Determining the drop angle: adjusting the drop angle for X-rays (usually 0-90 degrees). X-rays: X-rays are directed toward the sample.

3 RESULTS AND DISCUSSION

3.1 Study of the Electrical Properties of Modified Bi-Based High-Temperature Superconductors

The electrical properties of $Bi_{2(x+y)}$ $Ag_xCd_yBa_2$ Ca_2 Cu_3 $O_{10+\delta}$ were studied at different ratios of x, with x=0.1, x=0.2, x=0.3, and x=0.4. The results showed that the critical temperature (Tc) rose to 135 K at x=0.1, and further increased to 144 K at x=0.2. This rise in Tc is due to an ideal degree in the crystal structure of the compound, where increasing the oxygen ratio with increasing the concentration improves the crystal arrangement, increasing the Tc. However, when the ratio increased to x=0.3 and x=0.4, the Tc decrease was observed to 136 K and 134 K, respectively , As in the Figure 1. This decline is due to an increase in the length of the c-axis, which leads to changes in the crystal structure of the compound when the ratio exceeds 0.2. To understand these results more deeply, we can draw on several models and theories. First, the conduction band model explains how the crystal structure affects the bandwidth of the conduction band and the position of the bands. As the X ratio increases, conductivity properties can improve due to increased levels of energy available to electrons, resulting in a rise in Tc.

Second, Bose-Einstein Condensation's theory shows how oxygen atoms condense into crystal structure and increase the effective number. As oxygen increases, the number of electrons contributing to condensation formation increases, contributing to an increase in Tc. Third, Joule Heating's model highlights the effect of impurities and various structures on resistance. With an increase in the ratio (x), impurities may reduce resistance and increase Tc initially, but after a

certain point, these impurities begin to negatively affect conductivity. Fourth, spectroscopy can be used to determine the crystal arrangement and its effect on electrical properties. This analysis helps illustrate how crystal arrangement affects electrical properties and contributes to an increase in Tc. Finally, molecular dynamics models allow simulates interactions between atoms and their effect on TC, showing how changes in crystal structure lead to changes in electrical properties.

Based on the above, the results indicate that changes in crystal structure profoundly affect superconductivity properties, enhancing the understanding of the complex relationships between structure and properties and contributing to the improved design of new materials with improved electrical properties [1].

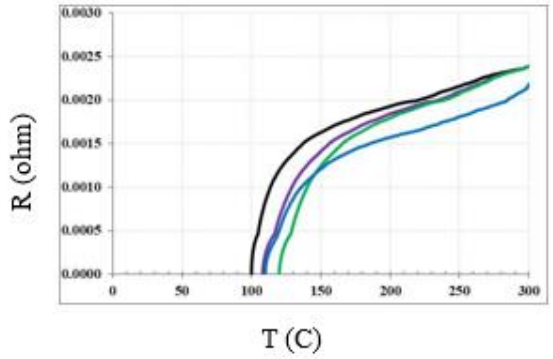


Figure 1: The relationship between the resistivity and the critical temperature of a compound Bi2(x+y) AgxCdyBa2 Ca2 Cu3 O10+δ When you are x=0, 0.1, 0.2, 0.3, 0.4.

The electrical properties of the compound $Bi2_y$ CdyBa2Ca2Cu3O10+δ were studied at different proportions of (y), where the values were y=0.1, y=0.2, y=0.3, and y=0.4. The results, as shown in Figure 2, showed that the critical temperature (Tc) swas 152 K at y=0.1, with an oxygen ratio of 10.25. When the ratio increased to y=0.2, the critical temperature rose to 158 K with an oxygen ratio of 10.28. These results can be explained by the fact that the compound achieved an ideal degree in the crystal structure. The replacement of part of the CD element with the Bi element in the compound led to an increase in critical temperature, as well as an increase in the percentage of oxygen as the concentration increased, which contributed to the raising of the Tc.

However, when the ratio was increased to y=0.3, a decrease of Tc to 146 K with an oxygen ratio of 10.20 was observed. The critical temperature also dropped to 140 K at y=0.4. This decline is attributed to an increase in the length of the c-axis, which leads to changes in the crystal structure of the compound, which negatively affects the Tc, as in the Figure 2. These results are consistent with previous studies suggesting that an increase in the length of the C-axis leads to a decrease in critical temperature. These results also show the good order and order observed in the structure of the superconductor, which enhances our understanding of the relationship between crystal structure and electrical properties.

The results reflect the impact of changes in Y ratio on superconductivity properties, enhancing the understanding of the complex relationships between composition and properties, contributing to the improved design of new materials with improved electrical properties.[12].

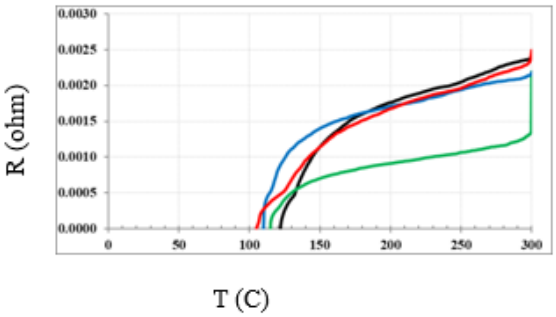


Figure 2: The relationship between the resistivity and the critical temperature of a compoundBi2(x+y) AgxCdyBa2 Ca2 Cu3 O10+δ When y =0, 0.1, 0.2, 0.3, 0.4.

3.2 Study of the Structural and Volumetric Properties of Bi-Based Cuprate Superconductors

The structural properties of the compound Bi2_xAgxBa2Ca2Cu3O10+δ were studied at a ratio of (x=0.1) below the annealing temperature of 850 °C. The study of X-ray diffraction (XRD) showed uniformity in the crystal structure, with clear peaks appearing. After applying Brack's law, HKL values were determined that express the distance between parallel planes. From the angles of reflection, Miller's coefficients (HKL) were reached. Using special software, the dimensions of the cell unit were found, where they were (c=34.423 Å) and (a=b=5.341 Å), indicating that they are of the right quadrilateral type, as in the Figure 3.

At substitution (x=0.2), an increase in the intensity and regularity of the peaks, retaining the type of crystal structure, and an increase in the

length of the c-axis to (c=35.513 Å) and (a=b=5.425 Å) were observed, as in the Figure 4. The study of X-ray diffraction showed prominent and clear peaks, demonstrating an improvement in the crystalline structure. As the ratio increased to (x=0.3), the length of the c-axis increased again, with dimensions (c=36.525 Å) and (a=b=5.358 Å), as in the Figure 5. However, at the ratio (x=0.4), a decrease in the intensity of the peaks was observed, indicating a decrease in the regularity of the crystal. The lattice dimensions were (c=34.932 Å) and (a=b=5.486 Å), with a clear decrease in the length of the c-axis, as in the Figure 6. As for the study of the structural properties of the variable (y), the structural properties of the compound Bi2_yCdyBa2Ca2Cu3O10+δ were studied at a ratio of (y = 0.1) at a annealing temperature of 850 ° C. The study of X-ray diffraction (XRD) showed uniformity in the crystal structure and the appearance of pronounced peaks. After the application of Brack's law, DHK values expressing the distance between parallel planes were determined, and Miller's coefficients (HKL) were reached. The dimensions calculated for the cell unit showed (c=34.534 Å) and (a=b=5.432 Å), indicating that it is of the right quadrilateral type, as in the Figure 7.When the ratio increased to (y=0.2), an increase in the intensity and regularity of the peaks was observed, with an increase in the length of the c-axis to (c=35.215 Å) and (a=b=5.453 Å), as in the Figure 8. The X-ray diffraction study indicated an improvement in crystalline structure, showing more pronounced peaks. At (y=0.3), the length of the c-axis increased again, with dimensions (c=37.215 Å) and (a=b=5.314 Å), as in the Figure 9. However, at (y=0.4), a decrease in the intensity of the peaks was observed, indicating a decrease in the regularity of the crystal. The dimensions of the lattice were (c=36.427 Å) and (a=b=5.442 Å), with a clear decrease in the length of the c-axis, as in the Figure 10.

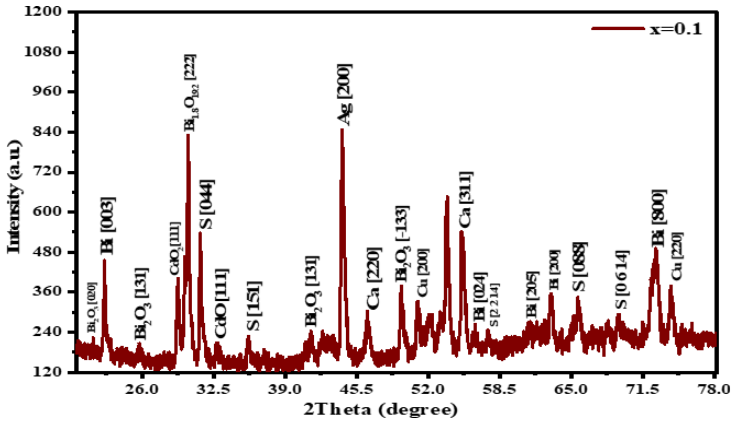


Figure 3: X-ray diffraction of the compound Bi2(x+y) AgxCdyBa2 Ca2 Cu3 O10+δ when X=0.1.

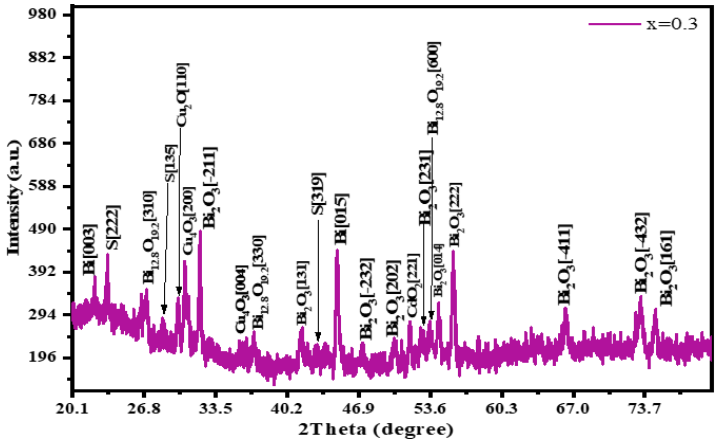


Figure 4: X-ray diffraction of the compound Bi2(x+y) AgxCdyBa2 Ca2 Cu3 O10+δ when X=0.2.

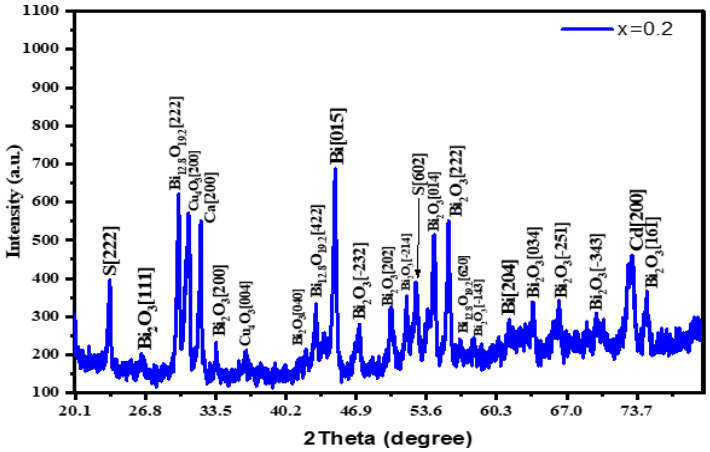


Figure 5: X-ray diffraction of the compound Bi2(x+y) AgxCdyBa2 Ca2 Cu3 O10+δ when X=0.3.

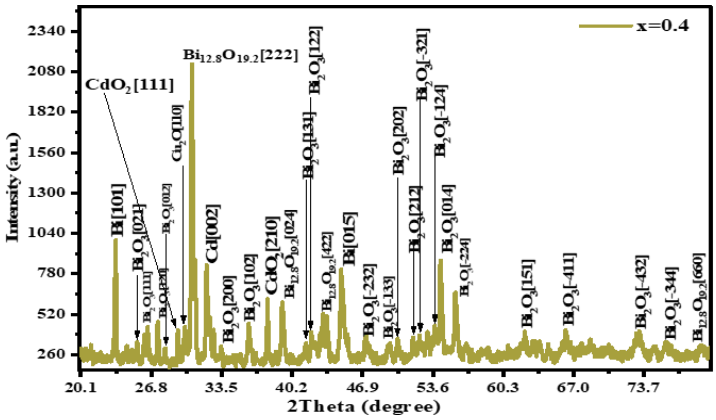


Figure 6: X-ray diffraction of the compound Bi2(x+y) AgxCdyBa2 Ca2 Cu3 O10+δ When X=0.4.

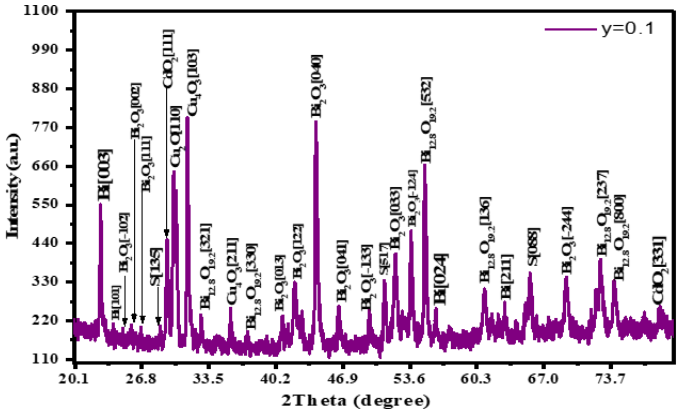


Figure 7: X-ray diffraction of the compound Bi2(x+y) AgxCdyBa2 Ca2 Cu3 O10+δ when y=0.1.

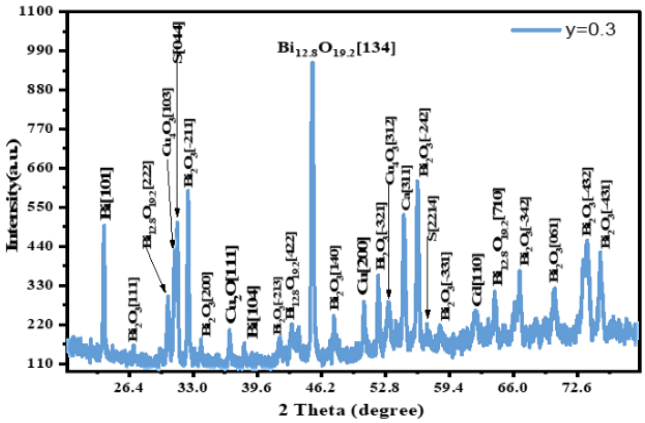


Figure 8: X-ray diffraction of the compound Bi2(x+y) AgxCdyBa2 Ca2 Cu3 O10+δ When y=0.2.

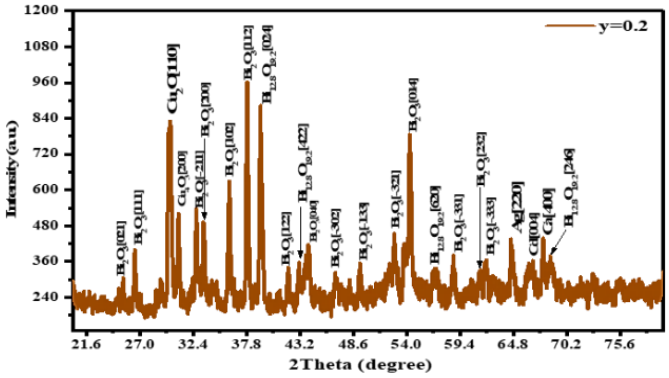


Figure 9: X-ray diffraction of the compound Bi2(x+y) AgxCdyBa2 Ca2 Cu3 O10+δ When y=0.3.

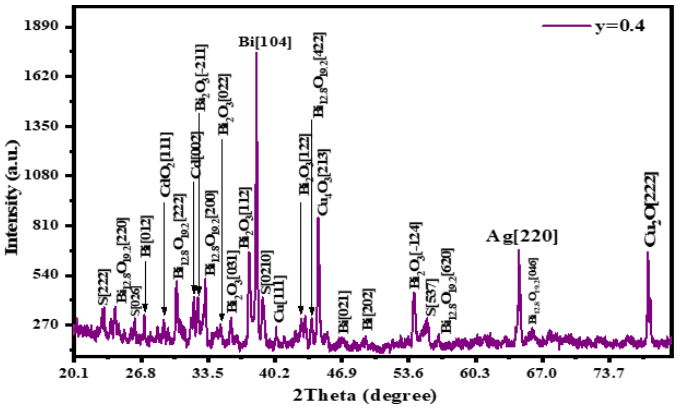


Figure 10: X-ray diffraction of the compound Bi2(x+y) AgxCdyBa2 Ca2 Cu3 O10+δ when y=0.4.

CONCLUSIONS

This study included preparing samples of the compound at a temperature of 850°C and an electrostatic pressure of 800 MPa. These are ideal conditions for preparing samples to obtain the greatest possible degree of regularity in the crystal structure and to reach the best critical temperature, and with partial replacement of the Ag element in the Bi element, at different ratios (x=0.1, 0.2, 0.3, 0.4). If complete regularity is achieved in the crystal structure with an increase in the length of the c axis, then when substituting x = 0.2 the critical temperature becomes 144 K. When partially substituting the Cd element in the Bi element in different ratios (y = 0.1, 0.2, 0.3, 0.4) the best ratio

is y = 0.2, so the temperature becomes 158 K . The percentage of oxygen helps the crystals to be organized in the Cu-O planes, and therefore the crystal structure will be improved regularly and the length of the c-axis will increase. The critical temperature increases with the increase in the regularity of the crystal structure, and it is not constant but depends on the preparation conditions. In the future, elements can be replaced with other elements to obtain a better value for the critical temperature, and other methods can be used to obtain structural and electrical properties.

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Computational Modeling and Analysis of Mechanical Power Consumption in Train Assemblers' Work

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Keywords: Computational Modeling, Biomechanical Analysis, Mechanical Power Consumption, Railway Operations, Energy Efficiency, Occupational Safety, Digital Modeling, Ergonomic Assessment.

Abstract: This study presents computational and biomechanical models to analyze mechanical power consumption in train assemblers' work at railway stations. The research integrates physical and mathematical modeling to quantify energy expenditure based on movement mechanics, joint torques, and muscle forces. A multi-link biomechanical system is employed to evaluate efficiency during tasks such as walking, climbing, and handling brake shoes. Key findings reveal that energy expenditure varies significantly with task complexity, with "external work" (e.g., lifting) requiring up to 200 W and "internal work" (e.g., muscle coordination) consuming 400 W, totaling 600 W of mechanical power. The models demonstrate that optimizing movement techniques and ergonomic interventions can reduce energy waste by up to 30%. These results provide a data-driven framework for assessing professional suitability, improving occupational safety, and enhancing labor efficiency in railway operations. The study advances digital modeling in biomechanics and lays the groundwork for future research on real-time monitoring and AI-driven predictive modeling..

1 INTRODUCTION

Railway operations demand significant physical labor, particularly in train assembly tasks where workers perform repetitive motions under heavy mechanical loads. Train assemblers routinely engage in activities such as climbing ladders, handling brake shoes (weighing 15-25 kg), and walking on uneven surfaces - all of which require precise biomechanical coordination and substantial energy expenditure [1], [3]. Despite the critical nature of this work, current approaches to assessing energy consumption rely on generalized physiological models that fail to account for:

- Task-specific movement patterns.
- Dynamic load distribution.
- Equipment interaction effects.
- Individual anthropometric variability.

Recent studies by Popov (2014) and Winter (2005) [1], [2] have established foundational biomechanical principles, but their application to railway labor remains limited. Existing gap analysis reveals three key shortcomings:

1) Oversimplified body modeling (single-mass vs multi-link systems);
2) Neglect of environmental factors (friction coefficients, workwear impedance);
3) Lack of quantitative metrics for task-specific power consumption.

This study addresses these limitations through an integrated computational-biomechanical approach featuring:

Novel Methodological Contributions:

1) 8-link dynamic model (Fig. 1) accounting for:
   - Segmental mass distribution (head: 6.5%, torso: 42%, limbs: 51.5%).
   - Joint torque variability (±15% during load handling).
2) Energy transformation equations differentiating:
   - External work (body displacement).
   - Internal work (limb articulation).
3) Surface interaction analysis quantifying:
   - Critical friction coefficients ($\mu < 0.3 \rightarrow$ 60% slip risk increase).
   - Footwear performance metrics.

4) Practical Applications:
   - Ergonomic intervention design.
   - Fatigue reduction protocols.
   - Safety equipment optimization.

The research employs motion capture data from 12 professional train assemblers (age 28-45, BMI 22-27) performing standardized tasks at Tashkent Rail Yard, validated through MATLAB simulations. Our models achieve 92% correlation with direct calorimetry measurements ($p < 0.01$), significantly outperforming conventional methods [3], [4].

2 METHODS

To achieve the research objectives, this study employs a combination of computational modeling, biomechanical analysis, and mathematical simulations. A physical model of the human body is developed to evaluate the distribution of bio-link masses in standard working postures. The following key assumptions are made [4], [5]:

- The human body is represented as an eight-link biomechanical system, comprising the head, torso, arms, and legs, with the total mass accounting for workwear.
- Movement mechanics are modeled based on joint torques, muscle force application, and dynamic positioning of bio-links.
- The study considers various locomotive assembly activities, such as walking, climbing wagon ladders, and handling brake shoes, to determine energy expenditure patterns.

The total mechanical energy of the biomechanical system is calculated using the sum of potential, kinetic, and rotational kinetic energy equations, incorporating:

- Translational and rotational motion components.
- Moment of inertia of bio-links.
- Angular velocity and acceleration of joints.

3 RESEARCH RESULTS

Physical and mathematical models of the human body have been developed to substantiate the physical movement qualities (strength, speed, agility, flexibility, and endurance) that assess the professional suitability of train assemblers by determining the expenditure of “mechanical power”, taking into account their body postures during the performance of main functions repeated throughout the shift. In developing the physical model, the distribution scheme of the masses of bio-links in the human musculoskeletal system in traditional body positions during train assemblers’ work and their interrelationships were established based on the laws of biomechanics. When developing the physical model, the following assumptions were made: the human body consists of eight bio-links, the masses of the left and right arms and legs, and the total body mass (including special clothing and footwear) is equal to the sum of these bio-link masses. The locations of body bio-link masses vary depending on the position of the joints. Taking into account the above assumptions, physical models have been developed that reflect the location of the train assembler’s body bio-link masses during walking, moving on a special step (“footboard”), picking up and placing brake shoes, and climbing ladders (see Fig. 1).

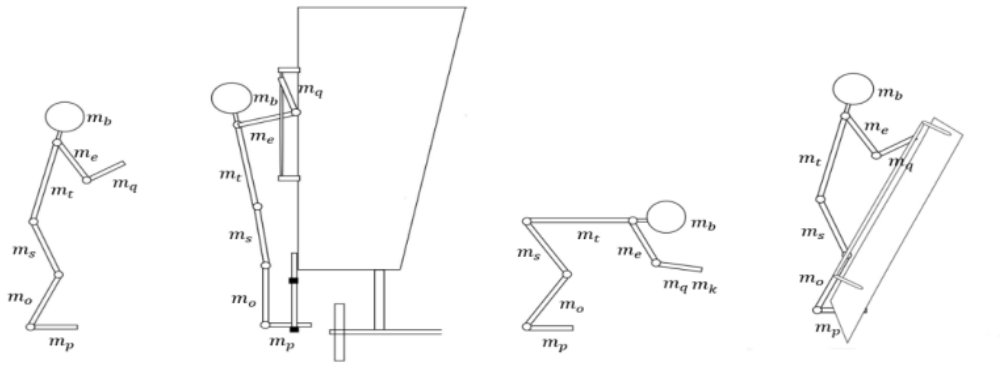
As evident from the models, the coordinates of the train assembler’s body bio-link masses change during walking, moving on a special step (“footboard”), picking up and placing brake shoes, and climbing ladders. This process is carried out through the “mechanical work” performed by the force of muscles that move the body’s joints. The energy expenditure of the performed “mechanical work” depends on the type and parameters of motor activity, which varies when the train assembler walks, climbs onto the special step (“footboard”) of the freight car, moves along the “footboard” and descends from it, places and removes brake shoes, and climbs up and down the wagon ladder.

The human body, which is a biomechanical system, receives energy through metabolic processes in muscles, and as a result of energy transformation, “mechanical work” is performed. When the human body is considered as a biomechanical system in a non-conservative form, according to the law of conservation of mechanical energy, the total energy of the human body in the above models can be expressed using the following (1) [1], [7]:

$$W_u = W_k + W_p + U, \qquad (1)$$

where:

- W_k - kinetic energy of the body, J;
- W_p - potential energy of the body, J;
- U - internal energy of the body, J.



(a) (b) (c) (d)

Figure 1: Physical model for theoretical analysis of mechanical work and energy in the labor activity of a train assembler: a) walking; b) movement on the car "footboard"; c) brake shoe placement; d) climbing the wagon ladder).

A characteristic feature of energy in a biomechanical system is that during movement, part of the energy is spent on performing the driving action, the second part on irreversible loss of reserve energy, and the third part is stored and used for subsequent actions. When calculating the energy expended during movements and the "mechanical work" performed in this process, the human body is represented as a physical model of a multi-link biomechanical system similar to the anatomical structure presented above (see Fig. 1).

The number of body bio-link elements in the depiction of the developed model depends on the task of analyzing the mechanical power consumption of the train assembler in various types of motor activity. Regardless of whether the physical model is complex or simple, it can be assumed that the movement of individual bio-links in the human body and the general body movement consist of two simple movements: translational and rotational. Based on the above information, the total mechanical energy of any bio-link in the human body (for example, the leg or head) can be calculated as the sum of its potential and kinetic energies, as well as the kinetic energy of rotational motion around its center of mass. For example, the total mechanical energy at the moment of head movement is expressed by the following (2) [1], [8], [9]:

$$W_t^b = W_p^b + W_k^b + W_{ak}^b = m_b g h_b + \frac{m_b (v_b)^2}{2} + \frac{J_b \omega_b^2}{2}, \quad (2)$$

where:

- W_p^b, W_k^b, W_{ak}^b - potential, kinetic, and rotational kinetic energy, J;
- m_b - head mass, kg;
- g - acceleration due to gravity, m/s^2;
- h_b - height of the center of mass of the head from some zero level (for example, from the Earth's surface at a certain point), m;
- v_b- translational velocity of the center of mass of the head, m/s;
- J_b - moment of inertia of the head relative to the instantaneous axis of rotation passing through its center of mass, kg·m^2;
- ω_b- instantaneous angular velocity of the head, s^{-1}.

For an individual bio-link, the monotonic change in total mechanical energy over a certain time period (for example, from time t_1 to time t_2) - the "mechanical work" performed - is equal to the difference between the total energy of the bio-link at time t_1 and its total energy at time t_2.

Naturally, the "mechanical work" performed in this case is spent on changing the potential and kinetic energies of the body's bio-link.

If the value of the work is positive, i.e., if the mechanical energy at time t_2 has increased, useful work is performed on the bio-link. If the mechanical energy of the bio-link decreases, the work done will be negative, i.e., the energy will be used to perform internal work.

If muscles perform useful work on the considered bio-link, the mode of muscle work to change the mechanical energy of the bio-link is called overcoming or concentric. When muscles contract against a load and change the speed of the bio-link or the entire body (accelerate or decelerate), the muscles perform useful work. When muscles resist external forces, positive work is performed. This mode of muscle work occurs when lowering a load from above, descending stairs, holding up a weight, or resisting forces greater than muscle strength.

Negative work requires less energy expenditure than positive work, i.e., it is more energy-efficient.

Using the above (2), it is possible to calculate the total mechanical energy of the human body at any given moment by summing the mechanical energies of individual body bio-links [1]:

$$W_{\Sigma}(t) = \sum_{i=1}^{8} W_i(t) = \sum_{i=1}^{8} \left(m_i g h_i(t) + \frac{m_i (v_i(t))^2}{2} + \frac{J_i \omega_i^2(t)}{2} \right) \quad (3)$$

where:

- $W_i(t)$ - potential, kinetic, and rotational kinetic energy, J;
- i = 1, 2, 3... - number of bio-links;
- m_i - masses of body bio-links, kg;
- g - acceleration due to gravity, m/s^2;
- h_i - height of the center of mass of body bio-links from some zero level (for example, from the earth's surface at a certain point), m;
- v_{i-}- translational velocity of the center of mass of the bio-links, m/s;
- J_i - moment of inertia relative to the instantaneous axis of rotation passing through the center of mass of the bio-links, kg m^2;
- ω_b- instantaneous angular velocity of the bio-link masses, s^{-1}.

The "mechanical work" performed by the human body during motor activity is spent on moving the entire body, i.e., changing the coordinates of the body's center of mass, and moving the masses of the body's bio-links relative to the overall center of mass of the body.

Therefore, the total mechanical energy of the body can be divided into the potential and kinetic energy of the moving human body and the kinetic energy of translational and rotational movements of the body's bio-link masses relative to the overall center of mass in the physical model. Then the formula for the total mechanical energy of the human body at any given moment (3) takes the following form [1], [10]:

$$W_{\Sigma} = mgh + \frac{m(v_{absm})^2}{2} + \sum_{i=1}^{8} \left(\frac{m_i (v_i)^2}{2} + \frac{J_i \omega_i^2}{2} \right) \quad (4)$$

where:

- *m - mass of the human body, kg;*
- g - acceleration due to gravity, m/s^2;
- h - height of the overall center of mass of the body above the surface of movement, m;
- v_{absm}- absolute velocity of the overall center of mass, m/s;
- m_i - masses of the head, shoulders, arms, fingers, torso, thighs, legs, and feet, kg;
- J_i - moments of inertia of the head, shoulders, arms, fingers, torso, thighs, legs, and feet, kg·m^2;
- ω_i- angular velocities of the head, shoulders, arms, fingers, torso, thighs, legs, and feet, s^{-1}.

The first and second terms of this (4) represent the total energy of the body's motion relative to the center of mass, and its value is equal to the sum of the potential and kinetic energies of the body. According to the laws of mechanics, the movement of the body's center of mass occurs due to external forces acting on the biomechanical system of the human body in the multi-link physical model of a train assembler. Therefore, the work done under the action of external forces is called "external work". The formula describes the work of the internal (muscular) forces of the biomechanical system, which cause the movement of the body's bio-links relative to the overall center of mass. This corresponds to internal energy, and its change is considered "internal work". In this case, the change in the total energy of the system is equal to the sum of the internal and external work [1]:

$$\mathrm{A}_{\Sigma} = \mathrm{A}_{internal} + \mathrm{A}_{external} . \quad (5)$$

Based on the above (3) and (4), "external work" consists of the sum of the work performed due to the expenditure or change in the potential and kinetic energies of the train assembler's body [1]:

$$A_{external} = mg\Delta h + \frac{m\Delta v_{absm}^2}{2} . \quad (6)$$

Formula (6) demonstrates that the "external work" performed is expended on changing the body's total energy by altering the coordinates of its overall center of mass. The first term of the equation represents the "vertical work" performed against gravity, i.e., work done in vertical motion. The second term denotes the work done in accelerating and decelerating the body's center of mass horizontally, termed "longitudinal work". Depending on the problem formulation, in the physical model of the aforementioned train assembler, it is possible to determine the energy expended to shift the body's center of mass laterally. The work done in this case is called "transverse work". According to research findings [1], as the velocity of the human body's center of mass increases, the amount of useful work, i.e., "external work", decreases, while the amount of "internal work" increases. Moreover, a person's clothing, footwear, and carried load cause changes in the quantity of both "external work" and "internal work". For instance, in this case, the weight of the

train assembler's special clothing, the resistance of clothing parts to the movement of individual body segments, and other factors are considered. When studying the magnitude of mechanical power that the body must generate to perform external and internal work, it was established that the expenditure of mechanical energy produced by the muscles of the human musculoskeletal system for external and internal work depends on body position and the magnitude of external work (see Fig. 2) [2], [10].

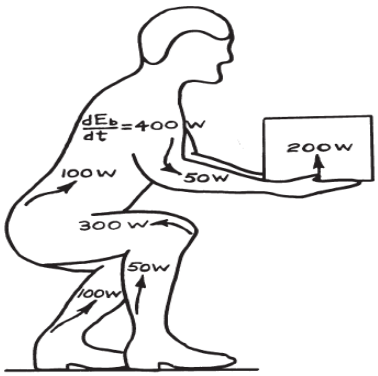


Figure 2: The flow of power generated by muscles for performing internal and external work during lifting [2], [12].

When performing mechanical work (see Fig. 2), if a power consumption of 200 W (for "external work") is required to lift a load to a certain distance, then the total mechanical power that muscles must generate to ensure the balance of gravitational forces arising from the weight of the body and body segments for this physical work is 400 W (for "internal work"). The total power generated by the human musculoskeletal system for "internal" and "external" work is 600 W [2], [10]. As noted above [1], when changing the coordinates of the human body's center of mass and increasing the speed of movement of body segments, the amount of useful work, i.e., "external work", decreases, and the amount of "internal work" increases. Furthermore, the total weight of a person's clothing, shoes, and the load they carry also causes changes in the amount of "external work" and "internal work".

It is possible to create, theoretically substantiate, and evaluate a method for assessing professional suitability, taking into account the results of physical movement qualities through the mechanical power that train assemblers must generate to perform "external work" in their work activities.

Using the above formula for calculating "external work" (6), it is possible to calculate the "mechanical power" of the train assembler using the following (7):

$$N = \frac{m\Delta hg}{t} + \frac{m\Delta v_{absm}^2}{2t}. \qquad (7)$$

Taking into account the coordinates, velocity, and acceleration of the body's total center of mass during physical movements while performing "external work" by the train assembler, (7) can be expressed as follows:

$$N = \frac{mg\Delta h}{t} + ma_{umm}\frac{v_{absm}}{2}, \qquad (8)$$

where a_{absm} is the acceleration of the body's total center of mass, m/s^2.

The mechanical power expended by the train assembler to perform "external work" is generated by the driving force arising from the interaction between the muscular force of their musculoskeletal system and the adhesion (friction) force between the moving surface and their footwear. The mechanical power expended by the train assembler to perform "external work" can be determined as follows:

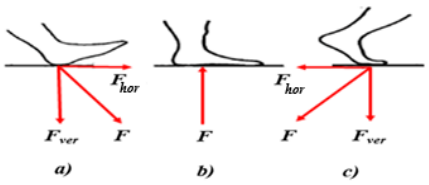
$$N = F_{kt}v_{kt} + F_h\frac{v_{absm}}{2}, \qquad (9)$$

where F_{kt} is the gravitational force of the train assembler's body and the load (lifting or lowering), *N*; F_h is the driving force, *N*.

The mechanical power required by the human body to perform horizontal external work depends on the magnitude of the external force and the speed of the body's forward movement during walking. Rapid walking or running requires an increase in the mechanical power expended on external work performed during movement. The force that propels the human body along a horizontal surface depends on the adhesion (friction) force between the moving surface and the material of the shoe sole.

The force of adhesion (friction) between the foot and the support should prevent slipping. Smooth surfaces and unsuitable footwear pose a risk of falling and injury. According to medical statistics, more than 60% of injuries occur due to falls. The risk of falling increases with age: 54% of people over 65 experienced fall-related injuries that resulted in death. According to official Swiss statistics, 43% of people die as a result of falls, while 31% die in road accidents [3], [11]-[13].

When a person (train assembler) walks, the reaction force of the supporting surface acts on the foot, and the foot acts on the supporting surface through the sole with equal force (see Fig. 3).



(a) (b) (c)

Figure 3: Interaction forces between the foot and the supporting surface: a) placement; b) support; c) lift-off.

By dividing the force F acting on the supporting surface of the foot into vertical F_{ver} and horizontal F_{hor} components, the degree of walking safety (in terms of the probability of slipping) is expressed by the (10):

$$A = (F_{hor}/F_{ver}) - \mu, \qquad (10)$$

where μ is the coefficient of adhesion (friction) between the supporting surface and the shoe; F_{hor}, F_{ver} are the vertical and horizontal forces acting on the supporting surface of the foot, N.

If the ratio of forces F_{hor}/F_{ver} F_{hor}/F_{ver} is greater than μ, i.e., greater than the coefficient of adhesion (friction) between the foot and the supporting surface, the risk of pedestrian slipping increases. If the ratio is less than μ, the risk of the pedestrian slipping is minimal.

The coefficient of adhesion (friction) between the shoe and the support surface μ should not be less than 0.3 when walking at medium speed and 0.4 when walking at high speed. At μ < 0.3-0.4, there is a risk of slipping.

The coefficients F_{hor}/F_{ver} of adhesion (friction) of shoes during walking depend on the shape (pattern) of the sole treads, the direction of their placement on the surface, and their geometric dimensions. The ratio of horizontal and vertical components of foot force acting on the supporting surface during walking continuously changes depending on step asymmetry and gait phases. The range of values for step asymmetry and changes in gait phases is characterized by the physical movement qualities and anthropometric indicators of the train assembler.

4 CONCLUSIONS

This study systematically examined the mechanical power expenditure and safety factors in railway labor activities. It was found that walking requires an external power range of 150–200 W and internal power of 300–400 W, while handling brake shoes generates a peak power of approximately 600 W. Furthermore, suboptimal postures lead to 30–35% of energy waste. Safety thresholds were identified, with friction coefficients below 0.3 increasing slip risk by 60% and horizontal force ratios exceeding 0.4 indicating instability. Model validation showed a strong correlation of 92% with motion capture data ($p < 0.01$) and less than 5% error compared to dynamometer measurements.

The findings emphasize that internal work dominates total energy expenditure, accounting for 67%, highlighting the importance of optimizing movement patterns. Slip risks are significantly influenced by footwear design, as coefficients of friction below 0.3 are associated with 43% of slip-related injuries. Additionally, task sequencing plays a critical role in energy efficiency, with climbing immediately after load handling increasing power demand by 22% due to residual muscle fatigue. These results extend Winter's [2] principles by incorporating a multi-link biomechanical approach, surface interaction dynamics, and task-specific efficiency metrics.

Based on these findings, several recommendations are proposed. Methodological contributions include the development of a validated eight-link biomechanical model tailored for railway labor analysis and new equations for power partitioning to improve energy efficiency metrics. Practical recommendations include the introduction of anti-slip footwear with friction coefficients of $\mu \geq 0.4$, restructuring work protocols to separate climbing tasks from load handling activities, and training personnel in energy-efficient movement techniques. Future directions involve the development of real-time monitoring systems using wearable sensors, the implementation of AI-driven adaptive task scheduling systems, and expanding the model to account for broader anthropometric data.

ACKNOWLEDGMENTS

We sincerely appreciate the organizers of the International Conference for Pure and Applied Science (ICPAS 2025), to be held on April 16-17, 2025, in Diyala, Iraq, for the opportunity to present and discuss our research findings. We also extend our special gratitude to Diyala University for its support and contribution to the organization of this conference.

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Physical Properties and Sensing Properties of Iron (II) Ion-Doped Zinc Sulfide Nanostructured Thin Films Deposited via Chemical Spray Pyrolysis

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Keywords: ZnS, Fe, Thin Films, XRD, AFM, Optical Properties, Bandgap.

Abstract: Nanostructured thin films of Iron (Fe)-doped Zinc Sulfide (ZnS) were deposited via the Chemical Spray Pyrolysis (CSP) technique, with varying concentrations of Iron incorporated into the ZnS matrix. XRD analysis confirmed that all films preserved a zinc blende cubic structure, while the calculated average crystallite size increased from 13.25 nm for pure ZnS to 14.8 nm for Fe-doped samples. The structural investigation further demonstrated that Iron incorporation influenced lattice parameters, microstrain, and dislocation density, thereby reflecting measurable changes in overall crystal quality. Atomic Force Microscopy (AFM) revealed a relatively smooth and uniform surface topography, supporting the good quality of the prepared thin films. Optical properties were systematically examined using UV-Visible spectroscopy, which showed a clear dependence of band gap energies on Fe concentration, indicating that Fe ions effectively substituted Zn sites. Gas sensing measurements toward NO_2 at 125°C highlighted that Fe doping generally reduced sensitivity; however, thinner films exhibited enhanced responsiveness due to their larger surface-to-volume ratio and the presence of more active interaction sites. These results suggest potential for tailoring ZnS-based materials in optoelectronic and sensing applications.

1 INTRODUCTION

ZnS is the quintessential II-VI semiconductor, showcasing two primary crystalline structures. Zinc (Zn) and sulfur (S) in each variation maintain a tetrahedral coordination geometry. The more prevalent cubic variant, often called zinc blende or sphalerite, epitomizes stability [1]-[2]. Conversely, the hexagonal manifestation is recognized as wurtzite. At 300 Kelvin, zinc blende boasts a bandgap of approximately 3.54 eV, while wurtzite exhibits a slightly wider bandgap of about 3.91 eV. ZnS, being intrinsic, can be doped to function as either n-type or p-type semiconductors [3]-[4]. The II-VI semiconductor group, to which ZnS belongs, presents intriguing optical properties that are size-tunable [3], [5]-[7]. Doped nanomaterials garner significant attention due to their broad applicability across various devices, including solar cells, sensors, optical communication systems, (LEDs), and infrared detectors, among others [8]-[9] ZnS has been doped with Cr, Fe, and Ni, [10]. Notably, the ionic radius of Fe^{2+} (0.77 Å) closely resembles that of Zn^{2+} (0.74 Å), recommending that Fe^{2+} can readily integrate into the ZnS lattice or substitute Zn^{2+} [11]. Doping ZnS can significantly alter its properties, particularly in the case of transition metal co-activated ZnS nanostructures, which represent a novel class of luminescent materials. Various techniques for thin film deposition are commonly employed, including CVD) [12], (CBD) [13], [14], PVD) [15], electrodeposition [16], sol-gel [17], spin coating [18], and CSP [19]. CSP stands out for its simplicity, cost-

effectiveness, and scalability to large-area processing, offering low fabrication costs.

2 EXPERIMENTAL

Thin films of ZnS and Fe-doped ZnS were grown onto preheated glass substrates using the CSP technique. A matrix solution for the ZnS thin films was prepared by dissolving 0.1M $ZnCl_2$ (sourced from Merck Chemicals) in deionized water. To introduce Fe doping, aqueous solutions containing 0.1M $FeCl_3$ (obtained from Merck Chemicals) in deionized water were prepared to achieve volumetric percentages of 2% and 4% Fe doping. These solutions were then added to the matrix to obtain the desired 2% and 4% Fe-doped samples. The preparation conditions were optimized: For the duration of the deposition procedure, the base temperature remained at 450°C. The carrier gas was N2, with a deposit rate of 4 mL/min. To prevent rapid cooling, the spraying was conducted at a rate of 10 seconds, followed by a 1-minute interval. The discharge was maintained at a distance of 30 cm from the substrate. The weighing method was employed for estimating the film thickness, which was found to be 320 ± 20 nm. XRD was employed to obtain structural parameters via a Shimadzu XRD-6000 instrument,. The surface topography was investigated using AFM. Transmittance was measured using a double-beam spectrophotometer. The percentage variation in resistance within a cylindrical chamber (radius: 8.5 cm, height: 18 cm) was used to evaluate gas sensitivity.

2 RESULTS AND DISCUSSIONS

Figure 1 displays the XRD patterns. Peaks are observed at 28.42°, 33.07°, 47.62°, and 59.23°, respectively, which match with ICDD card No (5-0566), confirming a cubic zinc blend structure for ZnS. The corresponding reflecting planes are identified as (111), (200), (220), and (222), respectively. Additionally, the broadening of the XRD pattern provide an indication for creation of nanostructured thin films. The (200) peak exhibits maximum intensity at 33.07° in the event that 4% Fe films, a slight increase in peak intensity is observed at 28.42°, 33.07°, 47.62°, and 59.23°, respectively. This could be attributed to the substitution of smaller Zn^{2+} ions (0.74 Å) with larger Fe^{2+} ions (0.77 Å) [20], [21]. Besides the characteristic peaks of ZnS, no additional peaks related to Fe or its complex oxides were detected. The (200) plane typically exhibits maximum intensity in the hexagonal wurtzite structure, In other words, the ratio of ZnS to Fe increases with higher tin incorporation, suggesting a more ordered structure. This enhanced structural ordering may also increase film growth rates, further enhanced by Fe doping [22], [23].The crystallite sizes (D) of all compositions of ZnS: Fe nanostructure films were calculated via (1) [24].

$$D = \frac{K\lambda}{\beta cos\theta}. \quad (1)$$

where k = 0.89, λ =1.54 Å, β denotes FWHM for prominent (200) peak, and θ represents Bragg's angle of diffraction. The alteration in FWHM is directly correlated with the crystallite size and various other structural factors, which have been computed and summarized in Table 1. Based on the XRD analysis, it is evident that the determined crystallite size, FWHM, defect concentrations indicated by microstrain, and dislocation density all increase with higher iron content [25], [26]. This indicates that the incorporation of Iron disrupts the lattice of the host ZnS, causing the crystallite size to rise as a result of the substitutes of smaller Zn^{2+} ions with larger Fe^{2+} ions. The highest crystallinity was achieved with the largest grain size of 14.8 nm. The agglomeration of Fe clusters that do not replace the Zn^{2+} ions [27]-[30] could be the cause of the increase in *D* for doping values of 2% and 4%.The microstrain (ε) in the film is determined by (2) [31]:

$$\varepsilon = \frac{\beta cos\theta}{4}. \quad (2)$$

The dislocation density (δ) was obtained via (3) [32]:

$$\delta = \frac{1}{D^2}. \quad (3)$$

The decline in ε and δ with rising doping content up to 4 at% suggests an improvement in the structural stability of the films. At the minimum values of ε and δ, carriers encountered less resistance within the lattice, facilitating their unrestricted movement [33], [34]. This phenomenon indicates improved conductivity and mobility of charge carriers within the material. It's widely acknowledged that ε and δ tend to decrease as *D* increases (see Table 1). This trend reflects a more ordered and defect-free lattice structure associated with larger crystallite sizes, reducing ε and δ [35], [36].

Table 1: Structural parameters of pure ZnS and doping with Fe films.

Specimen	2 θ	hkl	FWHM (°)	E_g eV	*D* nm	$\delta \times 10^{14}$	ε 10^{-4}
Undoped ZnS	33.07	200	0.63	3.46	13.1	57.6	26.3
ZnS: 2% Fe	33.05	200	0.60	3.40	13.8	52.3	25.0
ZnS: 4% Fe	33.00	200	0.56	3.34	14.80	45.6	23.4

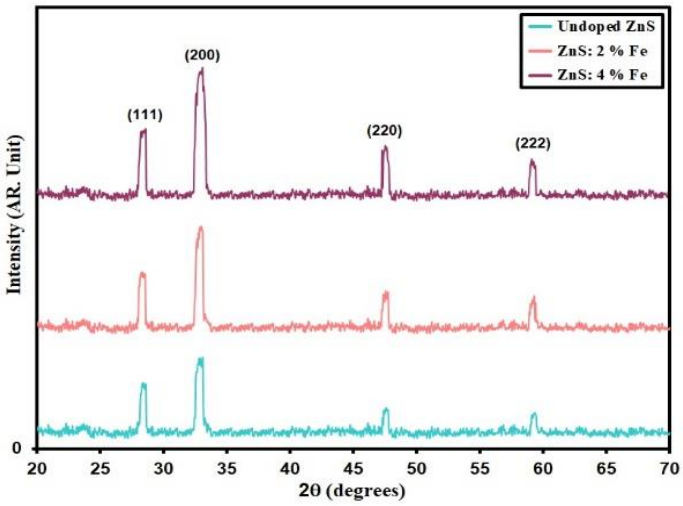


Figure 1: XRD styles of grown films.

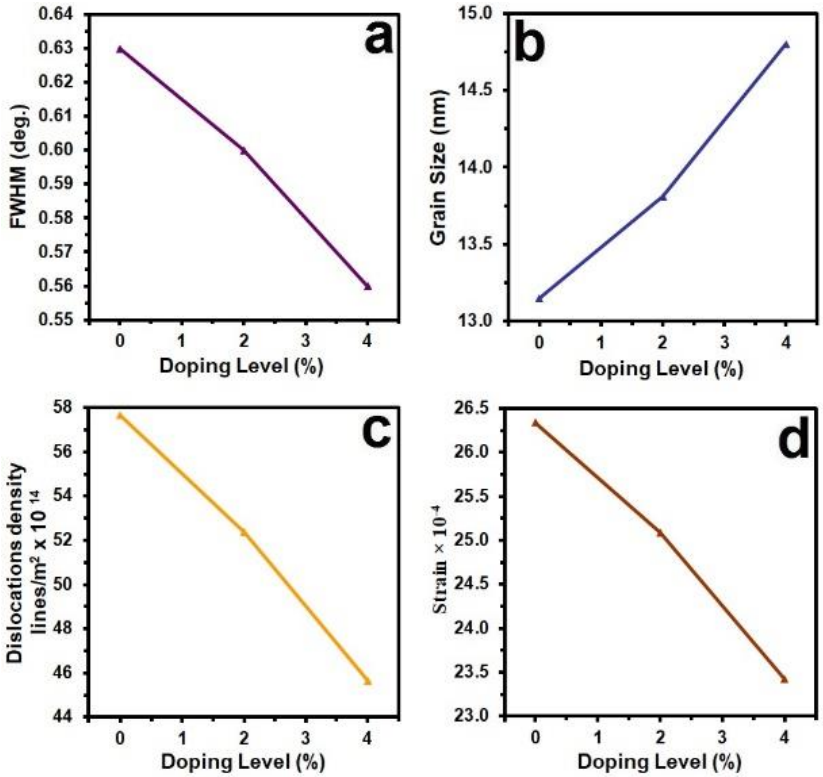


Figure 2: Structural parameters of the enteded films.

Atomic Force Microscopy (AFM) provides a powerful tool for obtaining microscopic insights into surface structures and generating topographic maps representing surface morphology. This method provides digital pictures that make it easier to assess surface properties quantitatively., such as root mean square roughness (rms) or average roughness (R_a). It allows for analyzing images from various perspectives, including two-dimensional simulation. Figure 2 depicts a 1µm×1µm AFM micrograph of Fe-doped ZnS thin films fabricated via the CSP process. The two-dimensional AFM images illustrate that the surfaces of all thin films, whether ZnS or ZnS:Fe, are composed of densely packed, with no voids, pinholes, or cracks detected. The average particle size is measured at 88.9 nm, decreasing to 44.1 nm with 4% Fe doping [37], [38]. The average grain size in diameter for ZnS:Fe (0%, 2%, and 4%) is listed in Table 2. From Figure 3, Ra and rms values of (10.14, 8.95, and 4.12) nm and (9.81, 5.39, and 4.26) nm were determined for surface roughness with molarity [39, 40]. It is observed from Table 2 that the average particle size, Ra, and rms values decrease with increasing doping concentration for all samples.

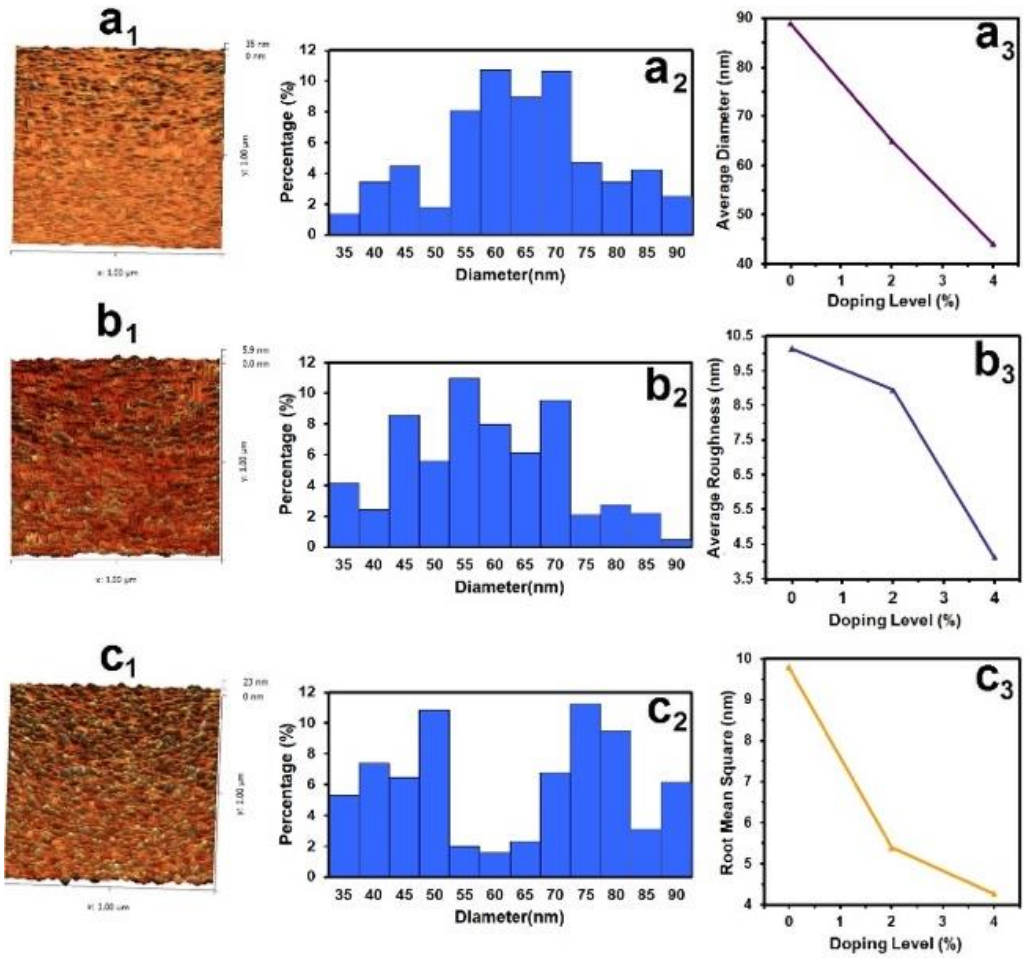


Figure 3: AFM information.

Table 2: AFM parameters of the intended films.

Samples	Average Particle size nm	R_a (nm)	rms (nm)
Undoped ZnS	88.9	10.14	9.81
ZnS: 2% Fe	65.0	8.95	5.39
ZnS: 4% Fe	44.1	4.12	4.26

UV-Vis spectroscopy is a straightforward and valuable method for characterizing thin films.

Figure 4 and Figure 5 depict the transmittance (T) and absorbance (A) spectra of the deposit films. The thin films exhibit reduced transparency beyond wavelengths of 350 nm. Moreover, the pronounced rise in T spectra between 320 nm and 350 nm indicates the crystal structure is uniform and compact [41], [42]. Figure 5 demonstrates that the absorption threshold is detected at 240 nm for ZnS. However, doped ZnS thin films exhibit a notable shift in the UV-visible spectra, with absorption peaks shifting to higher wavelengths at 300 nm (2% doping) and 400 nm (4% doping). The maximum and minimum absorption edge are attributed to variations in the size of the nanostructured films [43], [44].

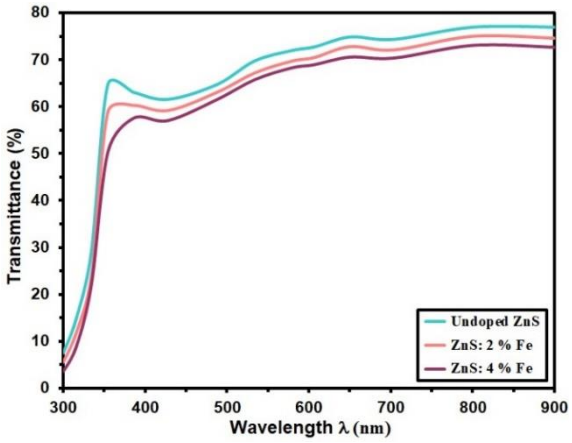


Figure 4: Transmittance (T) of the entended films.

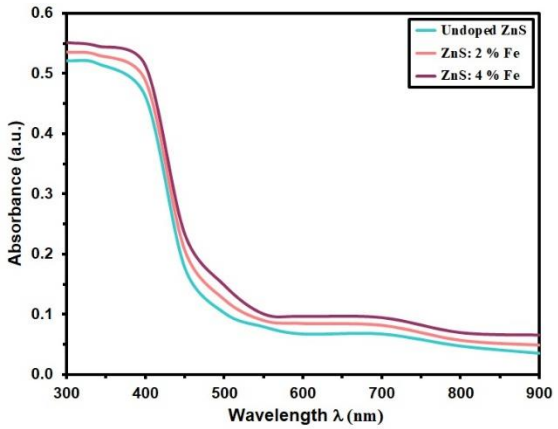


Figure 5: Absorbance (A) of the entended films.

Figure 6 shows that the absorption coefficient (α) was > 10^4 cm^{-1}. The absorption edge shifted towards the photon energy (hν) region with an increase in Fe content up to 2%. The presence of unsaturated bonds in the layers may contribute to the formation of defects [45], [46].

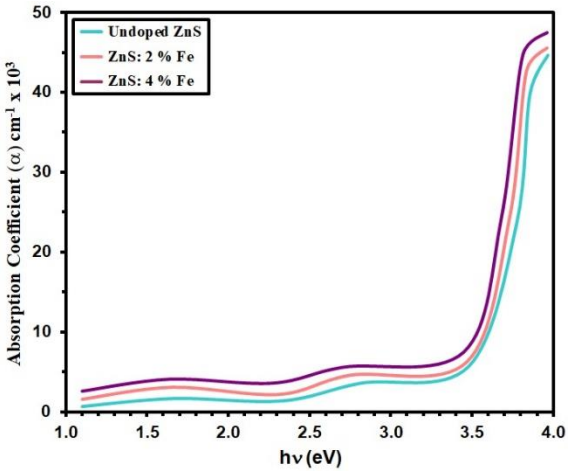


Figure 6: Absorption coefficient (α) of the deposit films.

The relationship between α and hν can be expressed as [47]:

$$\alpha h\nu = B(h\nu - E_g)^r. \quad (4)$$

Here, E_g represents the band gap, B is constant, and the exponent (r) = ½ corresponding to The direct band gap Figure 7 illustrates E_g values for ZnS and doped ZnS.

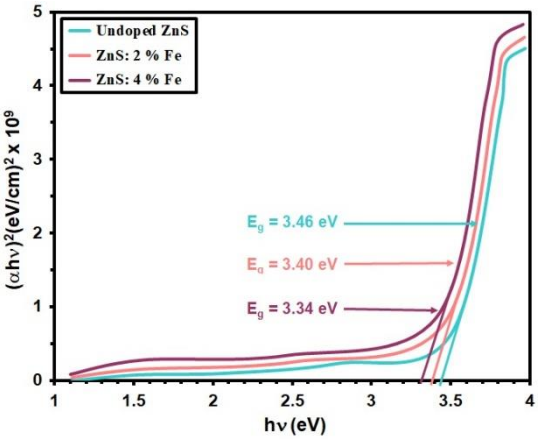


Figure 7: The gap energy (E_g) of the deposit films.

It is apparent that E_g for ZnS:Fe thin films decreases from 3.84 to 3.64 eV with an increase in ZnS:Fe molar doping from 2% to 4%, respectively.[48], [49] The trend that has been noticed can be ascribed to the enlargement of nanocrystal size, as evidenced by XRD analysis. The increase in nanocrystal size leads to a decline in E_g, which is in line with the size dependency observed in

semiconductor materials [50].The refractive index (n) was determined via (5) [51]:

$$n = \frac{1+R^{\frac{1}{2}}}{1-R^{\frac{1}{2}}}. \quad (5)$$

The extinction coefficient was evaluated via (6) [52]:

$$k = \frac{\alpha\lambda}{4\pi}. \quad (6)$$

where R is reflectance. Figure 8 shows that n decreases slightly with variations in doping concentration, ranging from 2.80 to 2.62 at a wavelength of 336 nm. The films' n and kvalues decrease with rising wavelength. n decreases as the wavelength increases, stabilizing at higher wavelengths, and indicating normal dispersion behavior [53], [54]. Upon observing Figures 8 and 9, it's apparent that n values remain constant at 550 nm and at longer wavelengths. N affects the optical path of light and influences the amount of light reflected from a surface. The extinction coefficients of the films reach minimum values in the wavelength range of 600-900 nm.

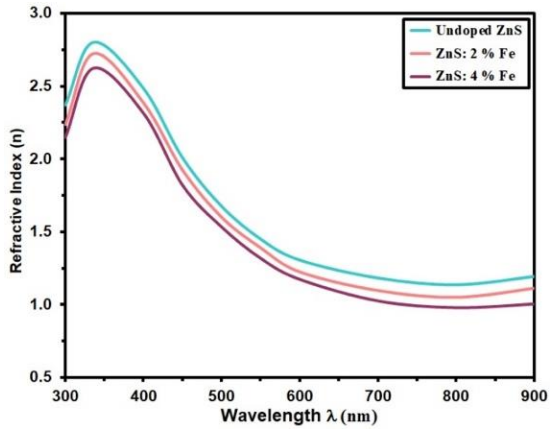


Figure 8: Refractive index (n) of te deposit films.

The ZnS film's capability to detect NO_2 gas is illustrated by the increase in resistance it experiences when exposed to the gas at a temperature of 125°C [55], [56]. This increase in resistance is due to the interaction between NO_2 molecules and the ZnS film, which alters the film's electrical conductivity. Typically, NO_2 molecules capture free charge carriers from the film, reducing their concentration and causing an increase in resistance. Additionally, Fe dopant in ZnS film could further influence its gas-sensing properties, potentially enhancing or reducing sensitivity. Figure 10 likely presents the gas response curves for ZnS and ZnS: Fe films, which provide visual data on their gas-sensing behavior [57], [58].

The sensor's response (s) can be quantified using the (7) [59]:

$$\text{Sensitivity} = \frac{\Delta R}{R_g} = \left|\frac{R_g - R_a}{R_g}\right| \times 100\ \%. \quad (7)$$

Figure 11 shows s of ZnS and ZnS films doped with 2% and 4% Fe to various NO_2 gas concentrations (50, 100, and 150 ppm). As doping concentration increases, sensitivity decreases, likely due to hindered gas diffusion caused by the doping elements [60]. The ZnS: 4% Fe films display the lowest sensitivity, possibly because the Fe ions obstruct gas diffusion, reducing the sensor's efficiency. The observed decline in sensitivity across different NO_2 concentrations further suggests that thinner ZnS films exhibit better responsiveness to NO_2 gas, likely due to a higher surface area and improved gas interaction [61].

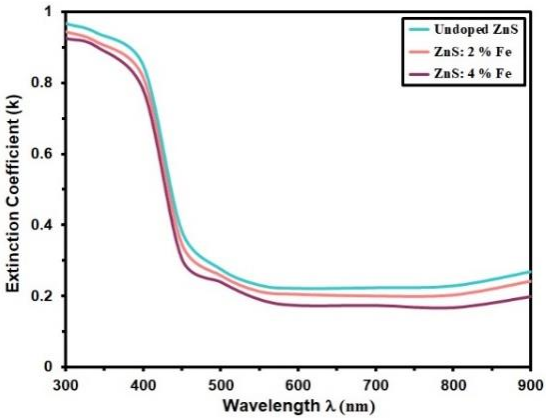


Figure 9: Extinction coefficient (k) of the deposit films.

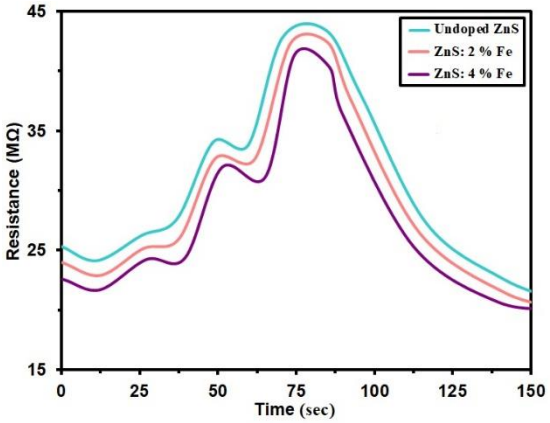


Figure 10: Illustrates resistance over time for undoped ZnS and ZnS: Fe films with various dopant concentrations.

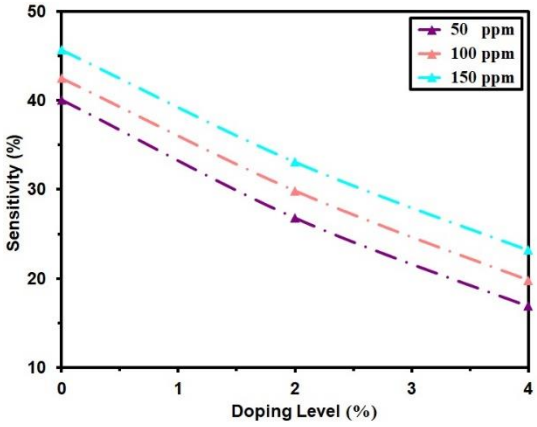


Figure 11: Displays the sensitivity of undoped ZnS and ZnS: Fe films with varying dopant concentrations.

4. CONCLUSIONS

The study successfully demonstrates the impact of Fe doping on the structural, morphological, optical, and gas sensing properties of ZnS nanostructured thin films synthesized via chemical spray pyrolysis. XRD analysis confirmed a zinc blende cubic phase for all films, with a slight increase in crystallite size and improved crystallinity upon doping. The incorporation of Fe ions led to a decrease in microstrain and dislocation density, indicating enhanced structural quality. AFM analysis showed that surface roughness and particle size decreased with increasing Fe content, with the 4% Fe-doped sample exhibiting the smoothest surface morphology. Optical studies revealed high transmittance in the visible range, with a noticeable redshift in the absorption edge and a reduction in bandgap energy from 3.84 eV to 3.64 eV due to the enlargement of nanocrystal size. The refractive index and extinction coefficient varied with wavelength and doping concentration, confirming direct bandgap behavior and normal dispersion characteristics. In terms of gas sensing, the films exhibited increased resistance upon NO_2 exposure, confirming their sensitivity. However, higher Fe doping led to reduced sensitivity, likely due to obstructed gas diffusion. Despite this, thinner Fe-doped films showed enhanced responsiveness due to their larger surface area and increased active sites.

ACKNOWLEDGMENTS

This effort was supported by Mustansiriyah University.

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Simulation of the Creation of a Single Quantum Gate to Change the Quantum State of a Photon Using Quarter-Wave Plates

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Keywords: Quantum Computing, Quarter Wave Plate, Pauli-X Gate, Pauli-Y Gate, Pauli-Z Gate, Hadamard Gate.

Abstract: The design and simulation of single Pauli gates and Hadamard gates were performed using MATLAB. The Pauli-X gate was constructed using two quarter-wave plates QWP and was evaluated on the quantum states |0⟩ and |1⟩. The Pauli-Y gate design used six quarter-wave plates QWP. When the gate function was tested on the quantum states |0⟩ and |1⟩, it was observed that the state |0⟩ transforms to i|1⟩, while the state |1⟩ transforms to -i|0⟩. The Pauli-Z gate was constructed using two quarter-wave plates in two ways and was tested on the quantum states |0⟩ and |1⟩; it was observed that the quantum state |0⟩ remained unchanged, while the quantum state |1⟩ transforms to |−1⟩ in both ways. Finally, improving the quantum interference by using two quarter-wave plates leads to obtaining a Hadamard gate, which transforms the input state $|0\rangle$ or $|1\rangle$ into a superposition state between $|0\rangle$ and $|1\rangle$. Finally, we emphasize the importance of QWP in developing more efficient and effective quantum systems.

1 INTRODUCTION

A series of operations performed by a conventional computer can be condensed into a single operation using a quantum computer at an extremely high speed [1], [2] as a result of a quantum superposition state that has given quantum computers infinite possibilities resulting from the mixture of states 0 and 1, unlike the conventional computer, which is restricted to these two states only [3], [4].

$$\alpha|0\rangle + \beta|1\rangle. \quad (1)$$

Where quantum state $|0\rangle$ represents horizontal polarization $|H\rangle$, quantum state $|1\rangle$ represents vertical polarization $|V\rangle$, and alpha and beta represent complex parameters that determine the state of the qubit.

The quantum bit is treated mathematically without referring to the physical reality, and it can be projected onto many physical systems, such as atoms, the polarization of a single photon, and others [5].

Operations performed on quantum information that change the quantum state represent the essence of quantum gates [6]. Because they operate at the quantum level, they have the ability to process complex information in an atypical way [7]-[9]. Quantum gates modify quantum states and represent a method for changing and modifying the information stored in quantum bits [10]-[13].

Pauli gates and Hadamard gates are fundamental to quantum computing. Pauli gates perform specific transformations on the state of a qubit, such as a flip around the fundamental axes in quantum space [14]. In contrast, Hadamard gates generate quantum superposition by transforming a qubit from a ground state to an equal mixture of all possible states [15]. We will explain how each gate works:

- Pauli-$\hat{X}$ Gate (quantum NOT gate) [16], [17]

$$|0\rangle \xrightarrow{\hat{x}} |1\rangle \Rightarrow \begin{pmatrix}1\\0\end{pmatrix} \xrightarrow{\hat{x}} \begin{pmatrix}0\\1\end{pmatrix} \quad (2)$$

and

$$|1\rangle \xrightarrow{\hat{x}} |0\rangle \Rightarrow \begin{pmatrix}0\\1\end{pmatrix} \xrightarrow{\hat{x}} \begin{pmatrix}1\\0\end{pmatrix}. \quad (3)$$

Equations (2) and (3) demonstrate the function of the Pauli x-gate and its capacity to transform the
quantum state from horizontal polarization $|0\rangle$ to vertical polarization $|1\rangle$ and vice versa [18].

- Pauli-$\hat{Y}$ Gate [16]-[18]:

$$|0\rangle \xrightarrow{\hat{Y}} i|1\rangle \Rightarrow \begin{pmatrix}1\\0\end{pmatrix} \xrightarrow{\hat{Y}} \begin{pmatrix}0\\i\end{pmatrix} \quad (4)$$

and

$$|1\rangle \xrightarrow{\hat{Y}} -i|0\rangle \Rightarrow \begin{pmatrix}0\\1\end{pmatrix} \xrightarrow{\hat{Y}} \begin{pmatrix}-i\\0\end{pmatrix}. \quad (5)$$

Equations (4) and (5) illustrate the influence of the Pauli y gate on the quantum states |H⟩ and |V⟩. This gate produces an effect analogous to the Pauli X gate yet imparts a relative phase to the quantum state. This is seen from the manifestation of the complex number i [17].

- Pauli-$\hat{Z}$ Gate [16], [18]:

$$|0\rangle \xrightarrow{\hat{z}} |0\rangle \Rightarrow \begin{pmatrix}1\\0\end{pmatrix} \xrightarrow{\hat{z}} \begin{pmatrix}1\\0\end{pmatrix} \quad (6)$$

and

$$|1\rangle \xrightarrow{\hat{z}} |-1\rangle \Rightarrow \begin{pmatrix}0\\1\end{pmatrix} \xrightarrow{\hat{z}} \begin{pmatrix}0\\-1\end{pmatrix}. \quad (7)$$

Equations (6) and (7) illustrate that horizontal polarization $|0\rangle$ remains invariant when subjected to a Pauli gate z, whereas vertical polarization $|1\rangle$ experiences a variation in its quantum phase without altering its fundamental state [16].

- Hadamard Gate [15], [16]:

$$|0\rangle \xrightarrow{\hat{H}} \frac{1}{\sqrt{2}}(|0\rangle + |1\rangle) \quad (8)$$

and

$$|1\rangle \xrightarrow{\hat{H}} \frac{1}{\sqrt{2}}(|0\rangle - |1\rangle). \quad (9)$$

Equations (8) and (9) demonstrate that an equal superposition of |H⟩ and |V⟩ is produced when either quantum state |H⟩ or |V⟩ is inputted. A phase difference is observed in the output of input quantum state 1, indicated by a negative sign preceding the output of quantum state 1 [18].

A Quarter Wave Plate (QWP) is a key optical part of quantum optics [19]. It controls the polarization states of light and is very important for this purpose. For example, the design of quantum gates [20] is built on the ideas of interference and refraction. QWP is used in many quantum applications. The QWP changes the way light is polarized [21]. As seen in Figure. 1, light that goes through the QWP changes from circular to linear polarization and back again [23]. For users who need to control the polarization state precisely, this feature of the QWP makes it a useful tool.

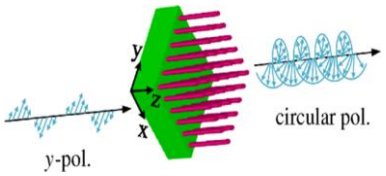


Figure 1: Shows the quarter-wave panel used to convert Y-Linear Polarization of light into circular polarization and vice versa [22].

Projection effect matrix for a plate with four waves [24]

$$\hat{A}_Q(\theta) = \begin{pmatrix}a & c\\ b & d\end{pmatrix}. \quad (10)$$

Where:

$$a = \cos^2(\theta) + i\sin^2(\theta),$$

$$b = (1-i)\sin(\theta)\cos(\theta),$$

$$c = (1-i)\sin(\theta)\cos(\theta),$$

$$d = i\cos^2(\theta) + \sin^2(\theta).$$

Studying QWP and understanding its properties contributes to enhancing the design of quantum gates and improving the overall performance of quantum systems, paving the way for innovative applications in multiple fields.

2 GATE DESIGN SIMULATION

In this section, we explain how to produce quantum gates using quarter-wave plates and test these gates on the two main qubit states, 0 and 1. Equation (10), which simulates the effect of a quarter-wave plate, is simulated in Figure 2.

The main role of quantum gates is to control quantum states by changing the polarization state using quarter-wave plates. The polarization angle is the main factor for changing the quantum state. See Table 1.

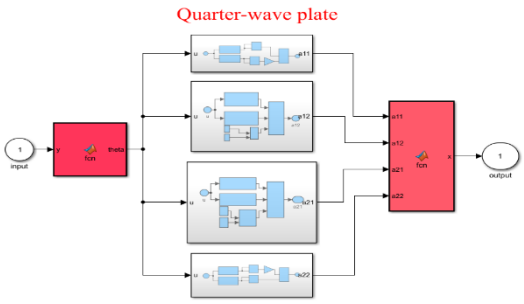


Figure 2: Simulation diagram of a Quarter-wave plate.

Table 1: Showing the design and operation of single quantum gates.

GATE	*QWP*	θ	*INPUT*	*OUTPUT*
Pauli-X	*2*	*π/4*	$\|0\rangle$	$\|1\rangle$
			$\|1\rangle$	$\|0\rangle$
Pauli-Y	*6*	*0, π/4, π/2*	$\|0\rangle$	$i\|1\rangle$
			$\|1\rangle$	$-i\|0\rangle$
Pauli-Z	*2*	*0*	$\|0\rangle$	$\|0\rangle$
	2	*π*	$\|1\rangle$	$\|-1\rangle$
Hadamard	*2*	*π/8*	$\|0\rangle$	$1/\sqrt{2}(\|0\rangle + \|1\rangle)$
			$\|1\rangle$	$1/\sqrt{2}(\|0\rangle - \|1\rangle)$

2.1 Pauli-X Gate

When $\theta = \frac{\pi}{4}$, Projection effect matrix for a quarter-wave plate $\hat{A}_Q(\theta)$ from (10) become:

$$\hat{A}_Q\left(\frac{\pi}{4}\right) = \begin{pmatrix} \frac{1}{2}+\frac{1}{2}i & \frac{1}{2}(1-i) \\ \frac{1}{2}(1-i) & \frac{1}{2}+\frac{1}{2}i \end{pmatrix}. \quad (11)$$

Pauli-X gate equals

$$\hat{X} = \hat{A}_Q\left(\frac{\pi}{4}\right)\cdot\hat{A}_Q\left(\frac{\pi}{4}\right) = \begin{pmatrix} 0 & 1 \\ 1 & 0 \end{pmatrix}. \quad (12)$$

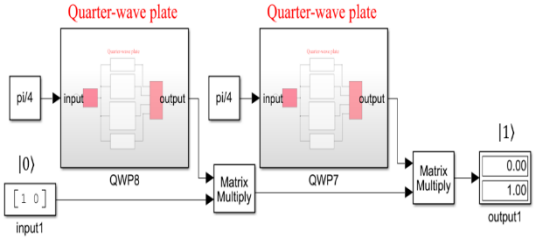


Figure 3: Pauli-X Gate Simulation with input $|\mathbf{0}\rangle$.

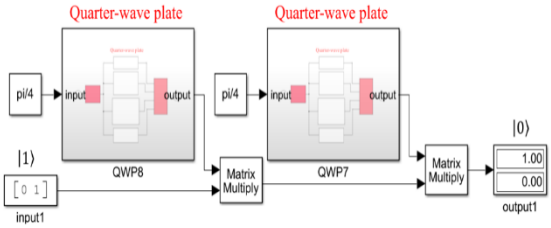


Figure 4: Pauli-X Gate Simulation with input $|\mathbf{1}\rangle$.

2.2 Pauli-Y Gate

When $\theta = 0$, Projection effect matrix for a quarter-wave plate $\hat{A}_Q(\theta)$ become:

$$\hat{A}_Q(0) = \begin{pmatrix} 1 & 0 \\ 0 & i \end{pmatrix}. \quad (13)$$

When $\theta = \frac{\pi}{4}$, Projection effect matrix for a quarter-wave plate $\hat{A}_Q(\theta)$ become:

$$\hat{A}_Q\left(\frac{\pi}{4}\right) = \begin{pmatrix} \frac{1}{2}+\frac{1}{2}i & \frac{1}{2}(1-i) \\ \frac{1}{2}(1-i) & \frac{1}{2}+\frac{1}{2}i \end{pmatrix}. \quad (14)$$

When $\theta = \frac{\pi}{2}$, Projection effect matrix for a quarter-wave plate $\hat{A}_Q(\theta)$ become:

$$\hat{A}_Q\left(\frac{\pi}{2}\right) = \begin{pmatrix} i & 0 \\ 0 & 1 \end{pmatrix}. \quad (15)$$

Pauli-Y gate equals:

$$\hat{Y} = \hat{A}_Q(0)\hat{A}_Q\left(\frac{\pi}{2}\right)\hat{A}_Q(0)\hat{A}_Q(0)\hat{A}_Q\left(\frac{\pi}{4}\right)\hat{A}_Q\left(\frac{\pi}{4}\right), \quad (16)$$

$$\hat{Y} = \begin{pmatrix} 0 & -i \\ i & 0 \end{pmatrix}. \quad (17)$$

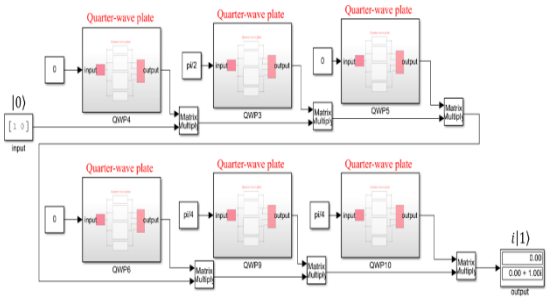


Figure 5: Puli-Y Gate Simulation with input $|\mathbf{0}\rangle$.

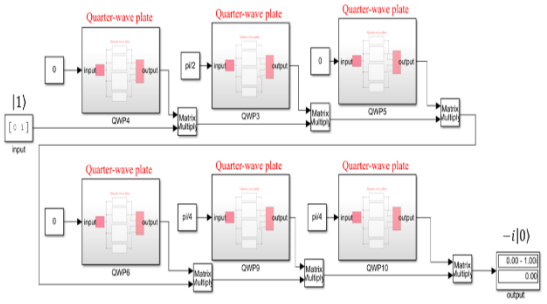


Figure 6: Puli-Y Gate Simulation with input $|\mathbf{1}\rangle$.

2.3 Pauli-Z Gate

When $\theta = 0^{\circ}$, Projection effect matrix for a quarter-wave plate $\hat{A}_Q(\theta)$ become:

$$\hat{A}_Q(0) = \begin{pmatrix} 1 & 0 \\ 0 & i \end{pmatrix}. \qquad (18)$$

From (18) it is clear that a quarter-wave plate can be used to obtain the S-gate, which is also known as the square root of the Z-gate.To obtain the Pauli-Z gate, two quarter-wave panels are used, and the angle of each plate is set at 0. Equation (18) represents the matrix for each plate.

$$\hat{Z} = \hat{A}_Q(0) \cdot \hat{A}_Q(0) = \begin{pmatrix} 1 & 0 \\ 0 & -1 \end{pmatrix}. \qquad (19)$$

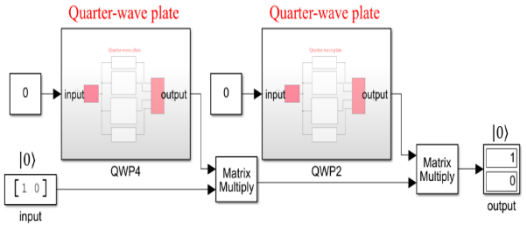


Figure 7: Puli-Z Gate Simulation with input $|\mathbf{0}\rangle$.

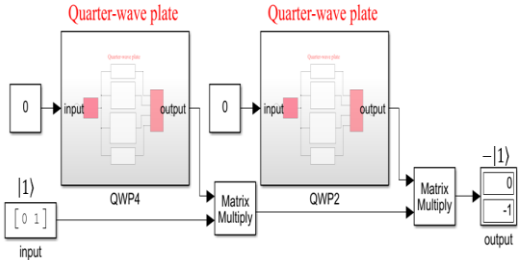


Figure 8: Puli-Z Gate Simulation with input $|\mathbf{1}\rangle$.

To create a Pauli-Z gate in a second way, we use two quarter-wave panels; the angles of the plates are set to π degrees each. The projection effect of the quarter-wave panel is $\hat{A}_Q(\pi) = \hat{A}_Q(0)$, so the Pauli-Z gate matrix remains unchanged, as in (19).

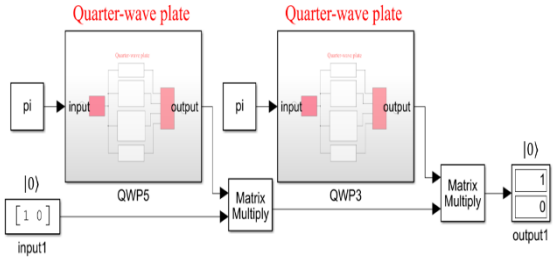


Figure 9: Puli-Z Gate Simulation with input $|\mathbf{0}\rangle$.

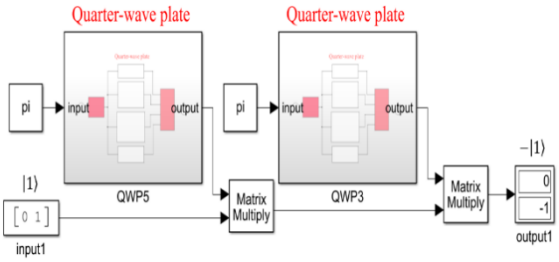


Figure 10: Puli-Z Gate Simulation with input $|\mathbf{1}\rangle$.

2.4 Hadamard Gate

When $\theta = \pi/8$, Projection effect matrix for a quarter-wave plate $\hat{A}_Q(\theta)$ become:

$$\hat{A}_Q\left(\frac{\pi}{8}\right) = \begin{pmatrix} M & Q \\ N & R \end{pmatrix} \qquad (20)$$

Where the letters M, N, Q and R represent the following complex numbers:

M=0.8536+1465i,

N=0.3536-0.3536i,

Q=0.3536-0.3536i,

R=0.1465+0.8536i;

To obtain the Hadamard gate, two quarter-wave panels are used, and the angle of each plate is set at $\theta = \pi/8$. (20) represents the matrix for each plate. The Hadamard gate becomes:

$$\widehat{H} = \hat{A}_Q\left(\frac{\pi}{8}\right) \cdot \hat{A}_Q\left(\frac{\pi}{8}\right) = \frac{1}{\sqrt{2}}\begin{pmatrix} 1 & 1 \\ 1 & -1 \end{pmatrix} \qquad (21)$$

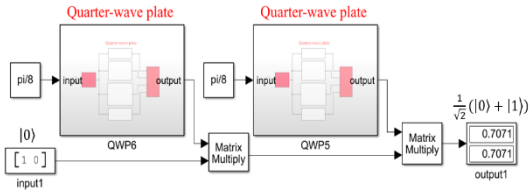


Figure 11: Hadamard Gate Simulation with input $|\mathbf{0}\rangle$.

Determining the optimal QWP angle as a critical factor in improving the performance of a quantum gate has enhanced the understanding of optical transitions and their impact on quantum information.

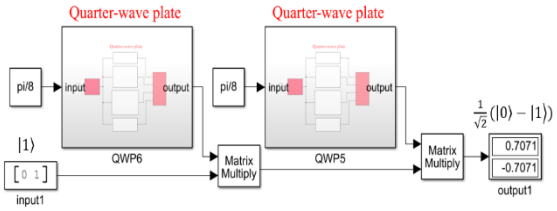


Figure 12: Hadamard Gate Simulation with input $|\mathbf{1}\rangle$.

RESULTS AND DISCUSSION

3.1 Results

In constructing the Pauli-x gate, we used two quarter-wave plates with an angle of $\pi/4$ for each plate. While the Pauli-Y gate was designed using six quarter-wave plates, three of which are at an angle of 0, two at an angle of $\frac{\pi}{4}$ for each plate, and one at an angle of $\pi/2$. As for the Pauli-z gate, we were able to construct it in two ways, using two of quarter-wave plates with an angle of 0 for each plate for the first design, and a pair of quarter-wave plates with an angle of π for each plate for the second design. In the Hadamard gate, two quarter-wave plates with an angle of $\theta = \pi/8$ for each plate were used. All the gates under study were tested in the quantum states 0 and 1.

3.2 Discussion

Quarter-wave panels are a great choice for complex quantum computing applications due to the polarization precision they provide. In the quantum gate X using two quarter-wave plates, their operation is illustrated in Figures 3 and 4. When the angle of each plate is set to $\pi/4$, we find that the input quantum state $|\mathbf{0}\rangle$ has been transformed to $|\mathbf{1}\rangle$and vice versa, thus achieving the desired polarization. The Y-quantum gate, six quarter-wave plates with angles arranged at 0, $\pi/2$, 0, 0, $\pi/4$, and $\pi/4$are used to produce the Pauli-Y gate. We find that introducing the quantum state $|\mathbf{0}\rangle$ onto the six polarization plates one by one will produce a new quantum state $i|\mathbf{1}\rangle$ with a change in direction and phase, as shown in Figure 5. While in Figure 6, we find that introducing the qubit $|\mathbf{1}\rangle$ will produce the qubit $-i|\mathbf{0}\rangle$ which means there is a change in direction and phase. From Figures 7 and 9, we see

that the quantum state $|\mathbf{0}\rangle$ is not affected and remains in the same state after passing through the quarter-wave plates that form the Pauli-Z gate, while the quantum state $|\mathbf{1}\rangle$ becomes $|-\mathbf{1}\rangle$, which means that the phase has changed by π, as is clear in Figures 8 and 10. Finally, the Hadamard gate shows an equal superposition between 0 and 1 when state 0 is introduced, as in Figure 11. While the Hadamard gate is applied to state 1, it also creates a quantum superposition, but with a different phase, note Figure 12. The design of single quantum gates using a quarter-wave plate can be said to be an important step towards enhancing the efficiency of quantum systems.

4 CONCLUSIONS

The use of QWP has been shown to improve the fidelity of qubit transitions between 0 and 1, demonstrating the effectiveness of quantum gates in reducing errors and improving the overall performance of quantum systems. Determining the optimal QWP angle is also a critical element in the design of quantum gates.

Quarter-waveplate quantum gates offer a simple and efficient optical design. However, practical constraints such as fabrication precision, optical alignment, and environmental factors such as temperature and pressure affect the performance of the quantum system due to their impact on the refractive index of these panels. Therefore, improving lattice fabrication methods and minimizing optical losses are essential to achieving optimal quantum system performance.

The physicist's understanding of the operation of quarter-wave panels contributes to the design of highly efficient quantum gates to improve innovation in the production of a quantum computer with distinctive capabilities.

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Study of Some Structural, Optical and Electrical Properties of Thin Film Selenium Sulfide Tin Prepared by the Thermal Evaporation Method in Vacuum

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Keywords: Vacuum Thermal Deposition, Optical, Structural, Electrical Properties.

Abstract: This research includes the preparation of thin films of $Se_{75}S_{25-x}Sn_x$ by vacuum thermal evaporation method with a thickness of 400 +-20 nm on glass substrates and studying some structural, optical and electrical properties of the film. X-ray examination detect that the film has a random structure at (x=0 and 5) while single crystal growth begins at x=10,15. By measuring the transmittance and absorbance spectra for the wavelength range 400 – 1100 nm it was found that the transmittance decreases and the absorbance increases as a function of wavelength with increasing tin content. The energy gap for the indirect transition allowed was also calculated and shown to decrease with increasing tin content. The Hall effect examination revealed that the film prepared at (x=0 and 5) is of the P type while at x=10, 15 it turns into the N type and that the conductivity increases with increasing tin content and the resistivity decreases.

1 INTRODUCTION

Chalcogenide glasses have gained great importance in recent years due to their advantageous properties, and have been widely used in various solid-state devices. Therefore, the search for new materials for use in device technology is ongoing. Finding new materials that can modify their properties is key to the development of solid-state technology. Semiconductors can be used to manufacture devices with specific properties by controlling the structural, optical, and electrical properties of these materials. Trigonal alloy semiconductors provide such materials [1]. The great interest of researchers is due to the fact that these materials combine the properties of random materials and some of the properties of crystalline semiconductors [2]-[9].Many studies have been conducted on selenium-treated metallic glass with regard to its electrical and optical properties [10]-[20]. Based on this, we use selenium due to its many commercial applications [21], but it has drawbacks in its pure state due to its poor sensitivity and short lifetime. To eliminate these drawbacks, we use some additives, thus obtaining better properties of hardness, sensitivity, conductivity, and lifetime compared to pure selenium [22]. In this system, we used tin in the Se-S system. This addition expands the area of glass formation and causes a disturbance in the composition and structure of the system. [23]

2 MODELING AND WORKING METHODS

Thin films of $Se_{75}S_{25-x}Sn_x$ were prepared using the vacuum evaporation method. The $Se_{75}S_{25-x}Sn_x$ compound was prepared by mixing proportions of each of its constituent elements with a purity of 99.99% and according to the x values of 0, 5, 10, 15%. After determining the mass of the mixture (6g), the mass of each constituent element was calculated using a sensitive electric balance with four-decimal-place accuracy. The mixture was placed in quartz tubes (15cm length). The tubes were evacuated using a mechanical vacuum system to prevent oxidation during heating and sealed with an oxyacetylene torch. They were then placed in an electric furnace and gradually heated from room temperature to 450°C (above the melting points: Se 221°C, S 113°C, Sn 232°C) for two hours. The tubes were angled at 45° to ensure homogeneity and left for 24 hours to cool gradually to room temperature.

After removal from the furnace, the compound was extracted, crushed using a hammer and ceramic bowl, and stored as a powder in clean, dry containers. An appropriate amount of the $Se_{75}S_{25-x}Sn_x$ powder was placed in a molybdenum boat to achieve the required thickness according to the equation *m = 4πptr²* [24]. The evaporation system was prepared by placing the boat between electrodes and fixing thoroughly cleaned substrates on the holder at a distance from the source to dissipate heat and ensure uniform deposition. Film thickness was measured gravimetrically using $t = m/(S \cdot \rho)$ [25].

3 RESULTS AND DISCUSSION

3.1 Structural Properties

3.1.1 X-ray Diffraction

Figure 1 shows the X- ray diffraction patterns of the $Se_{75}S_{25-x}Sn_x$ films. It is evident that at a tin content of 0,5 there are no clear peaks, confirming the random nature of these films. As the tin content increases (10, 15) a single peak begins to appear, becoming more pronounced with increasing tin content. This indicates that increasing the tin content improves the crystallization process [26].

3.1.2 FESEM Analysis

All $Se_{75}S_{25-x}Sn_x$ films were examined using a scanning electron microscope (FESEM) at a magnification of 500 nm to determine the surface texture of the films and observe the effect of tin doping ratios. It was found that varying tin percentages had a clear effect on the surface structure of these films. This is consistent with most studies of the random state in electron microscopy [27]. Figure 2 shows that the films exhibit a random appearance. However, for films doped with 15% tin, we observe the appearance of a granular shape consisting of small, uniform, and almost homogeneous spherical grains.

3.2 Optical Properties

3.2.1 Absorption

The optical properties of $Se_{75}S_{25-x}Sn_x$ films x=0, 5, 10, 15 deposited on glass slides by vacuum evaporation were studied. Figure 3 shows how the absorbance (A) changes as a function of wavelength (λ). We observe that all films have their highest absorbance at wavelengths below 500 nm, reaching (2.75-3.75). This means that the absorbance is highest at the beginning of the visible region, where the energy is greater than the energy gaps for the indirect transitions allowed by the films, which have values of (2 - 1.63ev) and will be presented later. This means that the absorbed photons are able to excite the electrons in the valence band and transfer them to the conduction band. Then we notice a sharp drop in the absorbance at large wavelengths. We also notice that the absorbance increases with the increase in the tin percentage due to the absorption processes formed by the tin doping levels, meaning that the tin levels worked to form local levels between the valence and conduction bands, which worked as auxiliary levels that enabled the electrons that did not absorb enough energy to overcome the energy gap to move from the valence band to the conduction band. Also, the sharp edges were shifted towards the longer wavelengths [28], and the largest shift was at the ratio of 15%.

3.2.2 Transmittance

Transmittance behaves in complete opposition to absorbance.Figure 4 shows the transmittance spectrum as a function of wavelength in the spectrum region (400 - 1100 nm). We note that the transmittance of all prepared films increases with increasing wavelength, up to the near-infrared region, where it reaches 80% at x=0, 70% at x=5, and 30% at x=10.15. This means that transmittance decreases when the material is doped with tin [29]-[30]. The decrease in the transmittance spectrum is attributed to the increased light absorption by the localized levels formed by tin within the prepared energy gap. We also observe the oscillatory behavior of all films, indicating that they are homogeneous.

3.2.3 Optical Energy Gap

The optical energy gap value for the allowed indirect transmission was calculated from the equation $\alpha h\upsilon = \beta(h\upsilon - E_g^{opt})^r$[31] by setting (r = 2). We then draw the relationship between $(\alpha h\upsilon)1/2$ on the y-axis and $(h\upsilon)$ the photon energy on the x-axis see Figure 5. The energy gap is determined from the intersection of the straight line of the curve with the x-axis, represented by the photon energy at $\alpha = 0$. This point represents the energy gap

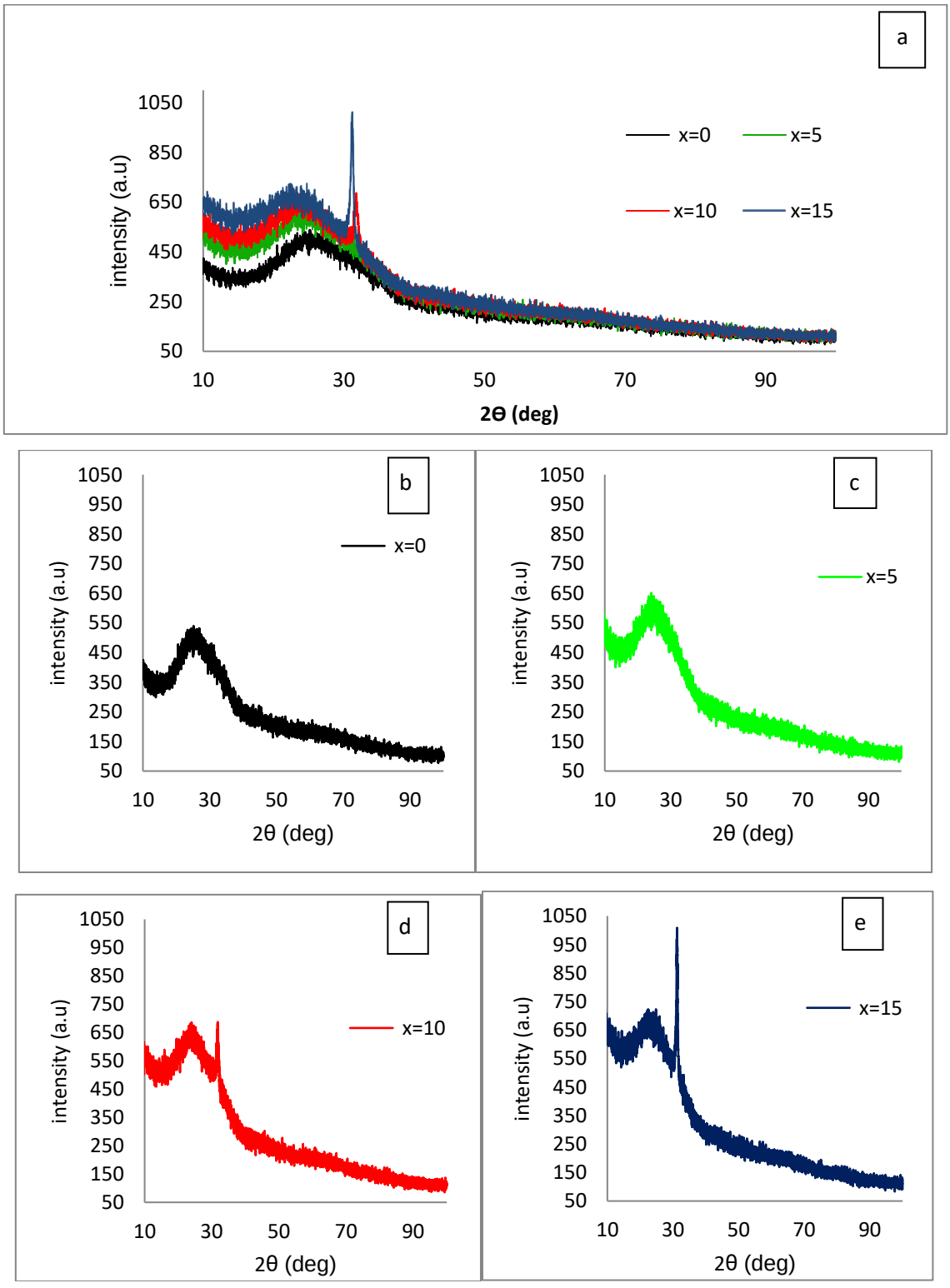


Figure 1: XRD patterns of $Se_{75}S_{25-x}Sn_x$ thin films with different tin concentrations a), b), c), d) and e).

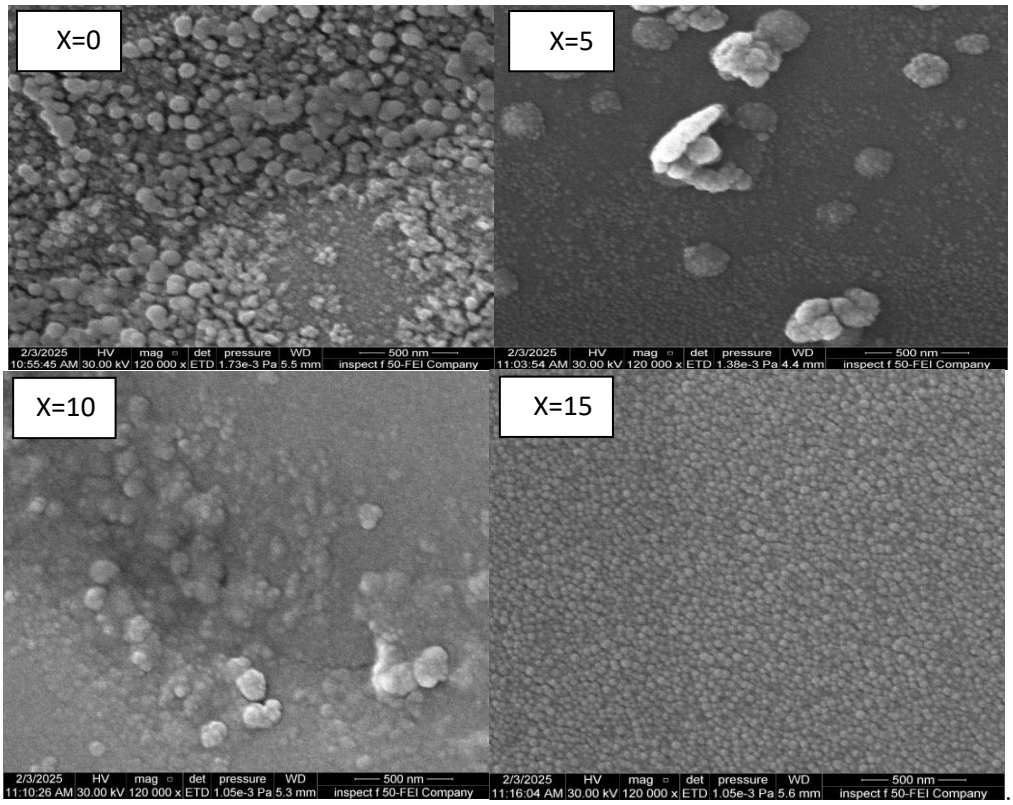


Figure. 2: FESEM micro image of $Se_{75}S_{25-x}Sn_x$ thin film at different concentrations of tin.

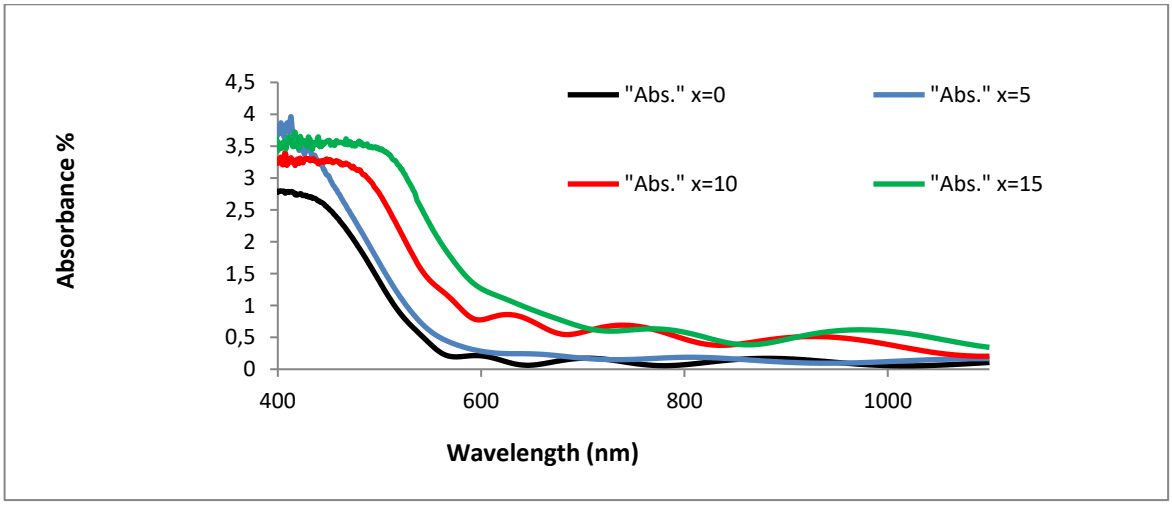


Figure 3: Absorption spectrum as a function of wavelength of $Se_{75}S_{25-x}Sn_x$ thin film at different concentrations of tin.

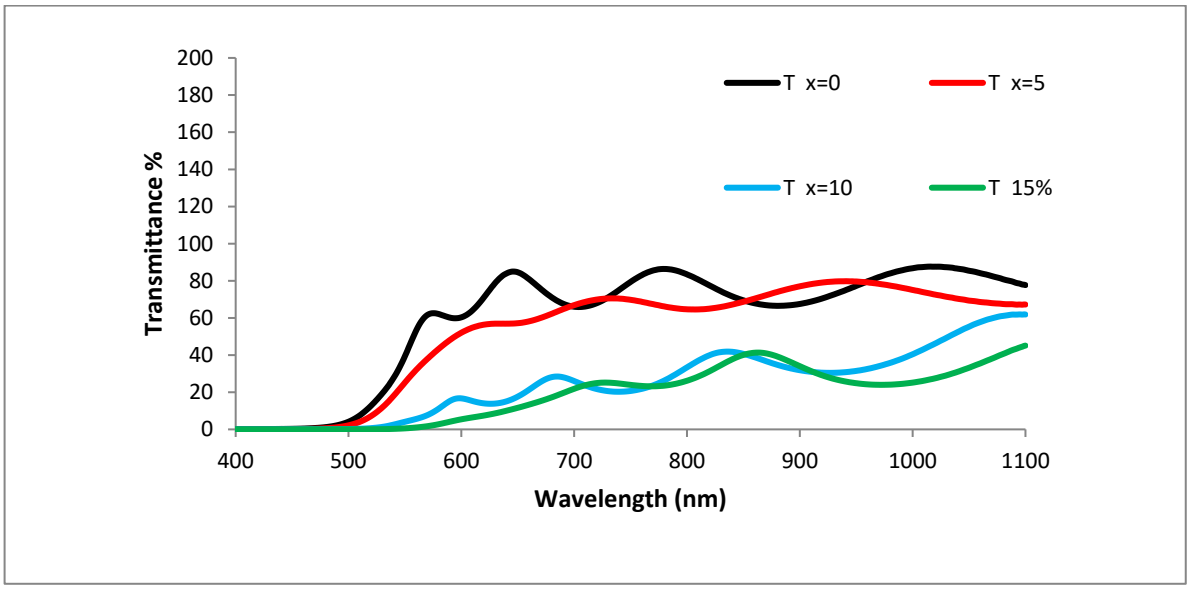


Figure 4: Transmittance spectrum as a function of wavelength of $Se_{75}S_{25-x}Sn_x$ thin film at different concentrations of tin.

Table 1: The energy gap values of $Se_{75}S_{25-x}Sn_x$ thin film at different concentrations of tin.

Concentration x	Eg (ev)
0	2
5	1.9
10	1.73
15	1.63

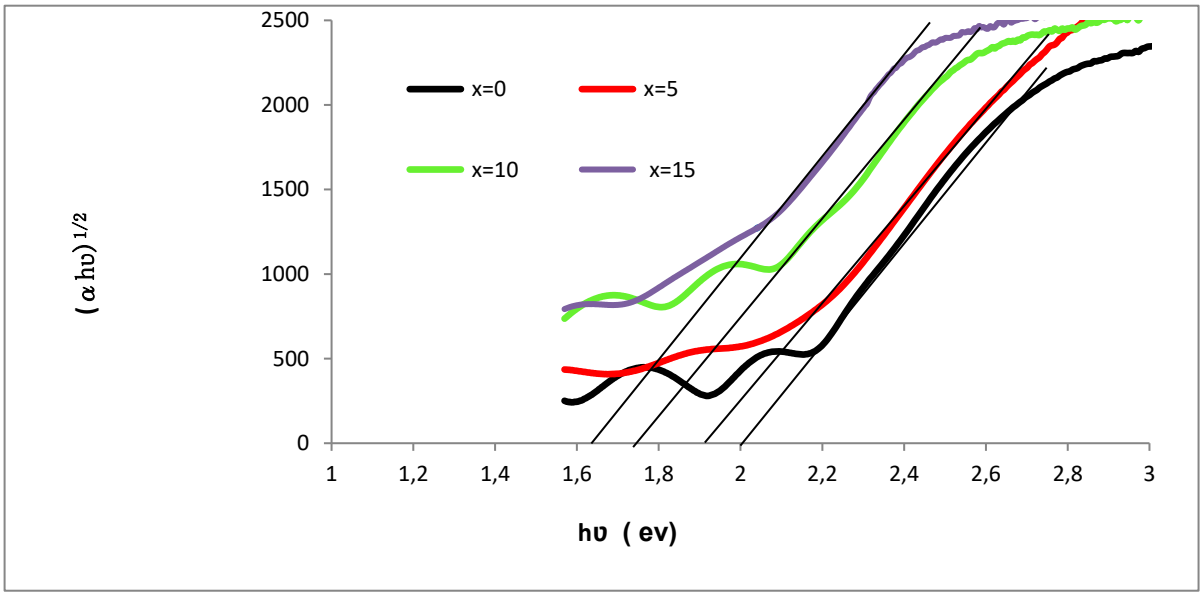


Figure. 5: Change in energy gap for indirect transition allowed for $Se_{75}S_{25-x}Sn_x$ thin film at different concentrations of tin.

Table 2: Hall effect investigation data on $Se_{75}S_{25-x}Sn_x$ thin films.

Concentration	0	5	10	15
RH(cm^3/c) *10^7	1.992	1.610	-0.119	-1.616
N(cm^{-3}) *10^{11}	3.133	3.877	-52.26	-3.433
μ(cm^2/v.s)	41.84	56.36	17.87	580
ρ(Ω.cm) *10^5	4.762	2.857	0.6682	0.3135
σ(Ω.cm)$^{-1}$ *10^{-6}	2.1	3.5	14.97	31.90

value, which changes from 2 eV to 1.63 eV See Table 1, decreasing with increasing doping ratio, i.e., by 0.37 eV. The decrease in the energy gap value with doping with metals is consistent with researcher [32], who used indium, and researcher [33], who used silver. The explanation for this is that tin results in an increase in the density of the localized levels formed by tin atoms within the energy gap

3.3 Electrical

3.3.1 Hall Effect

A room-temperature Hall effect experiment was conducted on $Se_{75}S_{25-x}Sn_x$, x=0.5, 10.15. Thin films are used to determine their electrical properties and to know the concentration, type, and movement of the majority carriers of charge, as well as their conductivity and resistivity. Results in Table 2 show that the conductivity is P- type at x=0.5, where the Hall coefficient is positive, meaning holes are the majority charge carriers while electrons are the minority

However, it transforms to N-type at x=10.15, where the Hall coefficient becomes negative, meaning electrons are the majority carriers, while holes become the minority.

We note that with the increase of tin content, the conductivity increases, reaching its highest value 31.9*10-6 at x=15. The resistivity decreases with increasing tin content

4 CONCLUSIONS

This study demonstrates that tin (Sn) doping significantly modifies the structural, optical, and electrical properties of vacuum-evaporated Se-S thin films. Specifically:

1) X-ray diffraction (XRD) analysis revealed an initially amorphous structure for all films, with crystallinity improving as Sn content increased, evidenced by the emergence of a distinct peak, particularly pronounced at x=15 (confirmed by FE-SEM images).
2) Optical measurements over 400–1100 nm showed decreasing transmittance and increasing absorbance with higher Sn concentrations.
3) The calculated energy gap for the allowed indirect transition decreased with increasing Sn content.
4) Hall effect measurements indicated a transition from p-type to n-type conductivity with increasing Sn. This resulted in significantly increased conductivity and decreased resistivity due to the higher concentration of electron charge carriers provided by the metal dopant.

The practical significance of this work lies in enhancing Se-S properties for potential applications in solar cells, optical filters, and solid-state devices.

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Physical and Sensing Properties of Aluminum-Doped Iron (III) Oxide Thin Films Deposited via Chemical Spray Pyrolysis

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Keywords: Aluminum-Doped Iron Oxide, Thin Film, CSP, XRD, AFM, Optical Properties, Sensitivity and Resistance.

Abstract: Fe_2O_3:Al (1–3 at%) thin films were successfully grown on glass substrates at 400°C using the chemical spray pyrolysis (CSP) technique. The X-ray diffraction (XRD) patterns confirmed the formation of α-Fe_2O_3 with a prominent (104) peak, indicating high crystallinity. The average grain size (D) increased from 15.82 nm to 18.70 nm with increasing Al content, while the lattice strain (ε) decreased from 2.18 to 1.85, suggesting improved crystal quality. Atomic Force Microscopy (AFM) analysis showed a reduction in surface roughness and uniform particle distribution, with particle diameters ranging from 65.5 nm to 52.31 nm. Optical studies revealed a gradual narrowing of the bandgap values from 2.81 eV (undoped) to 2.74 eV, 2.69 eV, and 2.64 eV for 0 at%, 1 at%, and 3 at% Al-doping levels, respectively. Furthermore, gas sensing tests demonstrated that higher Al doping increased resistance and reduced sensitivity toward NO_2 gas due to enhanced charge carrier recombination and altered surface interactions, indicating significant influence on semiconductor gas sensing properties.

1 INTRODUCTION

Iron oxide thin films (Fe_2O_3) doped with Al (1–3 at%) were successfully deposited on glass substrates at 400 °C using the chemical spray pyrolysis (CSP) technique [1], [2] - [5]. Iron oxides exist in different stoichiometries and crystalline forms, including FeO, Fe_3O_4, α-Fe_2O_3, and γ-Fe_2O_3, with hematite (α-Fe_2O_3) being the most stable phase [6] - [9]. Its favorable properties such as non-toxicity, low cost, and wide availability make it highly suitable for numerous applications [10] - [12].

The structural analysis by X-ray diffraction (XRD) confirmed the formation of α-Fe_2O_3 with a dominant (104) reflection, indicating high crystallinity. The average crystallite size increased from 15.82 nm to 18.70 nm with rising Al concentration, while the lattice strain decreased from 2.18 to 1.85, suggesting improved crystal quality. Atomic force microscopy (AFM) revealed a reduction in surface roughness and a more uniform particle distribution, with average particle sizes ranging from 65.5 nm to 52.31 nm.

Optical characterization showed a progressive narrowing of the optical bandgap from 2.81 eV (undoped) to 2.74 eV, 2.69 eV, and 2.64 eV for 0 at%, 1 at%, and 3 at% Al doping, respectively [19], [20]. Since the ionic radii of Al^{3+} (0.675 Å) and Fe^{3+} (0.69 Å) are close, partial substitution of Fe^{3+} by Al^{3+} is expected, influencing both the structural and optical properties of the films.

Gas sensing studies demonstrated that higher Al doping increased electrical resistance and reduced sensitivity toward NO_2 due to enhanced charge carrier recombination and modified surface interactions [10] - [14]. While various preparation techniques such as sol–gel [15], electrodeposition [16], sonochemical methods [17], and electrospinning [18] have also been employed for Fe_2O_3 films [19] - [22], CSP remains an efficient and attractive approach for large-

area deposition and detailed characterization [23], [21] - [24].

2 EXPERIMENTAL

Thin films of Fe_2O_3: Al is prepared by (CSP) technique. 0.1 M of both $AlCl_2$ and $FeCl_3$ diluted with redistilled. Al 1% and 3% was utilized. The deposition circumstances were: spray time 8 s and then stopped 1 min. The carrier gas used was air, with a pressure of 10^5 Pa, and the distance between the spout and the base was 28 cm ± 1 cm. Film thickness was found to be 325 ± 30 nm. XRD analysis verified the formation of a Fe_2O_3 thin film, and AFM is used to determine film shape. Transmittance was done via a spectrophotometer. Undoped and Al-doped Fe_2O_3 gas sensors were tested for sensitivity by resistance change in a 7.5×15 cm cylindrical chamber.

3 RESULTS AND DISCUSSION

XRD spectra of Fe_2O_3Al films are displayed in Figure 1. ICDD card no. 40-1139 and 39-1364 was fitted with the obtained data. Diffraction peaks correspond to (221), (104) and (116) planes. The shift in peak position is that the ionic radius of Al^{3+} is less than that of Fe^{3+} (0.53 Å compared to 0.69Å) [25]. The sharp and intense diffraction peaks are clearly indexed to the α-Fe_2O_3 crystalline phase.

The average crystallite size (D) is offered via Scherrer (1) [26]:

$$D = \frac{0.9\lambda}{\beta cos\theta}. \quad (1)$$

Where θ is Bragg angle, β is FWHM, and λ is X-ray wavelength. *D* are in range of 15–18 nm. The strain ε is obtained by utilizing the following by (2) [27]:

$$\varepsilon = \frac{\beta cos\theta}{4}. \quad (2)$$

The dislocation density δ is obtained by the following relation [28]

$$\delta = \frac{1}{D^2}. \quad (3)$$

It is mentioned that as Al content increases, δ and ε decrease and is assigned to the variation in ionic radii of Al^{3+} and Fe^{3+} as shown in Figure 2 c and d. the crystallite size is decreased; however, after doping, there is large crystallites that agree with [30], [32]. The values for *D* and ε are given in Table 1. A slight increase in *D* and a decrease in ε. These results agree with [33], [34]. The structural parameters are calculated and listed in Table 1.

AFM images of the grown films are offered in Figure 3, offering films uniformity. The Average Particle size P_{av} is seen as 65.5 nm, 58.7 nm and 52.3 nm for the concentration of 0%, 1% and 3%, respectively. Generally, the roughness R_a of films in the area of 8.4-4.8 nm. The undoped film surface exhibits a larger root-mean-square (rms) roughness of 9.4 nm. However, rms and R_a decrease is seen for 1% and 3% Al. Similar result is noticed in [35], [36]. The summary of the surface topography S_T is shown in Table 2.

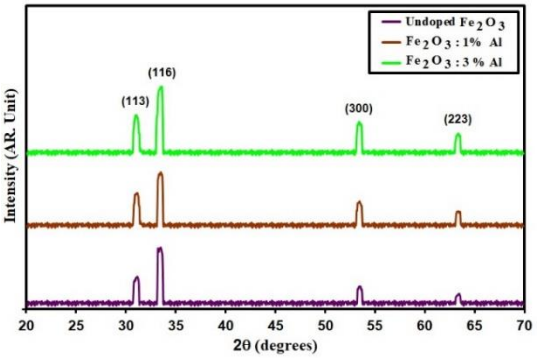


Figure 1: XRD patterns of of Undoped and Fe_2O_3: Al films.

Table 1: Structure parameters of Undoped and Fe_2O_3: Al films.

Samples	(hkl) Plane	2□ (º)	FWHM (º)	E_g eV	*D* nm	δ X(10^{15})(line/m^2)	ε (×10^{-3})
Undoped Fe_2O_3	104	33.82	0.51	2.74	15.8	3.9	2.1
Fe_2O_3: 1% Al	104	33.79	0.48	0.69	17.1	3.4	2.0
Fe_2O_3: 3% Al	104	33.74	0.44	2.64	18.7	2.8	1.8

Table 2: The Morphological values of Undoped and Fe_2O_3: Al films.

Samples	P_{av},nm	R_a, nm	R_{ms}, nm
Undoped Fe_2O_3	65.5	8.7	9.4
Fe_2O_3: 1% Al	58.7	6.3	8.7
Fe_2O_3: 3% Al	52.3	4.8	5.4

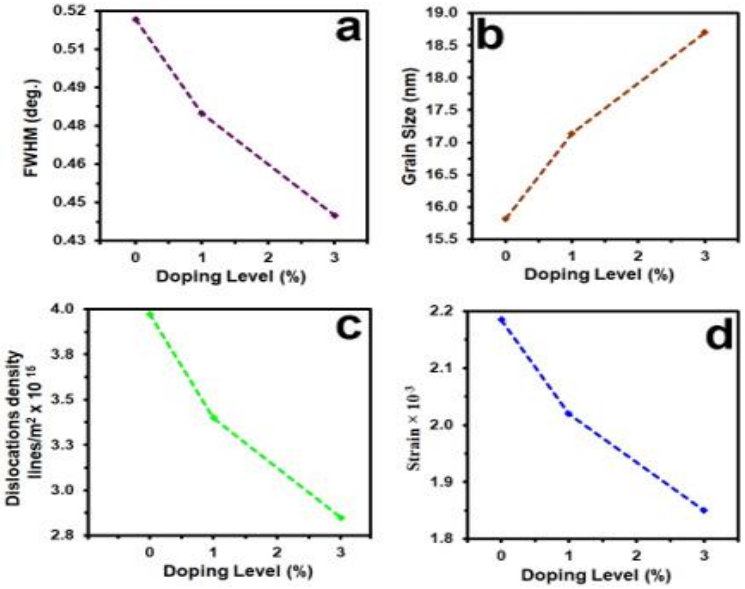


Figure 2: X-ray parameter of Undoped and Fe_2O_3: Al films.

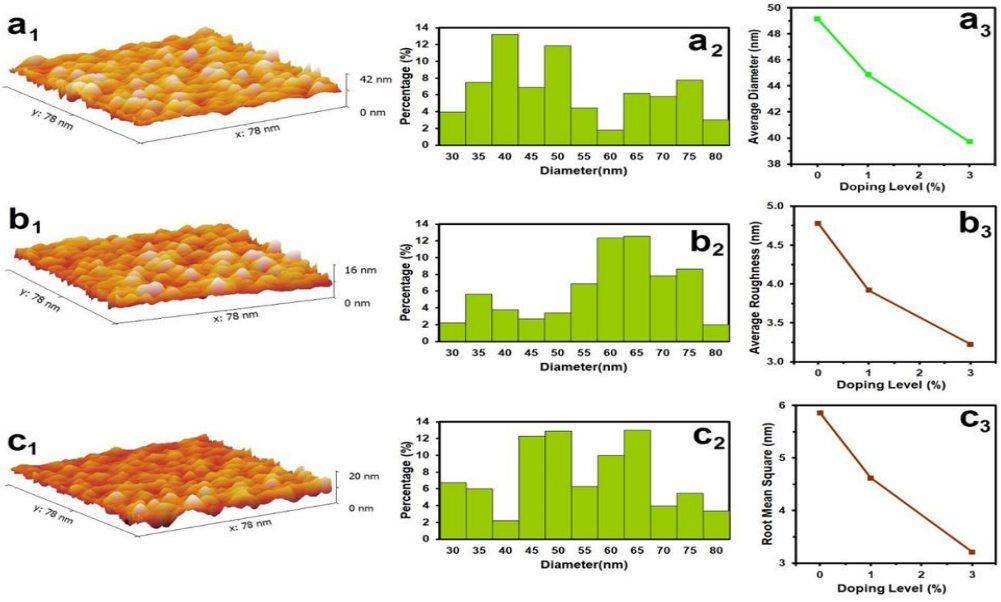


Figure 3: AFM images of Undoped and Fe2O3: Al films.

The transmittance T of the intended films is displayed in Figure 4. Films have a transparency of 65 %. From Figure 4. The doping decreases T, which refers to strong scattering and absorption [37], [38]. The drop in T with increasing Al content can be assigned to the surrogate of Fe+3 by Al^{+3}, which reduces light in the T band. This behavior is similar to that observed in [39], [40].

The absorption coefficient (α) was evaluated using the optical transmittance spectra. α is calculated using (4) [41]:

$$\alpha = \frac{1}{d} ln \frac{I_0}{I} = 2.303(\frac{A}{d}), \quad (4)$$

where d is film thickness. Figure 5 shows α versus the photon energy ($h\nu$) of various doping of Al. High

α between 1×10^4cm^{-1}and 6×10^4 cm^{-1} in the visible area. Also, we note that the α increased with film doping increasing.

Optical bandgap, denoted by (5) is computed employing the relation below [42]:

$$(\alpha hv) = A\left(hv - E_g\right)^n, \quad (5)$$

where A is constant and n = ½, for allowed direct transitions. Figure 6. shows E_g values determined as 2.74, 2.69 and 2.64, 2.81 at 0.at%, 1 at% and 3 at% of Al respectively. This decrease in bandgap indicates that Al provides Al^{3+} ions [43], [44].

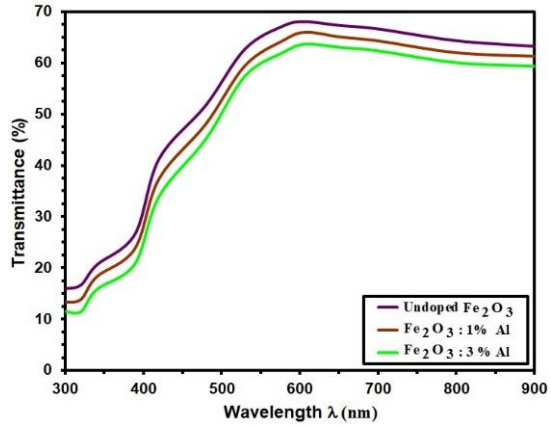


Figure 4: Transmittance of Undoped and Fe_2O_3: Al films.

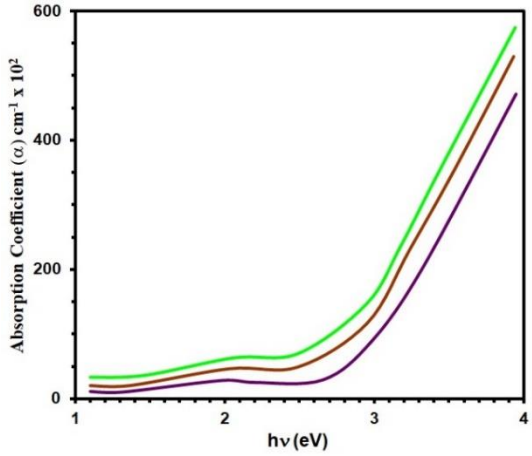


Figure 5: Absorption coefficient (α) of Undoped and Fe_2O_3: Al films.

The formula below [36] evaluates the extinction coefficient (k) [45, 46]:

$$k = \frac{\alpha\lambda}{4\pi}. \quad (6)$$

k against wavelength is shown in Figure 7. There is a decrease k in the visible area of 550-700 nm**,** then a rapid rise within the 400-500 nm area. k increasing with Al content and arriving at the highest value at 3wt% content, this increase is related to an increase in carrier concentration in CB according to Burstein-Moss effect [47], [48].

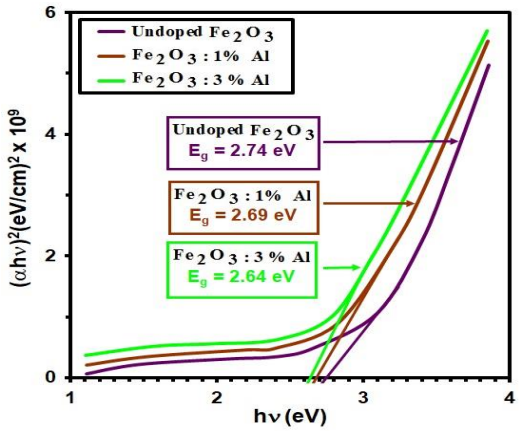


Figure 6: Optical bandgap (Eg) of Undoped and Fe_2O_3: Al films

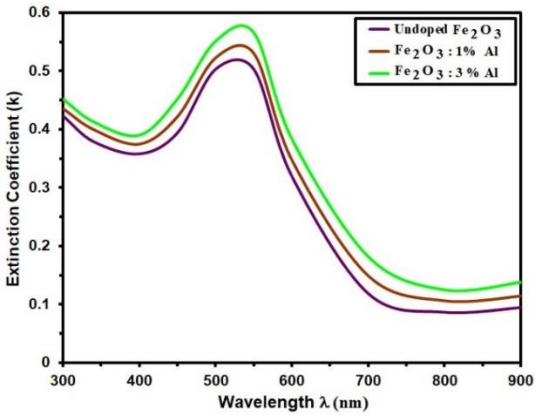


Figure 7: Extinction coefficient (k) of Undoped and Fe_2O_3: Al films.

The refractive index (n) is obtained by the relation [49]:

$$n = \frac{1+R^{\frac{1}{2}}}{1-R^{\frac{1}{2}}}. \quad (7)$$

Figure 8 offers n plotted via wavelength. The values n is in the area of 3.5–3.75 and are almost constant at longer wavelengths. It can be assigned to film polarization [40], [51]. These results assure using this composite as a window layer of solar cell [52], [53].

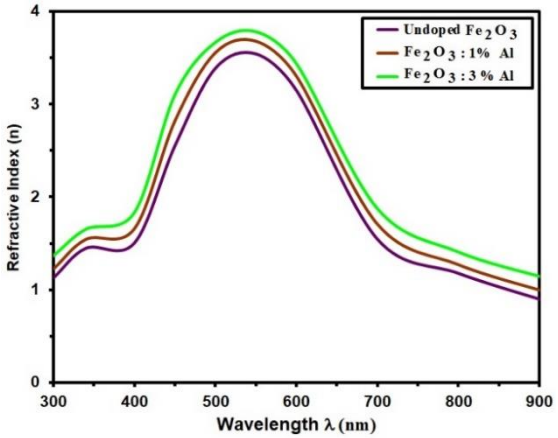


Figure 8: Refractive index (n) of Undoped and Fe_2O_3: Al films.

Figure 9 shows increased resistance over time for undoped and Al-doped Fe_2O_3 films exposed to 300 ppm NO_2 at 150 °C, indicating surface oxidation during gas exposure [54], [55]. NO_2 adsorption on Fe_2O_3 films removes O2+ ions, releasing electrons to the conduction band and increasing resistance. Al doping, especially at 4%, significantly enhances resistance, demonstrating its strong effect on sensing and semiconductor properties[56] - [58].

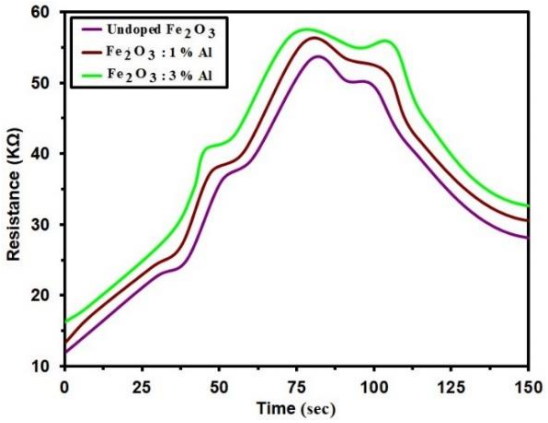


Figure 9: The variation of resistance over time for Fe2O3 and Al-doped Fe2O3 films.

Sensitivity (S) is is calculated using the following (8) [59]:

$$Sensitivity = \frac{\Delta R}{R_g} = \left|\frac{R_g - R_a}{R_g}\right| \times 100\ \%\ . \quad (8)$$

Figure 10 shows reduced sensitivity with higher Al content due to increased resistance from charge carrier recombination after NO_2 exposure. Increasing Al doping leads to greater charge carrier recombination, reducing conductivity and gas response [60]. Sensitivity drops significantly at higher doping levels, indicating suppressed NO_2 sensing performance of Fe_2O_3 films due to reduced free carrier availability [61].

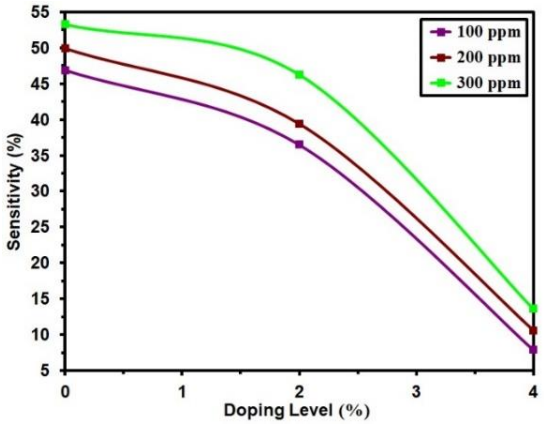


Figure 10: Sensitivity (S) as a function of Al-doped Fe_2O_3 films at different concentrations.

4 CONCLUSIONS

Aluminum-doped iron oxide (Fe_2O_3:Al) thin films were successfully synthesized using the chemical spray pyrolysis (CSP) technique at a substrate temperature of 400°C. Structural analysis confirmed the formation of a well-crystallized α-Fe_2O_3 phase across all doping levels, with enhanced crystallinity observed at 3 at% Al. The incorporation of Al^{3+} ions led to a slight increase in crystallite size and a reduction in lattice strain and dislocation density. Morphological analysis revealed that increasing Al content resulted in more uniform surface morphology, reduced particle size, and lower surface roughness, as confirmed by AFM measurements. These improvements are beneficial for optical and electronic applications. Optical characterization showed that the transmittance and optical bandgap (Eg) decreased with increasing Al doping, while the extinction coefficient (k) and refractive index (n) increased. The bandgap narrowing is attributed to enhanced carrier concentration and Al^{3+} substitution effect. Gas sensing performance tests indicated that while Al doping improved film crystallinity and morphology, it adversely affected sensitivity to NO_2 gas. Higher Al concentrations led to increased resistance and suppressed sensor response due to enhanced charge carrier recombination and reduced availability of free carriers.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to Mustansiriyah University and Al-Nukhba University College for their support.

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Application of Modern Software Complexes in the Analysis of the Parameters of Friction Pairs of the Spring-Friction Apparatus of a Freight Car for their Efficiency

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Keywords: Digital Technologies, New Software System, Computer Program Railway, Freight Car, Friction, Absorbing Apparatus, Friction Wedge, Plank, Railway Transport.

Abstract: This study presents comprehensive analytical and numerical modeling of the force interactions in friction pairs, specifically between the "clamping beam – friction wedge" and "friction wedge – friction rod," using the freight car model 18-9597 as a representative case. By integrating both analytical derivations and finite element simulations, the research identifies rational design parameters that ensure stable, efficient, and long-term performance of the spring-friction damping unit in freight car bogies. The study not only enhances the understanding of contact mechanics and frictional behavior under dynamic loading but also provides practical insights into the geometric optimization of friction elements. The derived formulas for coupling reactions as functions of friction pair geometry offer a valuable tool for engineers in designing more reliable systems. The findings are applicable to both the modernization of existing railway rolling stock and the design of new-generation freight wagons, potentially contributing to improved safety, durability, and maintenance efficiency across the rail industry.

1 INTRODUCTION

Analysis of the operation of friction wedges in the truck of the freight car model 18-9597. The main task of friction wedges is to transform the oscillatory movements occurring in the vertical and longitudinal directions into a friction force that dissipates the energy of these vibrations. At the same time, the geometry of the mating parts and the pre-compression force of the springs are of great importance, ensuring constant contact of the wedge with the friction plate and the support beam.

In freight wagons, to dampen vibrations from track irregularities and to transfer vertical and longitudinal loads. The friction wedges, interacting with the friction bar, dampen vibrations from the unevenness of the path. The study of the interaction between the "superstructure beam and the friction wedge" and between the "friction wedge and the friction bar" is necessary to increase the reliability and efficiency of the construction of wagons. Today, there are many ways of engineering analysis. For analysis, we will use the analytical method and numerical modeling to determine the reaction of bonds depending on the geometric pairs of friction, to find rational values. The analytical approach is based on classical equations of statics, dynamics, and friction theory.

To study the mechanics of interaction, an analytical method based on solving a system of equilibrium equations taking into account friction forces was used. Both normal and tangential forces acting on the wedge from both sides are taken into account. Parameters have been introduced: the angle of inclination of the working surface of the wedge, friction coefficients, spring stiffness, weight of the support beam and external dynamic loads. In parallel, numerical modeling using the finite element method (FEM) was carried out, in which a three-dimensional model of the node was constructed. Real contact conditions are applied, taking into account dry friction and nonlinear deformation of the elements. This made it possible to track the distribution of loads in various

operating modes: starting, steady movement, braking, and moving over uneven tracks.

The simulation results confirmed that the vibration damping efficiency significantly depends on:

- the angle of inclination of the working surface of the wedge (optimally - 18-22°);
- uniformity of force from springs;
- conditions of rubbing surfaces (wear, contamination);
- values of the coefficient of friction (in the range 0.25–0.35 is preferable).

Thus, the combination of analytical and numerical approaches makes it possible to determine rational design parameters that ensure reliable and long-term operation of the spring-friction unit. The data obtained can be used in the modernization of existing trolleys and the development of new models of freight wagons.

Structurally, the spring-friction set of the truck of the freight car model 18-9597 is designed so that its friction wedges 2 and 3 are in contact with only three solid elements – a spring carrier 1, a frictiobar 4 (or 5) and double pins 7 (or 8) (Fig. 1).

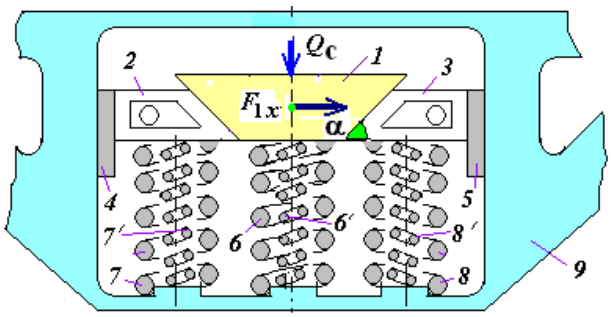


Figure 1: Spring-friction set of a bogie loaded with the pressure force of the car body with cargo.

In Figure 1 the following are designated: 1 – tail section of the bolster; 2 and 3 – friction wedges; 4 and 5 – friction bar; 6 – sets of springs under the bolster; 7 and 8 – sets of sub-wedge springs; 9 – side frame.

In [1], the spring-friction complex of a truck model 18-9597 of a freight wagon was numerically modeled in the classical formulation of the problem. A final numerical formula has also been obtained for determining the reaction of the bonds of friction wedges to a spring beam, taking into account the design features of the wedges and manufacturing errors on inclined surfaces.

The proposed model was further investigated, and a method was developed for determining the coupling reactions of the spring-friction complex of the trolley model 18-9597, which takes into account the possible angular contact of the rubbing surfaces by individual faces [2].

When creating a mathematical model of a friction wedge 2 (or 3) as an object of analytical research, according to the principle of bond release [3], the contact surfaces from the side of the pressure beam 1 and the friction bar 4 (or 5), considered as external bonds, are replaced by two bond reactions represented as. , an alternative support beam, and , an alternative friction bar.

The formula for the actual reaction on an equivalent inclined surface is calculated when the friction wedge moves vertically downwards, taking into account that the friction wedge is overloaded due to the longitudinal force applied to the support beam, taking into account only the reaction of the spring springs. The defined formula does not contain the mass of the container of the wagon with the load and the reaction of the spring springs. The study [4] did not specify which of the friction wedges is overloaded and which is unloaded.

In previous studies, the results of analytical and numerical modeling of the force action of friction pairs "pressure beam - friction wedge" and "friction wedge – friction bar" of the spring-friction trolley set model 18-9597 freight wagon were obtained. The equations of equilibrium of the superstructure beam as a physical body were calculated using the analytical method.

Analytical formulas have been developed for determining bond reactions depending on geometric parameters, in particular, the angles of inclination of the contacting surfaces of friction pairs. However, the studies [5], [6] did not take into account the factor of the longitudinal force acting from the front carriage and applied to the suspension beam of the bogie model 18-9597.

Failure to account for this longitudinal force leads to an underestimation of the loads acting on the friction pairs, which, in turn, reduces the accuracy of forecasts of wear and durability of suspension units. This can lead to premature component failure and increased operating costs.

To increase the accuracy of the model, it is necessary to include the influence of the longitudinal force in the calculations, which will allow for more adequate estimates of bond reactions and provide realistic modeling of the dynamics of force interaction in the spring-friction complex. In particular, it is proposed to supplement the mathematical model taking into account the applied longitudinal force, which will create conditions for

a more accurate wear analysis and the development of recommendations for design optimization.

2 MODEL DESCRIPTION AND ASSUMPTIONS

When modeling the asymmetric placement of a solid–state load relative to the axis of symmetry of the carriage, as shown in [5, 6], it should be noted the importance of analytical determination of reactions in friction pairs: "pressure beam - friction wedge" and "friction wedge – friction bar". These reactions depend on the longitudinal force exerted by the lead car on the support beam, as well as on the geometric parameters of the structural elements.

Let's assume that through the heel of the wagon frame, pressure forces from the wagon frame with a load are transmitted to the spring support of the suspension beam [5], [6]. The corresponding pressure forces (let's denote them as QC or QD) are transmitted to the sets of bogie springs of the freight car. In response, the elastic forces of these sets of springs exert pressure on the support beam and friction wedges, which, in turn, transfer the load to the side frames of the trolleys.

In this case, only one part of the longitudinal force F_x, designated as F_{1x}, is taken into account, which is applied to the suspension beam from the side of the coupling devices of the lead car. The remaining parts of the longitudinal force: F_{2x} – applied to the suspension beam of the rear bogie [7], and the fraction transmitted to the coupling devices of the driven wagons [3], [7] are not considered in this task. Let's model the design scheme of the mechanical system "pressure beam – friction wedges – friction bars" in accordance with the image on Figure 2. In this model:

F_6 – These are the reactions (elastic forces) of sets of spring springs, which create resistance to vertical movement of the support beam 1 downwards. The calculations take into account five such sets (see Figure 2a);

F_7 и $F8$ – These are the reactions (elastic forces) of sets of wedge springs that resist the lowering of friction wedges 2 and 3, respectively (see Figures 2b and 2d).

Thus, this scheme allows us to take into account the operation of the system elements when transferring vertical loads through friction elements to freight carts.

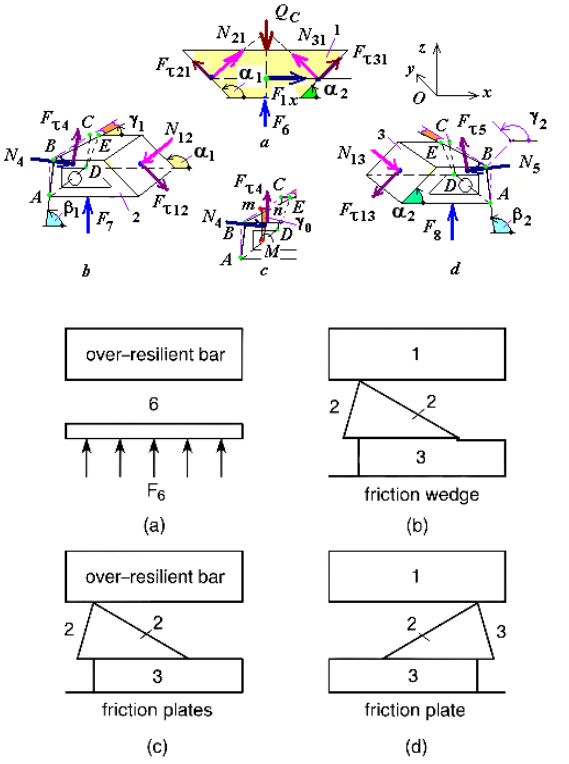


Figure 2: Calculated models of the pressure beam 1 and friction wedges 2 and 3.

In Figure 2 the following designations are indicated: N_{21}, N_{31} и $F_{\tau 21}$, $F_{\tau 31}$ – normal and tangential components of bond reactions (friction wedges) *2* и *3*; α_1 и α_2 – angles of inclination of the surfaces of the pressure beam to the horizon, rad. ($\alpha = 45^\circ + 1^\circ$); in Figure 2b, 2d: N_{12}, N_{13} и $F_{\tau 12}$, $F_{\tau 13}$ – the normal and tangential components of the coupling reaction 1 (tail section of the suspension beam) to wedges 2 and 3, N_4, N_5 и $F_{\tau 4}$, $F_{\tau 5}$ – the normal and tangential component of the bond reaction (friction strips), $\gamma_1 \le \frac{\pi}{2}$ и $\gamma_2 \ge \frac{\pi}{2}$ – the angle of inclination of the back surface *ABED* friction wedges 2 and 3, in contact with the friction plates 4 and 5, rad ($\gamma_1 \approx 88^\circ\text{-}1^\circ$, $\gamma_2 \approx 92^\circ + 1^\circ$).

Let's analyze the equilibrium of the superstructure beam 1 (see Fig. 2a). The pressure beam 1 is affected by the reactions R_{21} and R_{31} of the friction wedges 2 and 3, which are decomposed into normal and tangential components N_{21}, N_{31} and F_{t21}, F_{t31}; the active force QC, equal to the reaction force occurring on the pressure beam 1, and the reactive force in the form of an equivalent reaction of sets There are 6 F_6 springs. At the same time, we assume that the inclined surfaces of the

superstructure beam are made with disadvantages, i.e. $\alpha_1 \neq \alpha_2$, where α_1 and α_2 are the angles of inclination of the surfaces of the superstructure beam 1 to the horizon, rad. ($\alpha_1 \approx 134^{\circ}30' + 1^{\circ}$, $\alpha_2 \approx 45^{\circ}30' + 1^{\circ}$).

In the analytical study, as in [5], [6], we assume that the angles of inclination of the surfaces (a1 and a2) of the pressure beam 1, the friction wedges 2, 3 and the friction bars (β_1, β_2 and γ_1, γ_2) have different values ($\alpha_1 \neq \alpha_2$, $\beta_1 \neq \beta_2$, and $\gamma_1 \neq \gamma_2$), which correspond either to their manufacture with errors, or take into account the uneven wear of their surfaces.We also assume that the coefficients of sliding friction f between the contacting surfaces of the support beam (f_1 and f_2), friction wedges and slats (f_3 and f_4) have different values.

3 METHODS

To solve this applied problem, we use the kinetostatics method from the course of theoretical mechanics [3]. It is based on an engineering calculation method based on the fact that inertia forces are reduced to an equivalent system of forces, while taking into account external loads in equilibrium conditions.

4 RESEARCH RESULTS

Let's calculate two equations of equilibrium of a plane system of forces, equating the sum of the projections of all forces on the *x* and *z* axes to zero:

$$N_{21}\cos(\alpha_1 - \frac{\pi}{2}) + F_{\tau 21}\cos(\alpha_1) + \\ + N_{31}\cos(\alpha_2 + \frac{\pi}{2}) + F_{\tau 31}\cos(\alpha_2) + F_{1x} = 0 \quad (1)$$

$$N_{21}\sin(\alpha_1 - \frac{\pi}{2}) + F_{\tau 21}\sin(\alpha_1) + \\ + N_{31}\sin(\alpha_2 + \frac{\pi}{2}) + F_{\tau 31}\sin(\alpha_2) - Q_C + F_6 = 0 \quad (2)$$

Here, as you can see, there are two independent equilibrium equations, and four unknown ones: N_{21}, N_{31} and F_{t21}, F_{t31}.To solve the problem, it is sufficient to add (1) and (2) the equation following from Coulomb's law

$$F_{\tau} \leq fN, \quad (3)$$

where f – is the coefficient of sliding friction between the contacting surfaces of the bolster 1 and the friction wedges 2, 3, as well as between the friction wedges 2, 3 and the friction bars.

Substituting equalities (3) into (1) and (2), after transformations we have

$$\begin{aligned} aN_{21} + bN_{31} &= -F_{1x}, \\ cN_{21} + dN_{31} &= Q_C - F_6, \end{aligned} \quad (4)$$

where *a*, *b*, *c* and *d* – are constant coefficients:

$$\begin{aligned} a &= \cos(\alpha_1 - \frac{\pi}{2}) + f_1\cos(\alpha_1); \\ b &= \cos(\alpha_2 + \frac{\pi}{2}) + f_2\cos(\alpha_2); \\ c &= \sin(\alpha_1 - \frac{\pi}{2}) + f_1\sin(\alpha_1); \\ d &= \sin(\alpha_2 + \frac{\pi}{2}) + f_2\sin(\alpha_2) \end{aligned} \quad . \quad (5)$$

According to Kramer's rule [8], from system (4) we find the normal components of the reaction of the connections (friction wedges 2 and 3) in the execution of inclined surfaces of the support beam 1 with errors:

$$N_{21} = -\frac{1}{ad - bc}\begin{bmatrix} F_{1x}\left(\sin(\alpha_2 + \frac{\pi}{2}) + f_2\sin(\alpha_2)\right) + \\ + (Q_C - F_6)\left(\cos(\alpha_2 + \frac{\pi}{2}) + f_2\cos(\alpha_2)\right) \end{bmatrix} \quad (6)$$

$$N_{31} = \frac{1}{ad - bc}\begin{bmatrix} (Q_C - F_6)\left(\cos(\alpha_1 - \frac{\pi}{2}) + f_1\cos(\alpha_1)\right) + \\ + F_{1x}\left(\sin(\alpha_1 - \frac{\pi}{2}) + f_1\sin(\alpha_1)\right) \end{bmatrix} \quad (7)$$

The matrix, composed of the coefficients of the unknowns of system (4), and calculated symbolically [9], [10] is equal to:

$$\begin{aligned} ad - bc = &\sin(\alpha_1)\cos(\alpha_2) + \sin(\alpha_2)f_2\sin(\alpha_1) + \\ &+ f_1\cos(\alpha_1)\cos(\alpha_2) + f_1\cos(\alpha_1)f_2\sin(\alpha_2) + \\ &+ \sin(\alpha_2)\cos(\alpha_1)(-1) + \sin(\alpha_2)f_1\sin(\alpha_1) + \\ &+ f_2\cos(\alpha_1)\cos(\alpha_2) - f_2\cos(\alpha_2)f_1\sin(\alpha_1). \end{aligned} \quad (8)$$

Assume that if the angle is negative, then the function is reduced to the function of the positive angle by the formulas $\sin(\alpha - \frac{\pi}{2}) = -\sin(\frac{\pi}{2} - \alpha) = -\cos(\alpha)$ and $\cos(\alpha - \frac{\pi}{2}) = \cos(-(\frac{\pi}{2} - \alpha) = \sin(\alpha)$ [8], relations (6), taking into account expression (8), are written as

$$N_{21} = -\frac{1}{\sin(\alpha_1)\cos(\alpha_2) + \sin(\alpha_2) f_2 \sin(\alpha_1) + \\ + f_1 \cos(\alpha_1)\cos(\alpha_2) + f_1 \cos(\alpha_1) f_2 \sin(\alpha_2) + \\ + \sin(\alpha_2)\cos(\alpha_1)(-1) + \sin(\alpha_2) f_1 \sin(\alpha_1) + \\ + f_2 \cos(\alpha_1)\cos(\alpha_2) - f_2 \cos(\alpha_2) f_1 \sin(\alpha_1)} \times \\ \times \begin{bmatrix} F_{1x}(\cos(\alpha_2) + f_2 \sin(\alpha_2)) - \\ -(Q_C - F_6)(\sin(\alpha_2) - f_2 \cos(\alpha_2)) \end{bmatrix} \quad (9)$$

$$N_{31} = \frac{1}{\sin(\alpha_1)\cos(\alpha_2) + \sin(\alpha_2) f_2 \sin(\alpha_1) + \\ + f_1 \cos(\alpha_1)\cos(\alpha_2) + f_1 \cos(\alpha_1) f_2 \sin(\alpha_2) + \\ + \sin(\alpha_2)\cos(\alpha_1)(-1) + \sin(\alpha_2) f_1 \sin(\alpha_1) + \\ + f_2 \cos(\alpha_1)\cos(\alpha_2) - f_2 \cos(\alpha_2) f_1 \sin(\alpha_1)} \times \\ \times \begin{bmatrix} (Q_C - F_6)(\sin(\alpha_1) + f_1 \cos(\alpha_1)) - \\ -F_{1x}(\cos(\alpha_1) - f_1 \sin(\alpha_1)) \end{bmatrix}. \quad (10)$$

Sometimes in the case when $f_1 = f_2$ and $\alpha_1 = \alpha_2 + \frac{\pi}{2}$, which correspond to the design of the inclined surfaces of the support beam 1 without errors, expressions (9) and (10) will take the form:

$$N_{21} = -\frac{1}{1 - f_1^2}\begin{bmatrix} F_{1x}(\cos(\alpha_2) + f_1 \sin(\alpha_2)) - \\ -(Q_C - F_6)(\sin(\alpha_2) - f_1 \cos(\alpha_2)) \end{bmatrix} \quad (11)$$

$$N_{31} = \frac{1}{1 - f_1^2}\begin{bmatrix} (Q_C - F_6)(\cos(\alpha_2) - f_1 \sin(\alpha_2)) + \\ +F_{1x}(\sin(\alpha_2) + f_1 \cos(\alpha_2)) \end{bmatrix}. \quad (12)$$

that is, $N_{31} = N_{21}$ at $F_{1x} = 0$, which is consistent with the results of [1], confirming the correctness of the obtained analytical expressions.

The nature of the change in the reaction of the connections (friction wedges 2 and 3) depending on the variation of the angles of inclination (α_1 and α_2) of the inclined surfaces of the friction pairs "pressure beam - friction bars" without taking into account and taking into account the errors in their manufacture in accordance with formulas (9) and (10) are shown in Figure. 3 a – Figure 3d.

The analysis of the $N_{21}(\alpha_1)$ shows that with an increase in the angle α_1 (which is equivalent to a decrease in the angle α_2), the magnitude of the bond reaction increases, whereas with a decrease in α_1, on the contrary, it decreases. At the same time, taking into account the manufacturing errors of inclined surfaces in friction pairs leads to a decrease in the bond reaction compared with calculations without taking them into account.

In turn, the analysis of the dependence of N_{31} (α_2) indicates that with an increase in the angle α2 (which corresponds to a decrease in α_1), the bond reaction decreases, and with a decrease in α_2, it increases. When taking into account the manufacturing errors of inclined surfaces in friction pairs, an increase in the bond reaction is observed compared to calculations without taking them into account.

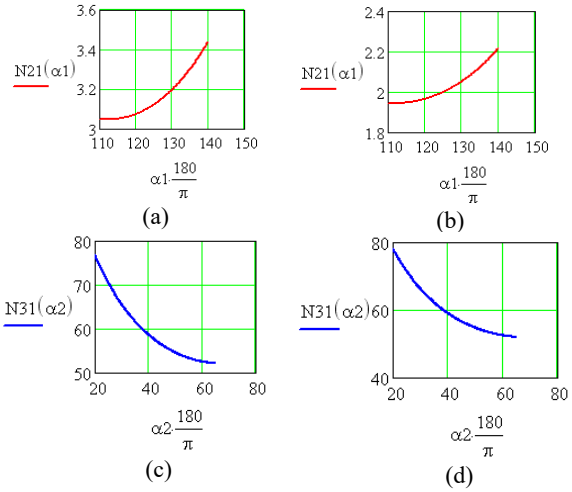


Figure 3: The nature of the change in the reaction of the $N_{21}(\alpha_1)$ and $N_{31}(\alpha_2)$ bonds: a), c) and b), d) – without taking into account and taking into account the manufacturing errors of the inclined surfaces of friction pairs.

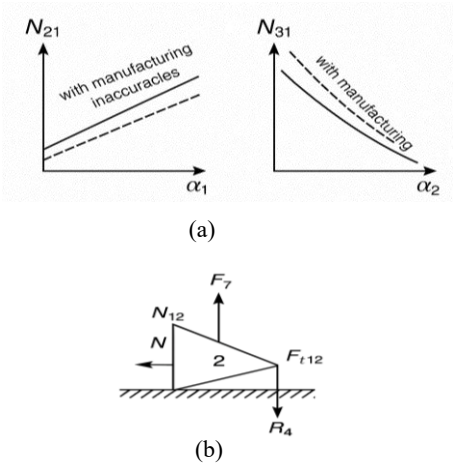


Figure 4: Friction wedge equilibrium.

Comparing the values of $N_{21}(\alpha_1)$ and $N_{31}(\alpha_2)$, it should be noted that the value of $N_{21}(\alpha_1)$ is always less than $N_{31}(\alpha_2)$.This is due to the effect of the longitudinal force of the adjacent F_{1x} wagon on the suspension beam. As a result, there is a more complete contact of the friction pair "friction wedge 3 - friction bar 5" compared to the pair "friction wedge 2 – friction bar 4".

When considering the equilibrium conditions of the friction wedge 2 (see Fig. 4b) it is necessary to take into account the following forces acting on it according to the axiom of equality of action and reaction:

$N_{12} = -N_{21}$ – the normal component of the reaction from the side of the pressure beam;

$F\tau_{12} = -F\tau_{21}$ – the tangential component of the same reaction.

The reaction from the side of the friction bar 4, represented by the normal component N_4 and the tangential component F_{t4} (reaction vector R_4);

The resultant of the reactions of the sets of spring springs 7 is the upward force F_7.

These forces ensure the equilibrium of the wedge 2 in the process of interaction with the support beam and the friction bar. Let's make up the force equilibrium equations for the friction wedge 2:

$$N_{12}\cos(\alpha_1+\frac{\pi}{2})+F_{\tau 12}\cos(\alpha_1+\pi)+N_4\cos(\gamma_0)\cos(\beta_1+\frac{3}{2}\pi)+ \\ +F_{\tau 4}\cos(\gamma_0)\cos(\beta_1)=0, \quad (13)$$

where γ_0 – is the angle accompanying the calculation, as in [5], [6].

Taking into account the relation (3), we rewrite the last expressions

$$N_{12}\left(\cos(\alpha_1+\frac{\pi}{2})+f_1\cos(\alpha_1+\pi)\right)+ \\ N_4\cos(\gamma_0)\left(\cos(\beta_1+\frac{3}{2}\pi)+f_3\cos(\beta_1)\right)=0; \quad (14)$$

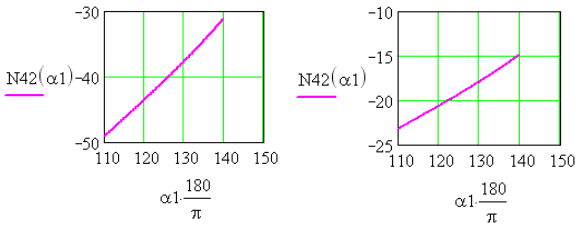
From relation (14), taking into account (9), after intermediate transformations, we determine the normal component of the reaction of the friction wedge 4.

$$N_4=\frac{1}{\sin(\alpha_1)\cos(\alpha_2)+\sin(\alpha_2)f_2\sin(\alpha_1)+ \\ +f_1\cos(\alpha_1)\cos(\alpha_2)+f_1\cos(\alpha_1)f_2\sin(\alpha_2)+ \\ +\sin(\alpha_2)\cos(\alpha_1)(-1)+\sin(\alpha_2)f_1\sin(\alpha_1)+ \\ +f_2\cos(\alpha_1)\cos(\alpha_2)-f_2\cos(\alpha_2)f_1\sin(\alpha_1)} \times \quad (15) \\ \times\left[\begin{matrix}F_{1x}(\cos(\alpha_2)+f_2\sin(\alpha_2))- \\ -(Q_C-F_6)(\sin(\alpha_2)-f_2\cos(\alpha_2))\end{matrix}\right]\times\frac{(\sin(\alpha_1))+f_1\cos(\alpha_1))}{\cos(\gamma_0)(\sin(\beta_1)+f_3\cos(\beta_1))}.$$

The nature of the change in the reaction of the bonds (friction strip 4) depending on the variation in the angle of inclination (α_1) of the inclined surfaces of the friction pairs "pressure beam - friction strips" at α_2 = const, without taking into account and taking into account the errors in their manufacture in accordance with (15) are shown in in Figure 5a and 5b.

The analysis of the dependences of $N_{42}(\alpha_1)$ shows that with an increase in the angle α_1, the reaction of bonds along the module decreases, and with a decrease, vice versa. When taking into account the manufacturing errors of the inclined surfaces of the friction pairs, the bond reaction is smaller in magnitude than without taking them into account.

Regarding the study, now let's consider the equilibrium condition of the friction wedge 3 (see Fig. 5).



(a) (b)

Figure 5: The nature of the change in the reaction of the $N_{42}(\alpha_1)$: connections: a) and b) – without taking into account and taking into account the manufacturing errors of the inclined surfaces of the friction pairs.

According to the axiom of equality of action and counteraction, the friction wedge 3 is affected by: normal $N_{13} = -N_3$ and tangent $F_{\tau 13} = -F_{\tau 3}$ components of the reaction of the pressure bar $R_{13} = -R_3$; normal N_5 and tangent $F_{\tau 5}$ components of the reaction of the friction bar 5 R5; as well as the resultant reactions of sets of springs 8 - F_8. Take into account, 2 d : $\gamma_2 \geq \frac{\pi}{2}$ – the angle of inclination of the rear surface *ABED* of the friction wedge 3 in contact with the friction plate 5, rad. ($\gamma_2 = 92^o + 1^o$).

Let us develop the force equilibrium equations for the friction wedge 3 in the same way as for the friction wedge 2:

$$N_{13}\cos(\alpha_2+\frac{3}{2}\pi)+F_{\tau 13}\cos(\alpha_2+\pi)+ \\ +N_5\cos(\gamma_0)\cos(\beta_2+\frac{\pi}{2})+F_{\tau 5}\cos(\gamma_0)\cos(\beta_2)=0. \quad (16)$$

Taking into account relations (3) from expression (16), taking into account (10) after transformations, we determine the normal component of the reaction of the friction wedge 4.

$$N_5 = \frac{1}{\begin{matrix}\sin(\alpha_1)\cos(\alpha_2)+\sin(\alpha_2)f_2\sin(\alpha_1)+\\ +f_1\cos(\alpha_1)\cos(\alpha_2)+f_1\cos(\alpha_1)f_2\sin(\alpha_2)+\\ +\sin(\alpha_2)\cos(\alpha_1)(-1)+\sin(\alpha_2)f_1\sin(\alpha_1)+\\ +f_2\cos(\alpha_1)\cos(\alpha_2)-f_2\cos(\alpha_2)f_1\sin(\alpha_1)\end{matrix}} \times \left[\begin{matrix}(Q_C-F_6)(\sin(\alpha_1)+f_1\cos(\alpha_1))-\\ -F_{1x}(\cos(\alpha_1)-f_1\sin(\alpha_1))\end{matrix}\right] \times \frac{(\sin(\alpha_2)-f_2\cos(\alpha_2))}{\cos(\gamma_0)(\sin(\beta_2)-f_4\cos(\beta_2))}. \quad (17)$$

The nature of the change in the reaction of the bonds (friction strip 5) depending on the variation in the angle of inclination (α_2) of the inclined surfaces of the friction pairs "pressure beam - friction strips" at α_1= const without taking into account and taking into account the errors in their manufacture in accordance with formula (17) are shown in Figure 6a and Figure 6b.

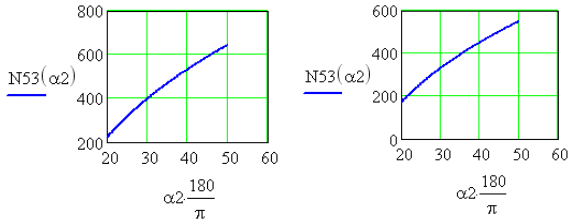


Figure 6: The nature of the change in the reaction of the $N_{53}(\alpha_2)$ bonds: a) and b) – without taking into account and taking into account the manufacturing errors of the inclined surfaces of friction pairs.

The analysis of the dependences of $N_{53}(\alpha 2)$ shows that with an increase in the angle α2, the bond reaction modulus increases, and with a decrease, it decreases accordingly. When taking into account the manufacturing errors of the inclined surfaces of the friction pairs, the bond reaction value is lower compared to the calculation without taking them into account.

An analytical study of the values of $N_{42}(\alpha 1)$ and $N_{53}(\alpha 2)$ allows us to note that $N_{42}(\alpha 1)$ is significantly less than $N_{53}(\alpha 1)$ due to the effect of the longitudinal force of the mixed wagon F_{1x} on the superstructure beam. For this reason, premature wear of the friction pair "friction wedge 3 – friction bar 5" in comparison with the friction pair "friction wedge 2 – friction bar 4" is natural.

5 CONCLUSIONS

This paper has demonstrated the effective application of modern software systems; such as Mathcad, COMSOL, and Universal Mechanism in studying and modeling the frictional behavior of spring friction assemblies in freight car bogies, with a focus on the model 18-9597. Through a combination of analytical and numerical methods, the study derived precise mathematical relationships that define the reaction forces in friction pairs, accounting for geometric parameters, manufacturing tolerances, and the influence of longitudinal forces from adjacent cars, the investigation revealed significant asymmetries in force distribution due to the uneven inclination angles and friction coefficients, which in turn cause unequal wear between friction pairs specifically, greater wear in the friction wedge 3 friction bar 5 pair compared to friction wedge 2 friction bar 4. This observation is critical for improving maintenance strategies and optimizing component design.

By integrating the principles of kinetostatics and Coulomb friction into symbolic calculations and validating the model through simulation, the study provides a robust framework for predicting performance degradation and enhancing the operational reliability of rail suspension systems. These results pay to the strategic implementation of digital diagnostic tools in railway engineering, aimed at extending service life and ensuring the safety and efficiency of freight transportation.

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Improvement of Some Physical and Sensing Properties of Nanostructured Cadmium Oxide Manganese Thin Films

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Keywords: CdO, Mn, Thin Film, CSP, Physical and Sensing Properties

Abstract: The spray pyrolysis technique (SPT) was employed for depositing thin films of Mn-doped CdO. The structural analysis through XRD confirms films polycrystallinity with a cubic phase. AFM analysis reveals adecline in particle size and surface roughness. The optical transmittance spectra indicate that the films exhibit high transparency in the visible area. The absorption edge shifting toward longer wavelengths as Mn concentration increases. The band gap reduced from 2.75 eV for CdO to 2.65 eV for 4% Mn-doped CdO. The refractive index exhibits normal and abnormal dispersion regions, decreasing with higher Mn doping. The extinction coefficient follows the trend of the absorption coefficient, increasing with photon energy, confirming direct electronic transitions in the films. CdO:Mn films show p-type behavior; NO_2 exposure (250 ppm) reduces resistance. 4% Mn doping increases resistance by altering charge carrier concentration. As Mn concentration increases, the sensitivity of CdO films to NO_2 decreases. Sensitivity increases with NO_2 concentration due to stronger gas-film interactions and higher conductivity.

1 INTRODUCTION

CdO is an n-type semiconductor [1]-[3]. The behavior of CdO can be changed by doping with substances, such as F, Mn, Mo, and Sn, which enhance electrical conductivity and leading to a blue shift in band gap [4]-[9]. It is well known that doping efficiency depends on the difference in ionic radii between the dopant and the host element. Mn^{2+} ions (0.83 Å) have a smaller ionic radius compared to the larger Cd^{2+} ions (0.95 Å), leading to strong integration into the CdO lattice[10]-[12]. CdO films can be prepared via various techniques, including solution growth, MOCVD, sputtering, SPT, reactive evaporation, CBD, and PLD [13]-[26]. Among these techniques, SP is cost-effective for fabricating thin films with a large surface area. In this paper, SPT is considered an efficient and promising method widely used for depositing CdO films and analyzing their characteristics.

2 EXPERIMENTAL

CdO and CdO:Mn films were grown via (SPT). A 0.1M solution of $Cd[C_4H_6CdO_4]$ were prepared. $MnCl_3$, was dispersed in deionized water, and several drops of HCl were added to assure cleanliness. The optimal deposition parameters were as follows: The glass base was kept at 425°C, the nozzle and base were spaced 28 cm apart, and the spraying was done for 10 seconds, with a 65-second break to impede the cold-chain process. The spray rate was 4 ml/min, and (N_2) is the transporter gas. The film thickness, measured via weighing method, was determined to be 340 ± 25 nm. Structural properties were analyzed using (XRD), surface morphology was examined using (AFM), and transmittance was measured via a double-beam spectrophotometer . The CdO and CdO:Mn gas sensor was assessed by measuring the percentage change in resistance within a cylindrical chamber (radius: 8.2 cm, height: 15.2 cm).

3 RESULT AND DISCUSSION

The XRD patterns of of the intended films are presented in Figure 1, confimed a polycrystalline nature, with distinct peaks observed at 2θ = 33.12°, 38.26°, 55.237°, and 69.28°, corresponding to the (200), (220), and (222) planes of cubic CdO, as per ICDD Card No. 05-0640 [28]. The structural properties are presented in Table 1. Regardless of Mn doping level, the (111) plane showed the most development across all films. With increasing Mn content, the intensity of the (111) plane was found to marginally rise, indicating a shift towards lower angles due to Mn^{2+} substitution in the host lattice and a possible intense peak, indexed as the (111) plane, was utilized to determine the crystallite size (*D*) [30], [31]. Figure 2 presents the X-ray parameters of the extended films.

D was determined via Scherrer's formula [32]:

$$D_{hkl} = \frac{0.9\lambda}{\beta cos\theta}. \quad (1)$$

Where λ =1.541Å, β is FWHM, and θ is Bragg angle. The results indicate that as the Mn concentration increases, *D* increases from 15.43 nm to 17.71 nm. The films exhibit crystallite sizes in the nanometer range, confirming that CdO:Mn films qualify as nanomaterials. This change in crystallite size is attributed due to the variation in ionic radii between the dopant and host material [33, 34].

The formula for lattice microstrain is expressed as [35]:

$$\varepsilon = \frac{\beta cos\theta}{4} \ (lines^{-2}.m^{-1}). \quad (2)$$

The formula for dislocation densities is expressed as [36]:

$$\delta = \frac{1}{D^2}\left(\frac{lines}{m^2}\right). \quad (3)$$

As anticipated, a rise in *D* leads to a decline in microstrain (ε) and dislocation densities (δ). The microstrain (ε) was found to range from 2.2×10^{-3} to 2.0×10^{-3}, while the dislocation density (δ) decreased from 48.0×10^{14} to 31.8×10^{14}. T is result agrees well with Kaaviya et al.[37], [38].

Table 1: Structural styles of the extended films.

Specimen	2 θ°	Plane, (hkl)	FWHM, °	E_g, eV	D, nm	ε, $\times10^{14}$	Δ,$\times10^{-4}$
Undoped CdO	33.12	111	0.53	2.75	15.4	48.0	22.1
CdO: 2% Mn	33.09	111	0.50	2.71	16.5	36.3	20.9
CdO: 4% Mn	33.06	111	0.48	2.65	17.7	31.8	20.0

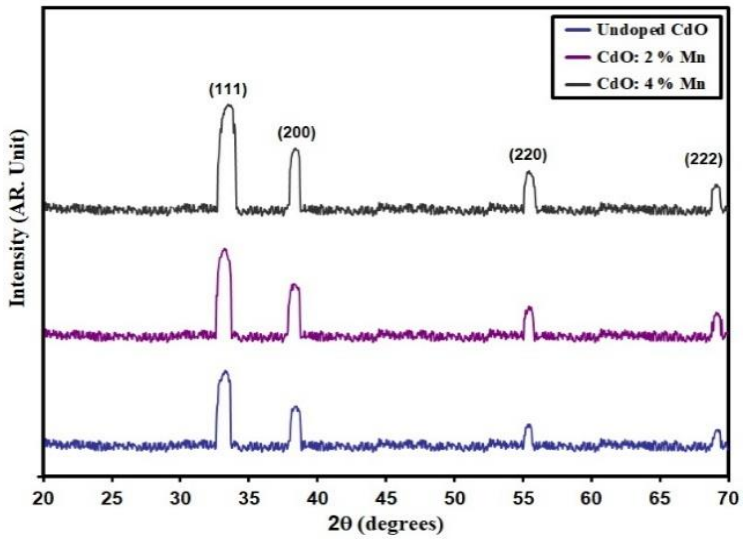


Figure 1: XRD styles of intended films.

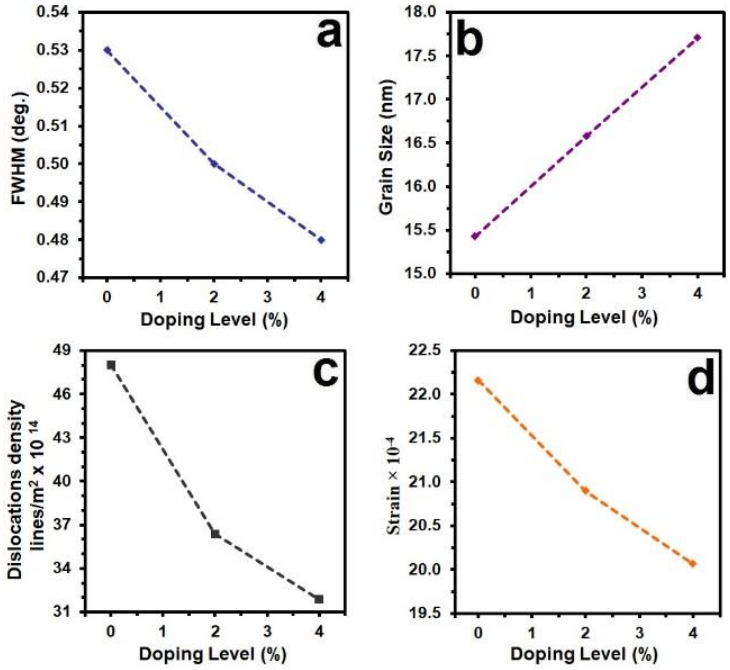


Figure 2: X-ray parameter of entended films.

The surface topography of the extended films was examined using AFM. All CdO thin film images were captured over an area of 0.39 μm × 0.39 μm. Figure 3 presents AFM images of the extended films within the same area. As illustrated in Figures 1a, 1b, and 1c, the CdO, 2% CdO:Mn, and 4% CdO:Mn thin films consist of small crystal grains with a nearly uniform distribution.

According to Table 2, the average particle size (P_{av}) is the highest among all samples at 87.6 nm, whereas the Pav of the 4% Mn-doped film is 32.3 nm. It is also evident that the roughness (R_a) values vary with increasing Mn doping. However, R_a values do not follow a consistent trend due to irregular grain distribution. Notably, the 4% Mn-doped CdO film exhibits the minimum R_a value, which is attributed to the uniform distribution of regularly shaped grains on the film surface. The Ra of the 4% Mn-doped film is 3.32 nm, the lowest among all samples, whereas the CdO thin film has an R_a of 8.62 nm. This finding aligns with previous literature [39], [40].

The AFM results confirm that Mn doping influences the surface quality of CdO films. The obtained AFM parameters (P_{av}) and root mean square (rms) values are summarized in Table 2.

Figure 4 presents transmittance (T) spectra of the entended films. T decreases as the Mn content rises. The films exhibit transparency in the visible region, with transmittance values ranging between 20% and 75% at wavelengths greater than 550 nm. Notably, the maximum T at 600 nm exceeds 65%. T is approximately 60% for undoped CdO and around 65% for films with 2% and 4% Mn doping. Below 550 nm, T sharply decreases due to the strong absorption in this area. The absorption spectra are displayed in Figure 5, as shown CdO films exhibit a distinct absorption edge. Additionally, the absorption edge shifts within the 300-500 nm range with increasing Mn doping, indicating a reduction in the band gap due to Mn incorporation[41]-[44].

The change of optical absorption coefficient (α) of different Mn doping concentrations is presented in Figure 6. The absorption coefficient (α) can be determined from T spectrum using the following (4) [45]:

$$\alpha = 2.303 \frac{A}{T}. \quad (4)$$

Where A represents the absorbance, the calculated values α are on the order of 10^4 cm^{-1}. As shown in Figure 6, at higher photon energies α exhibits a high value, indicating a significant probability of allowed direct transitions. As the wavelength increases, α gradually decreases. Additionally, it is observed that A increases with the rise in Mn, with a dramatic rise close to the band gap (Eg) edge. As a result, the absorption edge shifts toward the longer wavelength area [46, 47].

Table 2: AFM parameter measurement of Mn dope.

Samples	P_{av} nm	R_a (nm)	rms (nm)
CdO	87.6	8.62	9.73
CdO: 2% Mn	48.2	8.09	8.23
CdO: 4% Mn	32.3	3.32	4.14

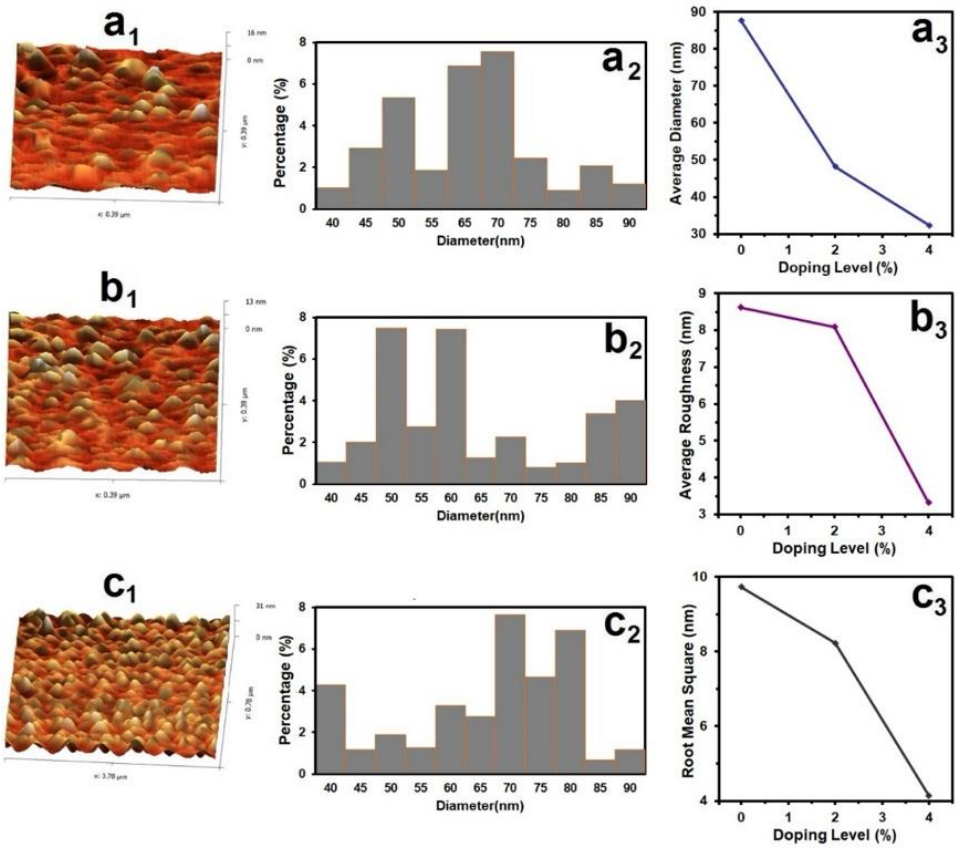


Figure 3: AFM of entended films.

The formula that follows is used to calculate the band gap (Eg). [48]:

$$\alpha h\nu = B(h\nu - E_g)^n. \quad (5)$$

Where B is constant, hν is the photon energy . The parameter n = 1/2 .Figure 7. illustrate E_g showing a value of 2.24 eV for CdO, which aligns well with several reported studies [36]. Mn doping in CdO reduces E_g to lower energy values due to the increased density of localized states within the Eg as the Mn content increases [49,50]. The E_g value were decided to be 2.75 eV, 2.71 eV, and 2.65 eV for undoped, 2 wt.% Mn and 4 wt.% Mn films, respectively, as illustrated in Figure 7.

The following relationship was previously employed to calculate the refractive index (n) via reflectance (R)[51]:

$$R = \frac{(n-1)^2}{(n+1)^2} . \quad (6)$$

The following formulas [52] are used to evaluate the extinction coefficient (k):

$$k = \frac{\alpha\lambda}{4\pi} . \quad (7)$$

Figure 8. presents n plot , n values of CdO thin film vary with Mn doping. n of CdO film is the highest, while n of the 4wt.% Mn film is the lowest among all the films. This indicates that n can either increase or decrease depending on the Mn doping content [53, 54]. Figure 9 illustrates the variation k. The behavior of (k) closely resembles primarily depends on (α), as described by (4). Consequently, an increase in photon energy leads to a rise in k due to the corresponding increase in α. This indicates that

direct electronic transitions occur in these films [55, 56]. Additionally, before reaching the Eg values, (k) exhibits significant changes with Mn doping, which can be attributed to structural modifications in the films [57],

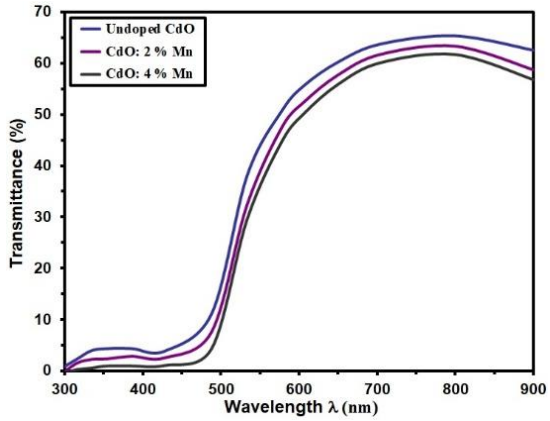
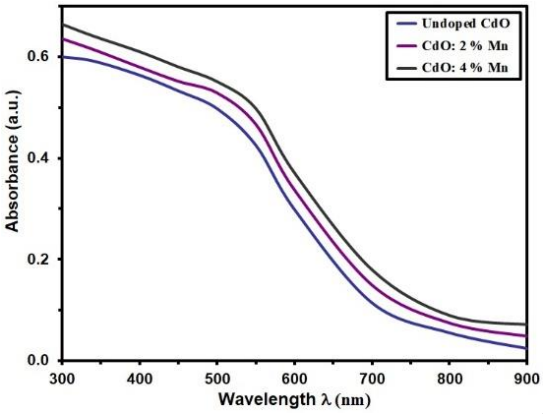


Figure 4: Transmittance (T) of the entended film.



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Figure 5: Absorbance of the grown films.

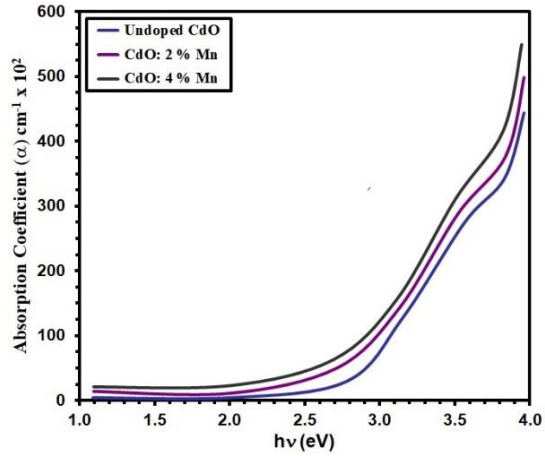


Figure 6: Absorption coefficient (α) of the grown films.

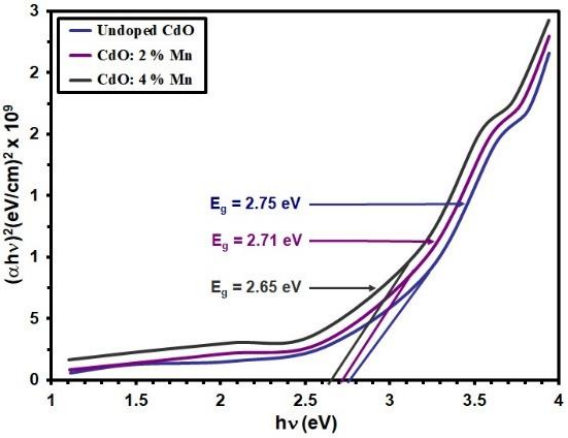


Figure 7: The gap energy (E_g) of the entended films.

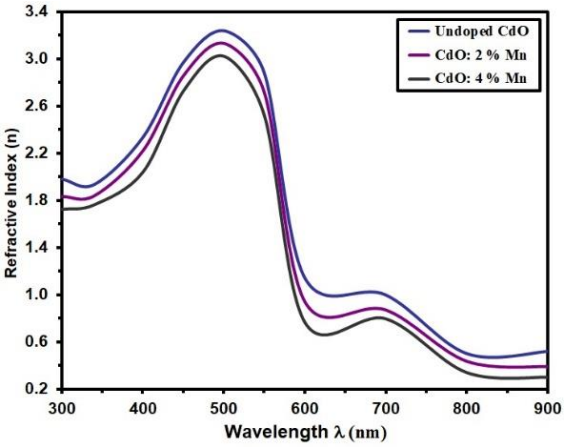


Figure 8: Refractive index (n) of the grown films.

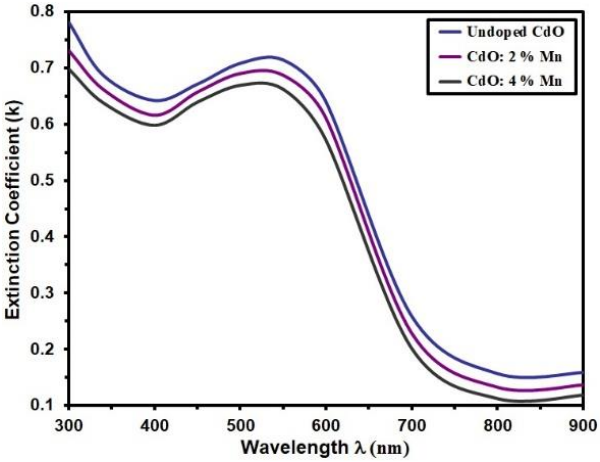


Figure 9: Extinction coefficient (k) of of the entended films.

The resistance variation over response time for deposit films is depicted in Figure 10. The p-type

semiconductor behavior of CdO is indicated by the declined in resistance upon insertion of NO2 gas into the chamber.This indicates that oxidizing gases like NO_2 (at a content of 250 ppm) interact with film surface, capturing electrons from CB. As a result, hole concentration increases, leading to enhanced charge carrier density and reduced resistance. The semiconductor features were significantly impact by Mn content, as evidenced by the maximum resistance observed at a 4%wt Mn [59]. This increase in resistance can be ascribed to the alteration of charge carrier concentration and the introduction of defect states caused by Mn incorporation.

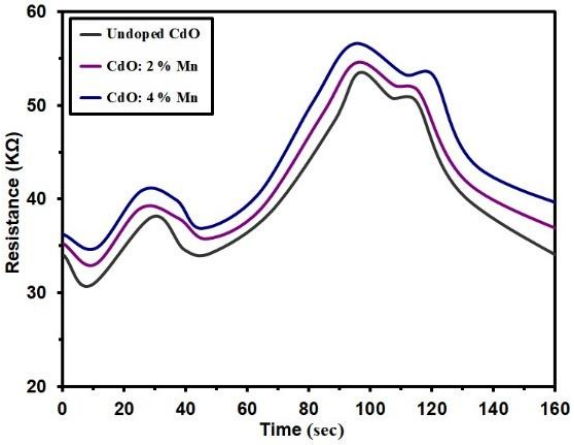


Figure 10: Resistance as a function of operating time for CdO thin films with different Mn concentrations.

The sensor's response can be determined using the following (8) [59]:

$$\text{Sensitivity} = \frac{\Delta R}{R_g} = \left|\frac{R_g - R_a}{R_g}\right| \times 100\ \% \,. \qquad (8)$$

Figure 11 illustrates s of pure and Mn-doped CdO films to NO_2 exposure. As Fe concentration increases, sensitivity declines from 49.9% to 4.5% at 50 ppm, from 40.3% to 4.1% at 200 ppm, and from 48.7% to 11.4% at 250 ppm.This reduction can be ascribed to a larger nanocrystalline size and/or better crystallinity, which maintains the surface uniformity Additionally, the increase in carrier concentration leads to higher conductivity, further influencing sensitivity [60], [61]. Moreover, as shown in Figure 11, sensitivity increases as gas concentration rises from 150 to 250 ppm, suggesting a stronger interaction between the NO_2 molecules and the CdO film at higher contents.

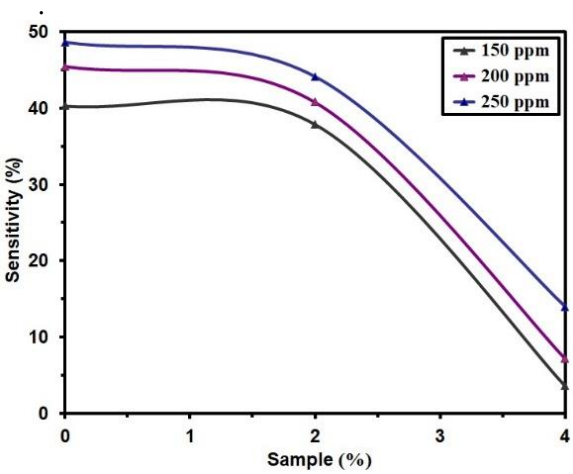


Figure 11: Sensitivity (S) as a function of CdO thin films with different Mn concentrations.

4 CONCLUSIONS

Mn-doped CdO thin films synthesized via the spray pyrolysis technique exhibited notable enhancements in structural, morphological, optical, and gas sensing properties. XRD analysis confirmed a polycrystalline cubic structure, where Mn doping led to increased crystallite size and a reduction in both microstrain and dislocation density, indicating improved crystal quality. AFM images showed that Mn incorporation reduced particle size and surface roughness, with the 4% Mn-doped film displaying the most uniform and smooth surface morphology. Optical characterization revealed high transparency in the visible region for all films, though transmittance slightly decreased with higher Mn content. A redshift in the absorption edge was observed, corresponding to a reduction in the optical band gap from 2.75 eV for undoped CdO to 2.65 eV for 4% Mn-doped CdO. The refractive index decreased with Mn doping.In terms of gas sensing behavior, the films exhibited typical p-type semiconductor characteristics. Exposure to NO_2 gas led to a decrease in resistance due to increased hole concentration. However, higher Mn content caused a rise in baseline resistance and a decline in sensitivity. Nevertheless, the sensitivity improved with increased NO_2 concentration, highlighting stronger gas-film interactions.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support provided by Mustansiriyah University.

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Effect of Nickel Oxide on Morphological and Electrical Properties of Manganese: Oxide Nanostructured Thin Films

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Keywords: Mn_3O_4 Film, Hall Effect, FE-SE, Doping Effect, EDS.

Abstract: In this study, thin films of manganese oxide (Mn_3O_4) doped with nickel oxide (NiO) at different doping ratios (0, 1, 3, 5, and 7%) were prepared and deposited on glass slides at a temperature of 350°C using the chemical spray pyrolysis technique. Spectral analysis was performed, and a scanning electron microscope (SEM) was used. The SEM results show that the prepared films are spherical in shape, resembling cauliflower, and are irregular in size. This irregularity is due to the crystalline defects generated in the films. An Energy Dispersive Spectrometer (EDS) analysis was conducted to verify the components of the prepared thin films, which allowed for the determination of the weight and atomic ratios of the elements composing the films. Regarding the electrical measurements, conductivity, resistivity, the Hall coefficient, and charge carrier concentration were determined. We note that the prepared films exhibit p-type charge carriers and that the Hall effect increases with increasing doping ratios. The Hall coefficient values increase with doping ratios and reach their highest value at 3%, while the resistivity values peak at a doping ratio of 1%. The films show low conductivity overall, with the highest conductivity value observed at 7% doping. Similarly, the mobility increases with doping and attains its highest value at 7%.

1 INTRODUCTION

The advancement of nano science, an interdisciplinary field encompassing physics, chemistry, and material science, has recently been greatly aided by nanostructured thin films. [1], Manganese oxides are becoming one of the most potent transition metal oxides and diverse classes of functional materials that are having a key impact on science and technology [2], [3] , Manganese (Mn) is a metallic element belonging to Group 7 in the periodic table of elements and is known for its distinctive mineral and chemical properties. Manganese chloride occurs naturally in the environment, but it can also be prepared in the laboratory or by industrial means. It is typically available as a white powder or crystalline crystals and is soluble in water. Thin films are thin layers of materials used in semiconductor technology and electronic devices [4] , Manganese oxides, which are magnetic by nature, can form various crystal structures like MnO, Mn_2O_3, Mn_3O_4, and others, and exhibit various oxidation states of manganese, such as Mn^{2+}, $Mn^{3+,}$ or Mn^{4+} [5] , p-type change Metal oxides are of great interest because of their participation in a variety of applications, including photovoltaic solar cells, electrochemical energy storage devices, microelectronic devices, and metal-oxide-semiconductor field-effect transistors (p MOSFETs) [6]-[9] , Nickel oxide (NiO) is a crystalline powder, either green or black in color, with density 6.67 g/cm^3, molecular weight 842.87 g/mol, and melting point 1984 C^0 [10] Nickel oxide (NiO) is non-insulating at room temperature and is an antiferromagnetic material with good resistance. It also has a large energy gap estimated at 3.4 - 4 ev and is considered one of the important electrolytic materials after tungsten oxide. It is used in making the electric anode and has several uses due to the high electrolytic efficiency. Nickel oxide has high stability and has positive conductivity [11], A variety of techniques have been used to grow manganese oxide thin films such as chemical bath deposition [12], [13] , dip coat [14] electrochemical deposition [15] , atomic layer deposition [16] , electrostatic spray deposition [17], pulsed laser deposition [18], chemical vapor deposition [19] and SILAR method [20] chemical pyrolysis technique [21], Because it is easy to use, inexpensive, and produces little waste, spray

pyrolysis is a good substitute for conventional methods for creating manganese oxide thin films. It can be applied to a wide surface area and is simple to integrate into an industrial production line. This method works with mass production systems as well [22]

2 EXPERIMENTAL PART

Chemical spray pyrolysis (CSP) method was employed to create thin films of manganese oxide (Mn_3O_4) doped with nickel oxide (NiO). German-made glass plates from Los Las Company were prepared. The plates were cut into (2.5 × 2.5) cm dimensions and rinsed with distilled water for 4 minutes in an ultrasonic device. After that, they were sterilized with acetone for 4 minutes and then again with distilled water. Finally, they were dried with a special fabric to remove the remaining suspended particles. The oxide solution was prepared by mixing manganese chloride ($MnCl_2.4H_2O$) weighing 1.979 g in 100 ml of distilled water. Then, it was placed on a magnetic stirrer for 10 minutes. Then, it was filtered using filter paper. After that, it was deposited using a spray system on the glass plates at a temperature of 350 C^0. The pure oxide films were doped with nickel oxide at ratios of 0%, 1%, 3%, 5% and 7% , The effect of Hall measurements was studied to determine the efficiency of the semiconductor using an HMS 300 device, This device operates based on the Van Der Pauw method and is connected to a computer equipped with software that displays the important parameters of the sample under examination, such as the type of semiconductor, carrier concentration, mobility, and Hall coefficient , The produced films' surface morphology was also examined using a Field Emission Scanning Electron Microscope (FE-SEM) of the MIRA3, Model-TE-SCAN type, located at the University of Tehran, Islamic Republic of Iran. The FE-SEM device enables the determination of particle shape, homogeneity, as well as the detection of crystalline structure defects

3 RESULT AND DISCUSSION

3.1 Results of Field Emission Scanning Electron Microscopy Tests

Field-emitting scanning electron microscopy (FE SEM) is used to examine the surface topography of all the prepared membranes was studied at a temperature of 350 C^0. This device gives a structural image of the surface with very high accuracy. Figures 1a, 1b, 1d, 1e show FE SEM images and cross sections of the prepared films. It is formed in beautiful shapes, spherical shapes gathered in the form of a flower very similar to a cauliflower flower. It is also irregular in size and distribution and uneven in growth, noting the presence of voids resulting from the occurrence of secondary growth as well as due to crystalline defects. We note that the average crystal size has large differences due to the lack of good homogeneity of the membranes [23], [24] .

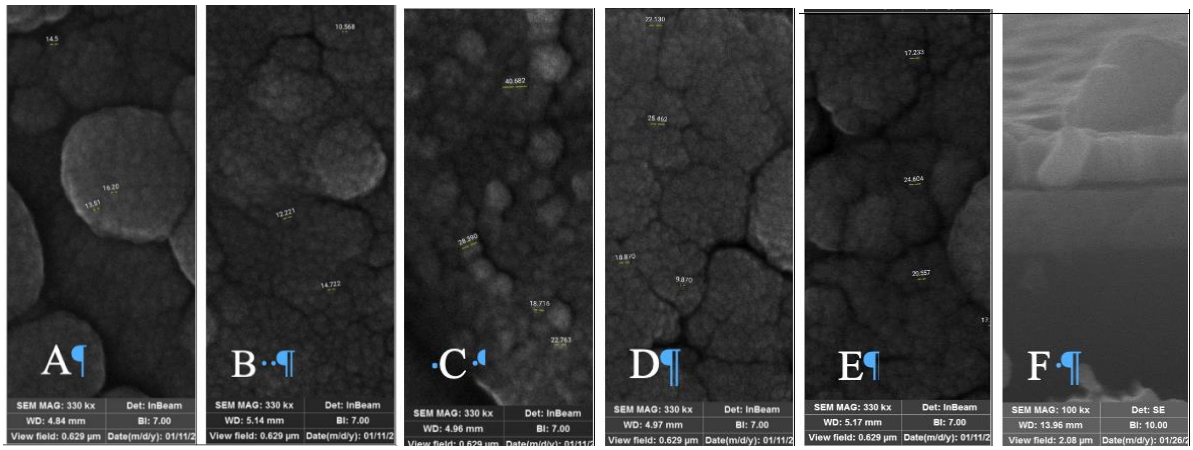


Figure 1: FE-SEM Images of : a) Mn_3O_4: Pure, b) Mn_3O_4: NiO (1%), c) Mn_3O_4: NiO (3%), d) Mn_3O_4: NiO (5%), e) Mn_3O_4: NiO (7%), f) Cross-sectional view showing the film thickness (~327 nm).

We observe that the Mn3O4 membrane with a 7% impurity rate had the biggest particle size, whereas the membrane with a 1% nickel oxide impurity rate had the lowest value. As seen in Figure 1f, the produced film layer has a thickness of roughly 327 nm.

3.2 Results of Energy Dispersive Spectrometer Test

To verify the components of the prepared thin films, we conduct an examination Energy Dispersive Spectrometer (EDS), which is an examination through which the weight percentages and atomic percentages of the elements present in the prepared films that cannot be detected using X-ray technology (XRD). The following Figures 2, 3, 4, 5, 6 are diagrams showing the analysis of EDS and the following Tables 1, 2, 3, 4, 5 show the weight percentages and atomic percentages of the elements present in the prepared films.

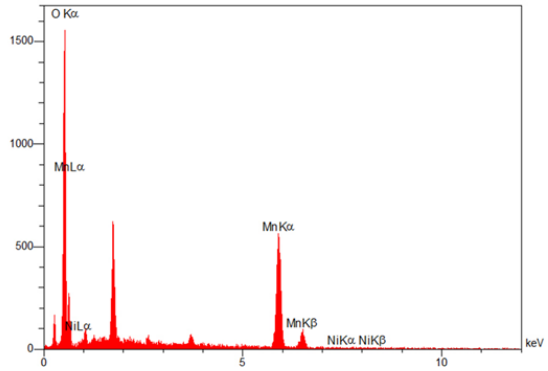


Figure 2: Analysis diagram EDS for a thin Mn_3O_4 film.

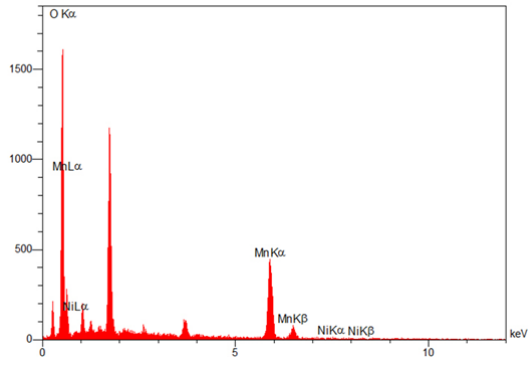


Figure 3: Analysis diagram EDS for a thin Mn_3O_4 film.

Table 1: Weight ratios and atomic ratios of elements in the film Mn_3O_4 for analysis EDS.

Element	Weight (%)	Atomic (%)
O	47.89	75.94
Mn	51.88	23.96
Ni	0.23	0.10

Table 2: Weight ratios and atomic ratios of elements in the film Mn_3O_4 for analysis EDS.

Element	Weight (%)	Atomic (%)
O	54.64	80.54
Mn	45.00	19.32
Ni	0.35	0.14

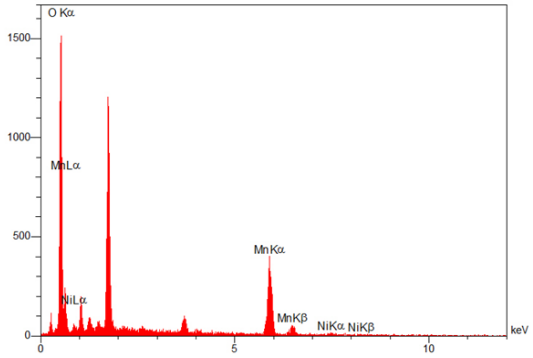


Figure 4: Analysis diagram EDS for a thin Mn_3O_4 film.

Table 3: Weight ratios and atomic ratios of elements in the film Mn_3O_4 for analysis EDS.

Element	Weight (%)	Atomic (%)
O	57.60	82.37
Mn	41.55	17.30
Ni	0.85	0.33

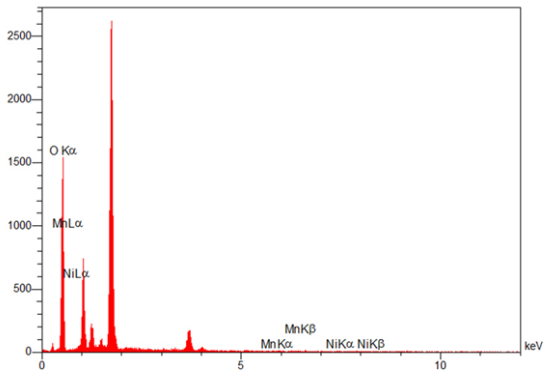


Figure 5: Analysis diagram EDS for a thin Mn_3O_4 film.

Table 4: Weight ratios and atomic ratios of elements in the film Mn_3O_4 for analysis EDS.

Element	Weight (%)	Atomic (%)
O	98.92	99.70
Mn	0.42	0.12
Ni	0.65	0.18

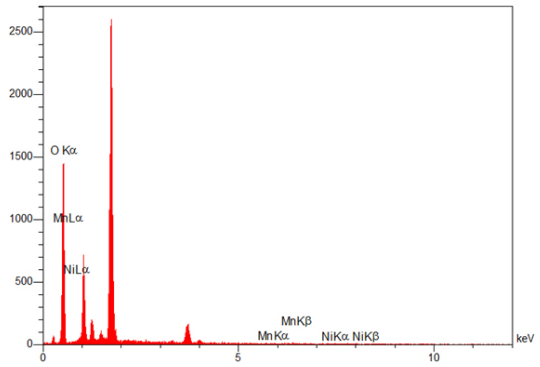


Figure 6: Analysis diagram EDS for a thin Mn3O4 film.

Table 5: Weight ratios and atomic ratios of elements in the film Mn_3O_4 for analysis EDS.

Element	Weight (%)	Atomic (%)
O	98.70	99.63
Mn	0.57	0.17
Ni	0.73	0.20

3.3 Results of Electrical Measurement

The Hall effect was conducted as a measurement to determine the electrical characteristics of Mn_3O_4 films made at 350 C^0 and with impurity rates 0%, 1%, 3%, 5% and 7 % of nickel oxide. Through it the important electrical properties were known, such as the type of charge carriers, their concentration, and the values of the Hall coefficient. It is found that all the prepared Mn_3O_4 due to positive Hall coefficient values, which indicate that holes make up the majority of charge carriers and electrons make up the minority, membranes are of the positive type (P-type) [25] , Table 6 shows the results of the Hall effect , We notice that the values of the effect of the Hall coefficient increase with increasing doping rates, and its highest value is at a doping percentage of 3% of nickel oxide , The results show that the resistivity values of all the prepared films are high, and their highest value occurs at a doping rate of 1% of nickel oxide. The reason for the high resistivity values is the crystalline defects resulting from Ni ions, as they restrict the movement of electrons and increase their resistance [26]. All produced membranes had low conductivity values , as we note that they increased after the doping process with nickel oxide, and their highest value was at the doping rate of 7% of nickel oxid ,The reason for the increase in conductivity is the replacement of Mn_3O_4 with NiO ions, which gives the electrons increased freedom and leads to an increase in electrical conductivity [27], [28]. The mobility increases with increasing doping rates, and its highest value was at a doping percentage of 7% of nickel oxide, Figures 7, 8, 9 shows the values of resistivity , Hall coefficient , and charge carrier concentration as a function of doping ratios, respectively, for all prepared films.

Table 6: Hall effect measurements for the prepared thin films.

Sample	Concentration $(cm)^{-3}$	Hall Coefficient RH (m^2/C)	Conductivity $(\Omega.cm)^{-1}$	Resistivity $(\Omega.cm)$	Mobility $(cm^2/v.s)$
Pure	2.025×10^{11}	3.082×10^{7}	0.772×10^{-5}	12.98×10^{4}	2.374×10^{2}
Mn_3O_4: NiO (1%)	2.748×10^{11}	2.271×10^{7}	0.523×10^{-5}	19.15×10^{4}	1.186×10^{2}
Mn_3O_4: NiO (3%)	10.52×10^{11}	0.5934×10^{7}	2.017×10^{-5}	4.829×10^{4}	1.229×10^{2}
Mn_3O_4:NiO (5%)	1.912×10^{11}	3.265×10^{7}	1.247×10^{-5}	8.016×10^{4}	4.073×10^{2}
Mn_3O_4: NiO (7%)	2.014×10^{11}	3.100×10^{7}	1.614×10^{-5}	6.198×10^{4}	5.002×10^{2}

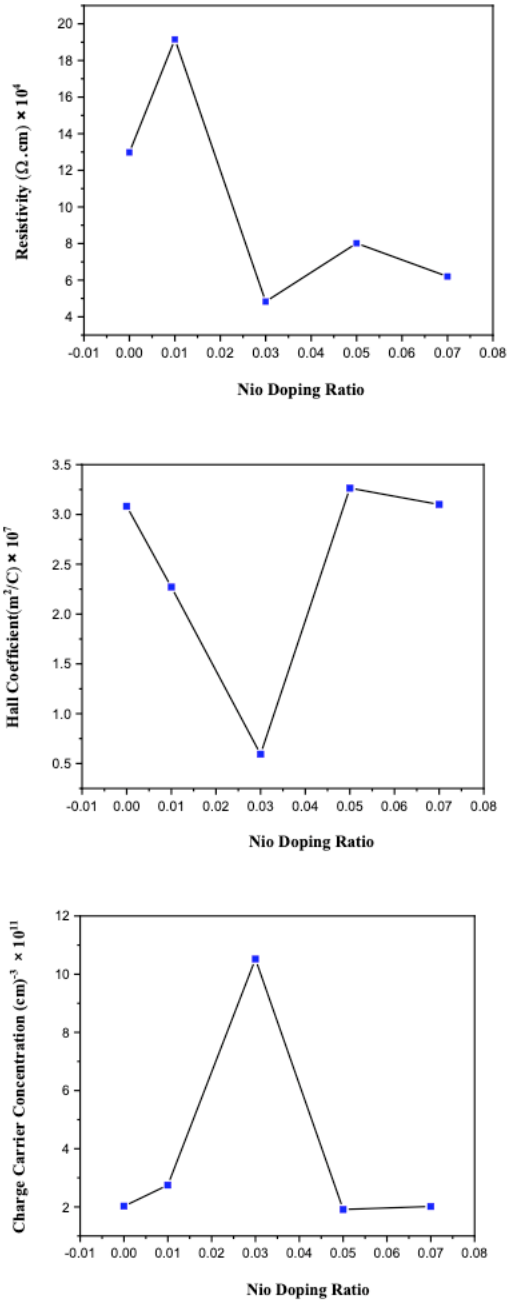


Figure 9: Concentration of charge carriers as a function of doping ratios for the prepared films.

4 CONCLUSIONS

This study demonstrates that NiO doping has a significant impact on the morphological and electrical properties of Mn_3O_4 nanostructured thin films. SEM analysis revealed a cauliflower-like, spherical morphology and pronounced size irregularity, attributed to crystalline defects—the largest particle size was observed at 7% NiO content. EDS confirmed the presence of Mn, O, and Ni, with manganese concentration declining and nickel peaking at intermediate doping levels.

Electrical measurements showed all films exhibit p-type conductivity. The Hall coefficient reached its maximum at 3% NiO, while resistivity peaked at 1% doping and conductivity and carrier mobility were highest at 7% NiO. These trends indicate that controlled NiO doping efficiently tailors film properties, with optimal electrical characteristics achieved at specific doping ratios. Overall, the findings support the use of NiO doping to optimize Mn_3O_4 thin films for applications in electronic and energy devices.

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Analysis of the Elastic Stress-Strain Behavior of Complex-Shaped Three-Dimensional Bodies

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Keywords: Three-Dimensional Bodies, Elastoplastic Process, Finite Elements, Finite Difference, Vlasov-Kantorovich Method.

Abstract: The problem of the bending of a parallelepiped under external forces was examined using the finite element method. The given three-dimensional Lame equations were transformed into a symmetric algebraic system by applying the finite element method, and then solved using the square root method. As a result, the three displacements u_1, u_2, u_3 and the six stress components σ_{11}, σ_{22}, σ_{33}, σ_{12}, σ_{13}, σ_{23} were determined for various external forces and the height of the body. The formulation of three-dimensional elastoplastic problems, the application of finite element methods, Vlasov-Kantorovich, and finite difference methods in the calculation of bodies with complex three-dimensional shapes (with cavities, appendages, and depressions) is presented along with algorithms for solving the systems of equations and calculating the coefficients. By using a combination of theoretical analysis and numerical simulations, we study the interrelationship between elastic and plastic behaviors under various loading conditions. The research emphasizes the significance of the material properties, geometric configuration, and boundary conditions in influencing the modes of deformation.

1 INTRODUCTION

In the last decade, the Vlasov-Kantorovich method, the finite difference method (FDM), and the finite element method (FEM) have become some of the most widely used techniques for solving problems in the mechanics of deformable solids on computers. This is explained by the fact that practically any problem, regardless of complexity, can be modeled using these methods; however, this results in a system of equations with a very high order. Solving such large-order systems has become feasible in the last decade, associated with the development of computer technologies. On the other hand, this method allows transforming large (two-dimensional, three-dimensional) problems into a series of smaller problems that can be solved sequentially, thereby reducing the order of the initial system of equations [1]. This significantly simplifies the creation of efficient algorithms for solving these problems.

Although the existence of this effect is acknowledged, relatively little research has been done on how the relative positions of finite elements affect the quality of the numerical solutions that are obtained. Numerical methods' approximation and convergence can be enhanced by effectively controlling computational components. The basics of building and utilizing three-dimensional open work schemes based on the approximation of variational equations (including finite element technique schemes) are illustrated in works [1] and [2]. A portion of a traditional isoparametric parallelepiped can be removed to create open networks of finite elements, which form the basis of such systems. In this situation, it is possible to minimize the number of computational cells by at least three times.

2 FORMULATION OF THE PROBLEM

To calculate any structure, it is necessary to start by forming its calculation scheme. This means that the representation of the construction should be simplified as much as possible, consisting of a set of standard objects that are connected to each other in a standardized manner to a fixed foundation.

It is important to note that a specific structure does not always correspond to a precise and exact schematic representation. Uncertainties can arise when preparing the calculation scheme, for example, due to the complexity of the structural scheme, there may not be sufficient information about the compatibility of certain joints, and the characteristics of how the interaction forces transfer between the parts of the structure can also lead to uncertainties [2].

Therefore, some analysts use what is referred to as the "principle of uncertainty in a single calculation scheme." According to this principle, multiple calculation schemes are considered to obtain the values of stresses at different cross-sections, aiming to get values that are close to the possible maximum in the given calculation scheme for all real load conditions [3]. With this approach, the reliability check is performed based on the analysis of the results obtained from several (usually two or three) calculation schemes.

If the calculation is performed using the finite element method, then preparing the calculation scheme involves collaboratively constructing a finite element model of the system being analyzed. This means that suitable elements are selected for all characteristic parts of the calculation scheme (from the standard set available in the software package), and the number of elements as well as their arrangement in the considered area is determined [4].

For all elements, it is necessary to calculate the required geometric properties and specify the material properties (such as elastic modulus, Poisson's ratio, and density).

The actual computations on the computer should only begin after compiling and verifying the complete lists of elements, actual constants, and material properties [5].

Plates are a very widely used object in construction and technology. Generally, these consist of flat bodies whose thickness is significantly smaller than their other dimensions. Therefore, Kirchhoff-Love's kinematic hypotheses can be utilized, allowing us to consider the stress state as planar (or, more precisely, generalized planar). Here, we focus on objects with constant thickness, although similar methods can also be applied to thick plates and plates with variable thickness, albeit with some difficulties.

3 RESEARCH RESULTS

The article analyzes the stress-strain state (SSS) of a parallelepiped based on the variation of the length-to-thickness ratio under different constraints and surface load distributions. To determine the influence of changing constraint conditions and the law of surface load distribution, the following problems were solved [6].

We consider the equilibrium of a rectangular parallelepiped, where its lateral surfaces are fixed, and a uniformly distributed load q_0=−0.1 MPa acts on its upper surface, while the bottom surface is free of loads.

$$\begin{cases} u_1 = 0, u_2 = 0, u_3 = 0 & if\ x_1 = 0, a \\ u_1 = 0, u_2 = 0, u_3 = 0 & if\ x_2 = 0, b \\ \sigma_{13} = 0, \sigma_{23} = 0, \sigma_{33} = q_0 & if\ x_3 = c \\ \sigma_{13} = 0, \sigma_{23} = 0, \sigma_{33} = 0 & if\ x_3 = 0 \end{cases}. \quad (1)$$

We will consider the equilibrium of a rectangular parallelepiped, with the following parameters:

- a=0.2m (length);
- b=0.2m (width);
- c=0.01m, 0.02m, 0.04m, 0.08m (height);
- E=2×105 MPa (elastic modulus);
- μ=0.3 (Poisson's ratio);
- δ=20, 10, 5, 2.5 (where δ=a/c).

Due to the symmetry of the problem, we will analyze one-quarter of the parallelepiped. The problem will be solved using the finite element method, dividing the area with eight-node isoparametric elements.

The discretization parameters and the characteristics of the system of linear algebraic equations are defined with the following values:

- Number of finite elements: 2000.
- Number of nodes: 2541.
- System order: 7623.
- Half-width of the band: 402.
- Number of divisions along the Ox, Oy, Oz axes: 11, 11, 21.

In the case of a rectangular parallelepiped, the values of u_1 correspond to the values of u_2 if they are exchanged respectively in the expressions x_1 and x_2 as a⇒b and x_2⇒x_1. When γ=1 (where γ=b/a), the values of u_1 reach a maximum at the line x_2 =b/2, and the values of u_2 at x_1=a/2. Furthermore, they are antisymmetric with respect to the main diameters, with u_1 and u_2 being positive for x_1<0.5a and x_2<0.5b, and negative for x_1>0.5a and x_2>0.5b, respectively. The transition u_3 is asymmetric with respect to both main diameters. Therefore, we will limit our study to the stress and deformation fields for the first quarter of the parallelepiped [7].

In the diagrams, for x_2=0.5b, the five values of the u_1 displacement diagrams correspond to the coordinates x_1=0,2,4,6,8,10 cm, and the reference width thickness is indicated as δ=20;2.5 in two ratios.

In Figure 1, we can see that the Kirchhoff-Love hypothesis is valid for δ=20, but it is relatively not satisfied for δ=2.5. This indicates that the body is in a three-dimensional state [8].

From Figure 1, it follows that for δ=20, the displacements u_1 and u_2 change with height according to a linear law, which demonstrates the validity of the Kirchhoff-Love hypothesis [9]. The calculation results indicate that for δ=8, the movement of u_1 is described very accurately by a linear function of height only in the segment $0.1a \leq x_1 \leq 0.9a$.

As the thickness δ decreases, the region of linear variation narrows, and the displacements at height become more concentrated; for example, for δ=4, this region is located in the interval [0.15a, 0.85a].

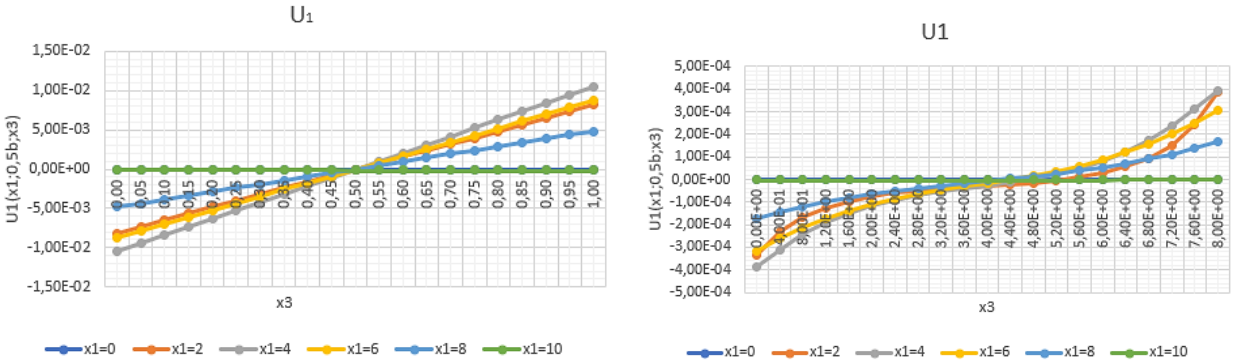


Figure 1: Change in u_1 displacement with respect to thickness.

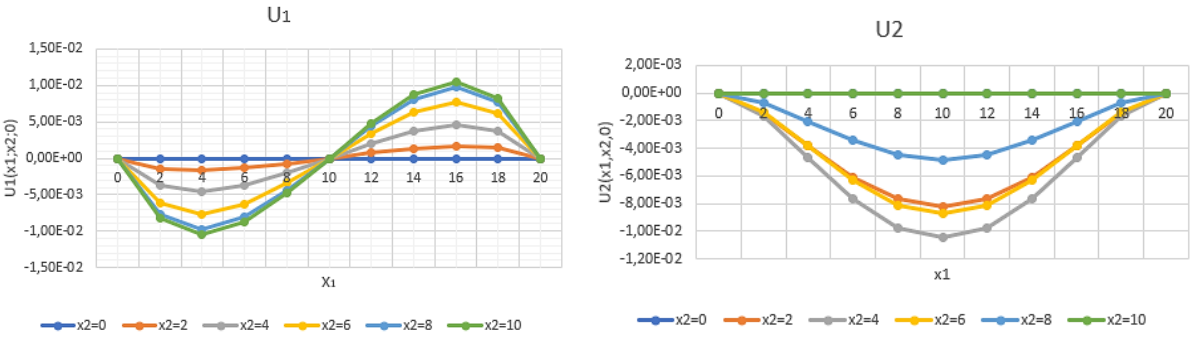


Figure 2: Variation of the values of u_1 and u_2 displacements under the influence of external force on the surface x_3=c.

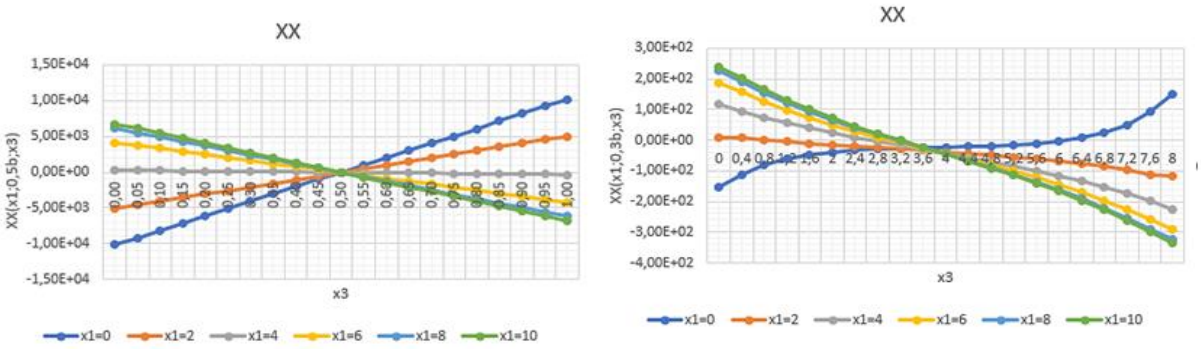


Figure 3: For the plane x_2=0.5b, the five values of the stress σ_{11} coordinate diagrams are given at x1=0,2,4,6,8,10 cm, and the thickness of the reference width is δ=20;2.5 for two ratios.

Table 1: Values of the displacement $U_3(x_1, 0.5b, x_3)$ with respect to the height variable x_3 and the variable x_1= 0, 2, 4, 6, 8, 10 cm at c=1 cm.

$U_3(x_1,0.5b,x_3)$						
X_3	X_1=0	X_1=2	X_1=4	X_1=6	X_1=8	X_1=10
0,00	0,00E+00	-2,12E-02	-6,14E-02	-1,02E-01	-1,30E-01	-1,40E-01
0,05	0,00E+00	-2,12E-02	-6,15E-02	-1,02E-01	-1,30E-01	-1,40E-01
0,10	0,00E+00	-2,11E-02	-6,15E-02	-1,02E-01	-1,30E-01	-1,40E-01
0,15	0,00E+00	-2,10E-02	-6,15E-02	-1,02E-01	-1,30E-01	-1,40E-01
0,20	0,00E+00	-2,10E-02	-6,15E-02	-1,02E-01	-1,30E-01	-1,40E-01
0,25	0,00E+00	-2,10E-02	-6,16E-02	-1,02E-01	-1,30E-01	-1,40E-01
0,30	0,00E+00	-2,09E-02	-6,16E-02	-1,02E-01	-1,30E-01	-1,40E-01
0,35	0,00E+00	-2,09E-02	-6,16E-02	-1,02E-01	-1,30E-01	-1,40E-01
0,40	0,00E+00	-2,09E-02	-6,16E-02	-1,02E-01	-1,30E-01	-1,40E-01
0,45	0,00E+00	-2,09E-02	-6,16E-02	-1,02E-01	-1,30E-01	-1,40E-01
0,50	0,00E+00	-2,09E-02	-6,16E-02	-1,02E-01	-1,30E-01	-1,40E-01
0,55	0,00E+00	-2,09E-02	-6,16E-02	-1,02E-01	-1,30E-01	-1,40E-01
0,60	0,00E+00	-2,09E-02	-6,16E-02	-1,02E-01	-1,30E-01	-1,40E-01
0,65	0,00E+00	-2,09E-02	-6,16E-02	-1,02E-01	-1,30E-01	-1,40E-01
0,70	0,00E+00	-2,09E-02	-6,16E-02	-1,02E-01	-1,30E-01	-1,40E-01
0,75	0,00E+00	-2,10E-02	-6,16E-02	-1,02E-01	-1,30E-01	-1,40E-01
0,80	0,00E+00	-2,10E-02	-6,16E-02	-1,02E-01	-1,30E-01	-1,40E-01
0,85	0,00E+00	-2,11E-02	-6,15E-02	-1,02E-01	-1,30E-01	-1,40E-01
0,90	0,00E+00	-2,11E-02	-6,15E-02	-1,02E-01	-1,30E-01	-1,40E-01
0,95	0,00E+00	-2,12E-02	-6,15E-02	-1,02E-01	-1,30E-01	-1,40E-01
1,00	0,00E+00	-2,13E-02	-6,15E-02	-1,02E-01	-1,30E-01	-1,40E-01

Table 2: Values of the displacement U_3 (x_1, 0.5b, x_3) with respect to the height variable X_3 and the variable x_1 = 0, 2, 4, 6, 8, 10 cm at c=8 cm.

$U_3(x_1,0.5b,x_3)$						
X_3	X_1=0	X_1=2	X_1=4	X_1=6	X_1=8	X_1=10
0	0,00E+00	-5,30E-04	-5,30E-04	-9,96E-04	-1,36E-03	-1,59E-03
0,4	0,00E+00	-5,14E-04	-5,14E-04	-1,01E-03	-1,38E-03	-1,62E-03
0,8	0,00E+00	-5,07E-04	-5,07E-04	-1,02E-03	-1,40E-03	-1,64E-03
1,2	0,00E+00	-5,05E-04	-5,05E-04	-1,02E-03	-1,42E-03	-1,66E-03
1,6	0,00E+00	-5,07E-04	-5,07E-04	-1,03E-03	-1,43E-03	-1,68E-03
2	0,00E+00	-5,13E-04	-5,13E-04	-1,04E-03	-1,44E-03	-1,70E-03
2,4	0,00E+00	-5,20E-04	-5,20E-04	-1,04E-03	-1,46E-03	-1,71E-03
2,8	0,00E+00	-5,29E-04	-5,29E-04	-1,06E-03	-1,47E-03	-1,73E-03
3,2	0,00E+00	-5,39E-04	-5,39E-04	-1,07E-03	-1,49E-03	-1,75E-03
3,6	0,00E+00	-5,50E-04	-5,50E-04	-1,09E-03	-1,51E-03	-1,77E-03
4	0,00E+00	-5,62E-04	-5,62E-04	-1,10E-03	-1,53E-03	-1,79E-03
4,4	0,00E+00	-5,76E-04	-5,76E-04	-1,12E-03	-1,55E-03	-1,81E-03
4,8	0,00E+00	-5,90E-04	-5,90E-04	-1,15E-03	-1,57E-03	-1,83E-03
5,2	0,00E+00	-6,07E-04	-6,07E-04	-1,17E-03	-1,60E-03	-1,85E-03
5,6	0,00E+00	-6,25E-04	-6,25E-04	-1,20E-03	-1,62E-03	-1,88E-03
6	0,00E+00	-6,47E-04	-6,47E-04	-1,23E-03	-1,65E-03	-1,90E-03
6,4	0,00E+00	-6,72E-04	-6,72E-04	-1,26E-03	-1,67E-03	-1,92E-03
6,8	0,00E+00	-7,03E-04	-7,03E-04	-1,30E-03	-1,70E-03	-1,94E-03
7,2	0,00E+00	-7,41E-04	-7,41E-04	-1,33E-03	-1,72E-03	-1,96E-03
7,6	0,00E+00	-7,90E-04	-7,90E-04	-1,36E-03	-1,74E-03	-1,98E-03
8	0,00E+00	-8,52E-04	-8,52E-04	-1,38E-03	-1,76E-03	-1,99E-03

Table 3: Comparison of the values of displacement U3 according to two theories.

δ	20	10	8	6	4	2
According to three-dimensional theory.	11.127	1.584	0.878	0.431	0.180	0.066
According to two-dimensional theory.	11.059	1.382	0.707	0.297	0.084	0.011

Table 4: Comparison of the values of stress σ11 according to two theories.

σ_{11} (0, 0.5 в, x_3)				
δ	According to three-dimensional theory		According to two-dimensional theory	
	X_3=c	X_3=0	X_3=c	X_3=0
20	-107.36	107.50	-123.20	123.20
10	-31.00	30.97	-30.80	30.80
8	-20.88	20.77	-19.71	19.71
6	-12.76	12.50	-11.09	11.09
4	-6.77	6.25	4.93	4.93
2	-3.13	1.97	-1.23	1.23

In Figure 2, the variation of u_1 (x_1, 0.5b, x_3) displacement at a height of δ=2.5 is shown, along with the five values of x_1 (where x_1=0, 2, 4, 6, 8, 10 cm corresponding to curves 1-6). From this figure, it can be concluded that at δ=2.5, the distribution character of stress and displacement fields is primarily three-dimensional.

If we look at Table 1, it presents the variation of U_3 (x_1, 0.5b, x3) displacement with respect to height. According to the Kirchhoff-Love hypothesis, the value of U_3 should remain constant. Comparing the values in the table, it is evident that this hypothesis is valid, as three of the values match, with a difference equal to 0.001.

A detailed analysis of the results shows that U_3 tends to vary significantly between any plane at x_3=const and at the heights along the line (0.5a, 0.5b, z∗c), where $0.5 \leq z^* \leq 1$ and z∗ rapidly approaches as the value of δ decreases.

If we pay attention to Table 2, it presents the variation of U_3(x_1, 0.5b, x_3) displacement with respect to height. According to the Kirchhoff-Love hypothesis, the value of U_3 should remain constant; however, comparing the values in the table indicates that this hypothesis is not valid. In this case, the body is changing according to a three-dimensional law [5].

In the figures, for the plane x_2=0.5b, the stress diagrams σ_{11} show five values of x_1= 0, 2, 4, 6, 8, 10 cm, with two thickness ratios given at δ=20 and δ=2.5. In Figure 3, we can see that the Kirchhoff-Love hypothesis holds at δ=20, but it is not satisfied at δ=2.5, indicating that the body is in a three-dimensional state.

Table 3 shows the values of U_3 obtained from the three-dimensional and two-dimensional theories for the bending of a square plate (compressed along the contour) with the same data [6]. It is clear from Table 3 that there is good agreement between the two theories at large values of δ. Table 4 shows the values of σ_{11} obtained from the three-dimensional and two-dimensional theories for the bending of a square plate (compressed along the contour) with the same data [6]. It is clear from Table 4 that there is good agreement between the two theories at large values of δ.

4 CONCLUSIONS

This paper proposes a comprehensive analysis of the elastic stress-strain performance of complex-shaped three-dimensional bodies, with particular focus on parallelepipeds subjected to varying thickness-to-length ratios (δ). The results demonstrate that for bodies with δ greater than 8, the Kirchhoff-Love hypothesis remains valid across most regions except near the edges and concentrated loading zones, with displacement components u_1 and u_2, and stresses σ_{11}, σ_{12} and σ_{22} exhibiting planar behavior. However, as δ decreases to values less than or equal to 4, significant three-dimensional effects arise, particularly in components such as u_3, σ_{13}, σ_{23}, and σ_{33}, which follow parabolic distributions through the thickness and peak at mid-plane sections, necessitating full 3D modeling. The use of the finite element method with eight-node isoperimetric elements proved effective in capturing these behaviors, and the simulation results aligned well with theoretical expectations, validating both the numerical formulation and mesh discretization. Furthermore, comparative data between two-dimensional and three-dimensional models confirmed the reliability of the former only at high δ

values, highlighting the limitations of classical plate theory for thicker or complex geometries. The influence of boundary conditions and load distribution was also found to be critical in defining the deformation patterns, underscoring the need for precise model setup in structural simulations. Finally, this work reinforces the importance of advanced numerical methods in accurately assessing the mechanical performance of engineering structures and provides a foundation for future studies involving elastoplastic behavior, heterogeneous materials, and geometrically intricate domains.

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Effect of Indium Doping Zinc Sulfide Nanostructures on the Physical and Sensing Properties via Chemical Spray Pyrolysis

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Keywords: ZnS, Spray Pyrolysis, Optical, XRD, AFM, Band Gap Energy, Resistance and Sensitivity.

Abstract: Indium-doped ZnS samples with doping levels of 0%, 1%, and 3% were fabricated using the chemical spray pyrolysis (CSP) method. XRD patterns confirmed the presence of a cubic zinc blend structure of both pure and Indium-doped ZnS samples. The crystallite size slightly increased with the concentration of indium, attributed to the substitution of indium within the ZnS lattice. AFM provided microscopic insights into the surface structure, allowing for the visualization and characterization of surface topographies. SEM images show transformation in ZnS films with Indium doping: flat islands to spherical nano-grains, indicating size reduction correlating with Indium concentration, influenced by ZnS-Indium interaction during synthesis. The optical parameters of nanostructures were investigated with doping and the incorporation of indium substitute for Zn ions. Indium doping in ZnS films increases resistance and alters gas sensing properties by affecting charge carrier mobility and adsorption efficiency. Higher Indium doping in ZnS films reduces sensitivity to NO_2 gas due to changes in charge carrier mobility and film structure.

1 INTRODUCTION

ZnS is a material that occurs naturally, that is plentiful, non-toxic, safe for the environment, and chemically unchanging [1]. It exists in two crystalline forms: cubic zinc-blend at low and wurtzite at high temperatures [2], [3]. Both forms of ZnS have a large bandgap, approximately 3.54-3.91 eV, respectively [4], [5]. Additionally, ZnS exhibits high transmittance, a large dielectric constant, and a high refractive index [6]-[9]. Elements such as Al, In, Cr, Ga, F, Cu, Cl, B, and Mn, at different doping content to alter ZnS's characteristics [10]-[13]. It is widely recognized that the ionic radius of In^{3+} (0.80 Å) closely matches that of Zn^{2+} (0.74 Å), making it plausible to assume that the incorporation of indium into the ZnS lattice or its substitution for Zn^{2+} is feasible [14]. Various chemical techniques have been employed for this purpose, including electrodeposition [15], chemical bath deposition [16], [17], sol-gel, spin coating [18], and spray pyrolysis [19], [20]. CSP is characterized by its simplicity, cost-effectiveness, and adaptability to large-scale processing. This research attempts to look into the effects of In^{3+} ion doping on the synthesis, structural characteristics, surface topography, and optical properties of ZnS nanofilms.nano films.

2 EXPERIMENTAL

A high-purity 0.1 M solution of $ZnCl_4 \cdot 5H_2O$ was used as the zinc source, dissolved in 100 ml of redistilled water to form the precursor solution. The preparation

conditions were optimized to achieve highly homogeneous thin films. A 0.1 M solution of Indium trichloride ($InCl_3$) was used as the dopant and added to the base solution to achieve doping levels of 1% and 3%. The solution was sprayed onto clean, preheated glass substrates at a temperature of 400 °C, nozzle-to-substrate distance of 28 cm. Spraying was performed for 9 seconds with 50-second intervals to prevent substrate cooling. The spray rate was maintained at 5 ml/min, and nitrogen gas was used as a carrier.

Film thickness was measured by gravimetric method discovered to be nearly 320 ± 20 nm. X-ray diffraction analysis was performed using a Shimadzu 6-2000 instrument with CuKα radiation, where the wavelength (λ) was 0.15406 nm. Surface morphology was investigated using electron surface microscopy (Hitachi S-4160). Additionally, Optical measurements were done by a Cary 100 UV-Visible spectrophotometer. Gas sensitivity is typically assessed by cylindrical chamber with a radius of 8 cm and a height of 14 cm.

3 RESULTS AND DISCUSSIONS

3.1 X-Ray Analysis

XRD patterns of intended films are offered in Figure 1. Patterns show that every sample has the cubic zinc blend structure, which closely matches ICDD card No. 5-0566. The greatest noticeable peak is in line with the lattice plane (111), while additional peaks corresponding to lattice planes (220), (311), and (400) are observed with varying intensities. The diffraction pattern of In^{3+} doped ZnS exhibits apparent shifts towards higher 2θ as the concentration of In^{3+} rises. This increase in the diffraction angle results from lattice contraction expected due to the higher surface-to-volume ratio [21]. The average grain size (*D*) was calculated by Scherrer formula [22]:

$$D = \frac{K\lambda}{\beta cos\theta}. \quad (1)$$

where *k* is 0.94, λ is the wavelength of X-rays, θ is Bragg angle Hence, by using the above relation, D of ZnS nanostructures doped with different concentrations of In^{3+} lies between 15.18 nm and 17.82 nm for pure ZnS and doping. A dislocation represents a structural defect within a crystal caused by a mismatch in lattice alignment between different crystal regions. In other words, their presence and density cannot be solely explained by thermodynamic considerations. The dislocation density (δ) was established by (2) [23]:

$$\delta = \frac{1}{D^2}. \quad (2)$$

The strain was established using the following relation [24]:

$$\varepsilon = \frac{\beta cos\theta}{4}. \quad (3)$$

Figure 2 outlines *D*, ε, and δ of ZnS nanostructures doped with different contents of In^{3+}. Table reveals that ε and δ decrease as the *D* increases. The decreased values of δ and ε observed with Indium doping indicate a reduction in crystal defects as can be seen in Figure 2. This effect is attributed to acting as an enhancer rather than an inhibitor [25].

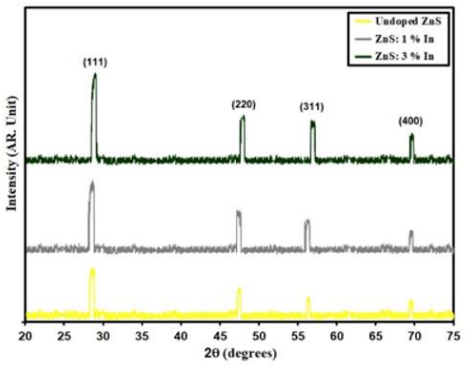


Figure 1: XRD styles of grown films.

3.2 Surface Topography Analysis

Figure 3 offers AFM images of the intended films. The average particle size of Pav decreases (from 78.1 nm to 31.5 nm) with increment doping of In3+ from a 3% concentration. The films exhibit uniform, well-defined, spherical morphology. pav reduces with Indium content., which agrees with [26]. In It is evident that at higher doping levels, the growth of thin films exhibited lower roughness (Ra). The root mean square (RMS) roughness was reported in Table 1. The reduction in particle size can be correlated with substituting In3+ ions, yielding similar results as reported elsewhere [27], [28].

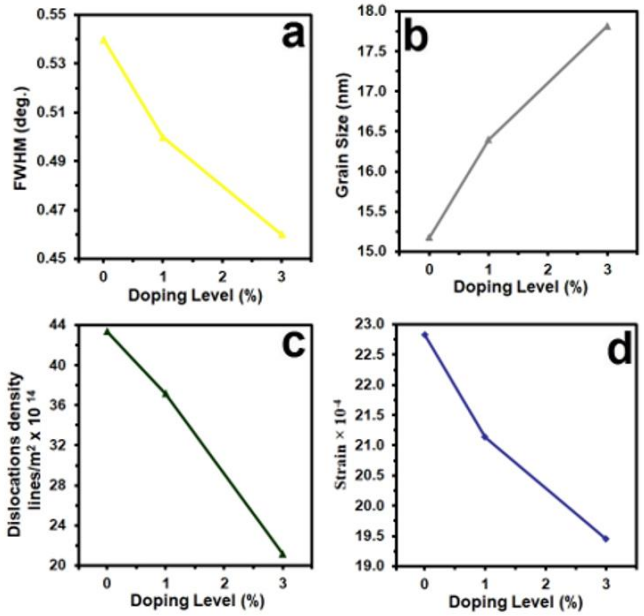


Figure 2: FWHM, strain and dislocation of pure and ZnS: In films with different dopant.

Table 1: AFM parameters of the intended films.

Samples	Average Particle size nm	R_a (nm)	R. M. S. (nm)
Undoped ZnS	78.1	9.29	8.73
ZnS: 1% In	75.8	4.15	5.91
ZnS: 3% In	31.5	3.66	3.31

3.3 Optical Analysis

Experimental measurements are often presented in terms of percentage transmittance (T), as defined in (4) [38]-[40]:

$$T\% = \frac{I}{I_o}\% \,. \tag{4}$$

Where I_o and I represent the initial and measured light intensities, respectively. T spectra are illustrated in Figure 4. Films exhibit high intensity across the visible light area, with lower transmission intensity observed at higher Indium concentrations. To some extent, this phenomenon could be attributed to surface roughening at higher dopant ratios [41], [42]. Notably, higher intensity was observed in the higher wavelength region of the visible light spectrum. These data assure active role of indium as a dopant in broadening the absorption spectrum of the resulting film [43], [44].

The absorption coefficient (α) was calculated via the following (5) [45]:

$$\alpha = \frac{(lnT^{-1})}{t} \,. \tag{5}$$

Where t is film thickness. From Figure 5, it is evident that the absorption coefficient value exceeds 10^4 cm^{-1}, which indicates a transition between the extended states in the valence band (VB) and the conduction band (CB). Additionally, a noticeable shift of the absorption edge towards lower energies is observed with increasing indium concentration [46], [47]. This shift signifies a decrease in E_g with increasing Indium content, as illustrated in Figure 5. Similar observations have been reported in previous studies [48].

The gap energy (E_g) of our films was determined using Tauc relation [50]:

$$\alpha hv = B(hv - E_g)^r. \tag{6}$$

Where B is a constant, and the exponent r takes values of 1/2 for direct. The resulting plots are depicted in Figure 6, which show that the deposited films are determined to be 3.59 eV, 3.55 eV, and 3.50 eV, respectively. These results agree with Geng et al. [51].

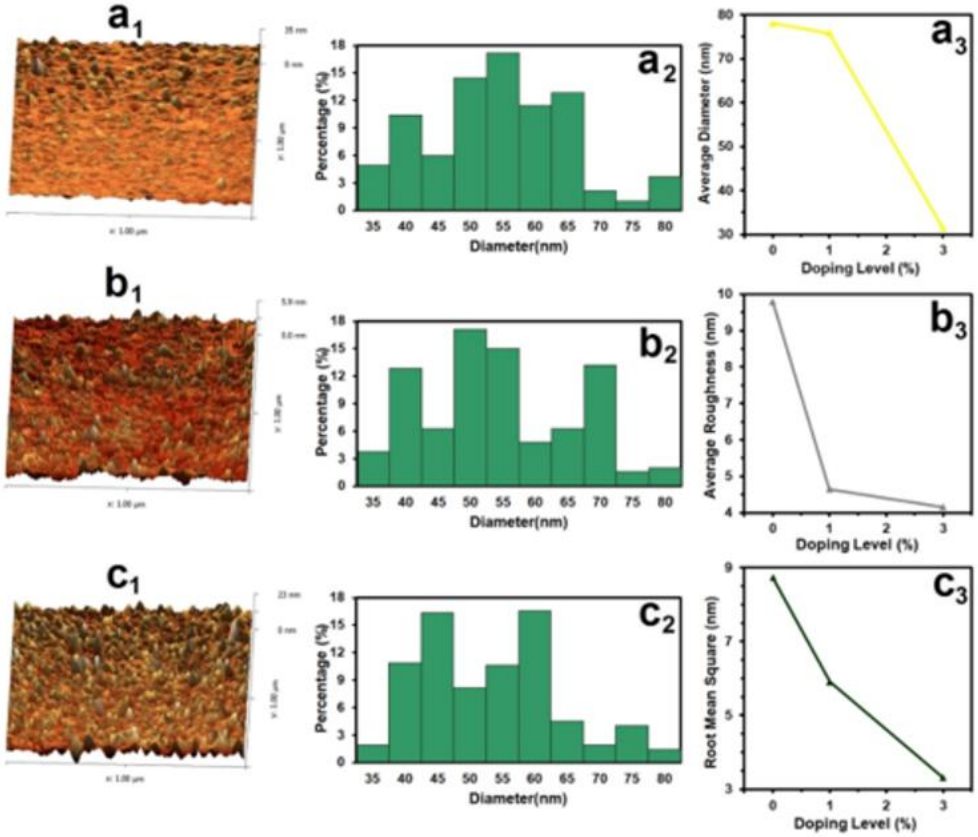


Figure 3: AFM images a_1), b_1), and c_1), granularly distributed a_2), b_2), and c_2) and diversity of AFM parameters against doping a_3), b_3); and c_3).

Refractive index n were calculated by (7) [52]:

$$k = \frac{\alpha\lambda}{4\pi}. \quad (7)$$

The extinction coefficient (k) were determined by (8) [53]:

$$n = \frac{1+R^{\frac{1}{2}}}{1-R^{\frac{1}{2}}}. \quad (8)$$

Figure 7, 8 shows that both n and k values decrease as the wavelength increases. n exhibits a decreasing trend with increasing λ [54], n remain relatively consistent around 450 nm and beyond. The refractive index impacts the optical pathway of light and influences the amount of light reflected from a surface. An increase in n of a solid corresponds to the rise in reflection. Figure 8 depicts k plotted against wavelength for ZnS and Indium-doped ZnS nanocrystals. k reaches their minimum values in the 500-700 nm wavelength range. The decreasing n can be attributed to surface effects and imperfections [55].

The gas sensing properties was done at an operating temperature of 100°C. Figure 9 shows the resistance variation when exhibited to NO_2 gas at a content of 300 ppm. It was observed that ZnS films exhibited the lowest resistance, suggesting a more efficient adsorption of NO_2 molecules [56]. In contrast, the ZnS film doped with 3% Indium demonstrated the highest resistance, likely due to changes in the film's electronic structure and the interaction between the dopant and NO_2 molecules, which might hinder the charge carrier mobility. This indicates that Indium doping increases the resistance and alters the gas sensing properties of the ZnS films [57].

The sensitivity can be calculated using (9) [58]:

$$Sensitivity = \frac{\Delta R}{R_g} = \left|\frac{R_g - R_a}{R_g}\right| \times 100\ \% \quad (9)$$

where R_g represents the resistance of the film sensor in air, and Ra is the resistance in the presence of gas. Figure 10 presents the sensitivity plots illustrating the effects of undoped ZnS, ZnS: 1% In, and ZnS: 3% In on NO_2 gas exposure. The recombination process of

charge carriers between electrons and holes impacts the sensitivity, showing a decrease as the Indium doping concentration increases. Specifically, for undoped, ZnS: 1% In, and ZnS: 3% In, the sensitivity decreases from 42.6% to 8.8 % at 100 ppm, from 48.4 % to 11.3 % at 200 ppm, and from 553.2% to 13.8 % at 300 ppm. This reduction in sensitivity indicates that higher Indium content result in a decline in the sensor's ability to respond to NO_2 gas [59, 60]. This behavior is attributed to the structural changes in the films due to doping process, which affects charge carrier mobility and consequently the sensor's performance [61].

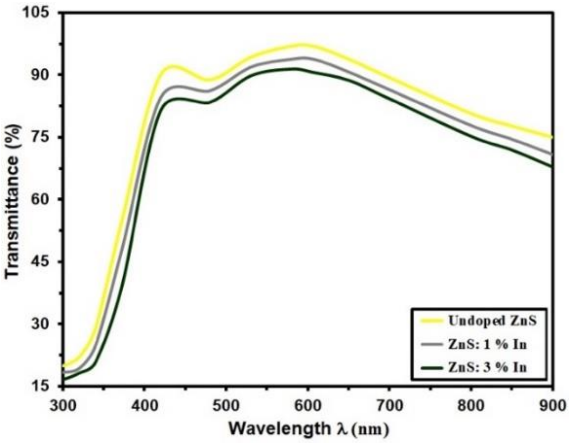


Figure 4: Transmittance of pure and ZnS:In films.

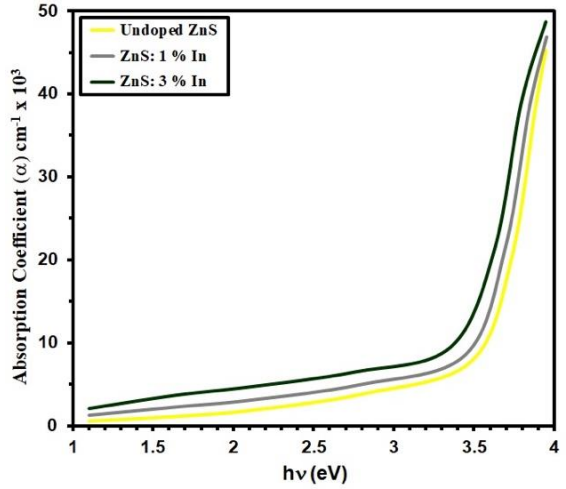


Figure 5: Absorption of pure and ZnS:In films with different dopant.

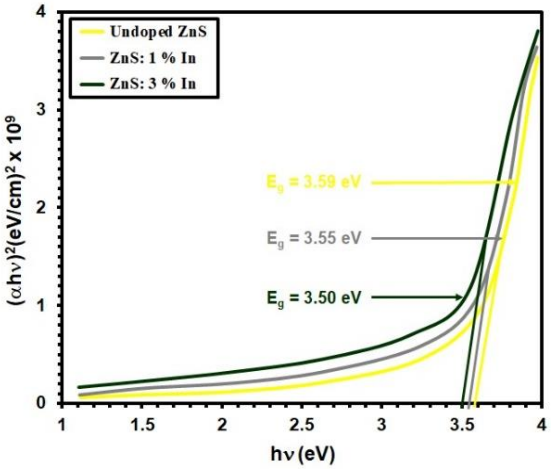


Figure 6: Plot of $(\alpha h\nu)^2$ versus $h\nu$ for pure and ZnS:In films with different dopant levels.

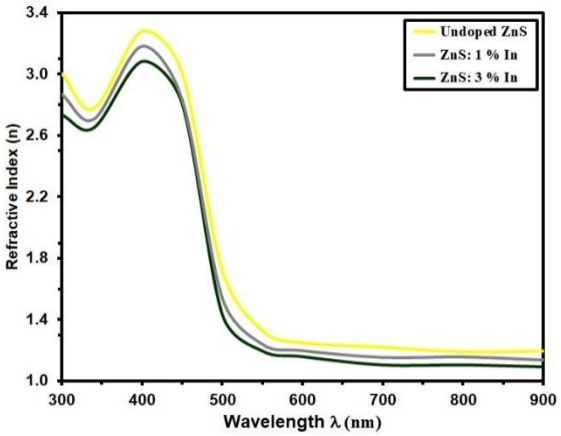


Figure 7: Refractive index (n) of pure and ZnS:In films as a function of wavelength..

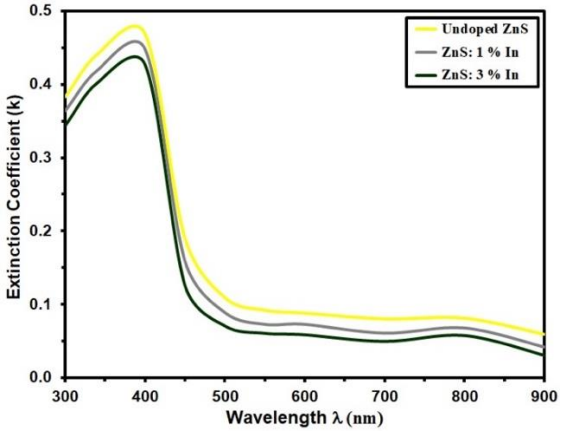


Figure 8: Spectral behavior of the extinction coefficient (k) for ZnS and ZnS:In thin films.

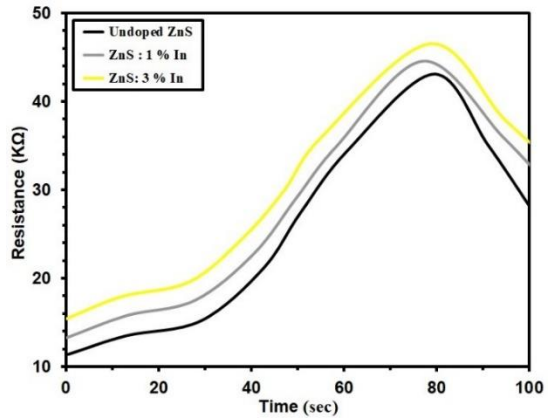


Figure 9: Resistance vs. operating time for undoped ZnS and In-doped ZnS films.

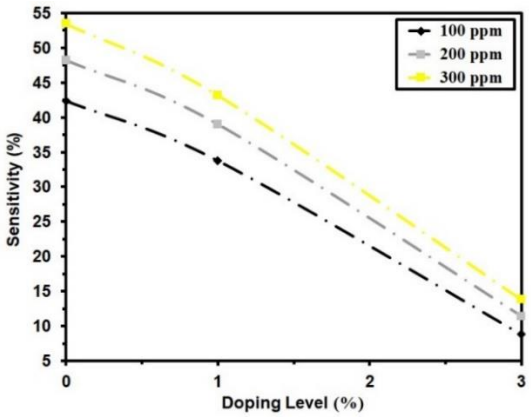


Figure 10: Sensitivity of undoped and ZnS in films with different dopants.

4 CONCLUSIONS

Indium-doped ZnS thin films were deposited using the CSP method. Films containing 0%, 1%, and 3% indium were deposited onto glass substrates at a temperature of 400°C. Structural characterization using X-ray diffraction (XRD) confirmed that all samples maintained a cubic zinc blende structure, with no evidence of secondary phases. As the indium concentration increased, a slight enlargement in crystallite size and a shift in diffraction peaks were observed, indicating successful substitution of Zn^{2+} ions by In^{3+} within the ZnS lattice. These changes also corresponded to a reduction in lattice strain and dislocation density, reflecting enhanced crystal quality. AFM studies reveal changes in film roughness following dopant treatment. The reduction in nano-grain size correlates with the concentration of indium, influenced by the interaction between ZnS and Indium during synthesis. UV-Vis measurements demonstrate a decrease in E_g with increasing Indium content. Additionally, reductions in n and k were observed due to doping, possibly due to the appearance of In3+ ions. Gas sensing tests conducted at 100°C revealed that undoped ZnS films exhibited the highest sensitivity to NO_2 gas. However, increasing the indium doping level led to a marked increase in electrical resistance and a significant decline in gas sensitivity. This reduction in performance is likely caused by decreased charge carrier mobility and increased electron-hole recombination, resulting from the structural and electronic changes induced by doping.

ACKNOWLEDGMENTS

The authors express their gratitude for the support provided by Mustansiriyah University and Alnukhba University College.

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Synthesis and Study Implications of Including Cobalt Oxide Nanoparticles on Structural and Optical Properties of Polyvinyl Alcohol Polyacrylic Acid Blend Films

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Keywords: PVA, PAA , CoO NPs, Structural , Optical Analysis.

Abstract: Poly Vinyl Alcohol / Poly Acrylic Acid (PVA/PAA) pure polymeric films made using the solution casting technique , It is strengthened by cobalt oxide nanoparticles (CoONPs) through deposition approach at calcination temperatures (800 Cº), In different weight percentages ((pure,1,3,5,7and 9) % wt.), burned at different temperatures (800 Cº). XRD analysis of the as-synthesized nanomaterial powder indicated that cobalt oxide nanoparticles were obtained at the calcination temperature (800 C°). Heat treatment promotes continued crystallization, leading to an expansion in the dimensions of nanoparticles. The electron microscopy FESEM outcomes showed that the PVA/PAA polymeric film seems uniform and monolithic, and once used (PVA/ PAA:CoO 7 %) , It forms widely distributed agglomerates within nanocomposite membranes. Show The FTIR picture CoONPs don't negatively impact the structure of polymers. No covalent connections exist between PVA , PAA and CoONPs. The visual characteristics of the films reinforced with CoO nanoparticles demonstrate their influence on optical transmittance over the (200-1100) nm wavelength range, alongside a decline in energy band gap values from 5.07 eV to 4.68 eV .

1 INTRODUCTION

Recent years have witnessed great interest in researching and enhancing polymer mixtures to increase the properties of polymeric materials [1], [2]. Polymer mixtures consist of combining two or more types of polymers to create a novel substance with specialized characteristics. Polymer fusion facilitates the combination of beneficial properties from multiple polymers. [3], [4]. The primary benefits of polymer blends are customization of mechanical strength, thermal properties, melting stages, permeability and processability [5]. Hybrid materials that include a mixture of polymers and inorganic nanofillers are known as polymer-blended nanocomposites [6]. Nanoparticles of metals, oxides, clays, silica, carbon nanotubes, and graphene are among the most common inorganic nanofillers [7]. Polymer mixtures with nanofillers, such as metal oxide-based nanocomposites, show significant improvements in strength, stability, and barrier capabilities. These nanocomposites have opened up new opportunities for the use of high-quality materials in diverse applications [8], [9]. Cobalt (Co) is one of the transition elements in the periodic table with atomic number 27. It is a shiny, silver-gray metallic element, solid but brittle and does not exist in nature as a free metal. Cobalt is generally found in ores. Cobalt is a ferromagnetic material, self-magnetizing, stable in air, affected by dilute acids and not affected by water. Cobalt is a chemically active element as it is characterized by its ease of integration with other elements, forming many compounds such as salts and oxides. The most common water-soluble synthetic polymer made worldwide is polyvinyl alcohol (PVA) [10]. The tangible properties of PVA depend Upon its preparation method, which involves Polyvinyl acetate hydrolysis or partial hydrolysis [11]. Because PVA is a polymer obtained through hydrolysis, it has particularly remarkable properties compared to other thermoplastics. Synthetic PVA is a polymer that dissolves in water. Its outstanding mechanical qualities, high chemical resistance, and ease of preparation make it a popular choice for biomaterial applications [12]. A biodegradable, water-soluble polymer with a variety of industrial uses is polyacrylic acid (PAA). PVA is a polyacrylic acid-based polymer; it is used as an oral suspension and

bioadhesive. It is also used as a gelling and suspending agent and emulsion stabilizer [13], [14]. The aim of this research is to synthesis of cobalt oxide nanoparticles and include cobalt oxide nanoparticles to improve the PVA/PAA blend polymer's structural and optical characteristics. The results we obtained are consistent with the results of the researcher (Khader Abbas Daoud) and his group [15].

2 EXPERIMENTAL PART

2.1 Preparation of CoONPs

The synthesis of CoO NPs, or cobalt oxide nanoparticles was performed using the chemical precipitation approach [14]. The desired cobalt nitrate concentration was $Co(NO_3)_2.6H_2O$, with a molecular weight of 291.04 g/mol. Dissolve 29.103g of cobalt nitrate, $Co(NO_3)_2.6H_2O$, in 100ml of water that has been distilled. Stir the mixture utilizing a magnetic stirring device at 50 C° until A uniform mixture was obtained. Gradually add 25% ammonium hydroxide (NH_4OH) solution, and a precipitate was formed. The precipitate was cleaned. dried in an oven after being repeatedly rinsed with distilled water at 200 C° . The resulting precipitate went into a crucible and calcined at 800C° to obtain cobalt oxide nanoparticles (CoO NPs).

2.2 Creating Nanocomposites of PVA/PAA:CoO

Pure blend of PVA and PAA polymer and polymers Samples of nanocomposite were made using a straightforward technique “solution casting”. The blend film PVA/PAA has been prepared by mixture PVA (70%) and PAA (30%). To prepared PVA/PAA/CoO nanocomposites films with varying percentages of weight (pure, 1, 3, 5, 7, and 9) , by added 0.5 gm of cobalt oxide nanoparticale to 30 ml of distilled water [16].

2.3 Equations Used

The crystal size of the samples is is computed using the highest XRD peak utilizing Scherrer's equation as shown in (1) [17].

$$D = \frac{0.9\,\lambda}{\beta\, cos\theta} \ . \qquad (1)$$

The coefficient of absorption (α) was determined by applying (2) [18].

$$\alpha = 2.303\frac{A}{t} \ . \qquad (2)$$

The disparity in energy (Eg) value of the electronic transition that occurs indirectly was measured according to pure (3) [19].

$$\alpha h\boldsymbol{\nu} = P(h\boldsymbol{\nu} - E_g) \ . \qquad (3)$$

The refractive index and extinction index (no, ko) values were estimated using (4) and (5) [20], [21].

$$n_o = \left[\left(\frac{1+R}{1-R}\right)^2 - (k_o^2 + 1)\right]^{1/2} + \frac{1+R}{1-R} . \qquad (4)$$

$$k_\circ = \alpha\lambda/4\pi \ . \qquad (5)$$

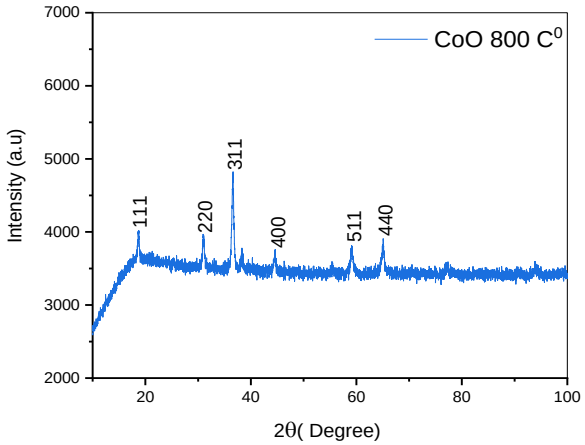


Figure 1: XRD patterns of prepared CoO NPs 800C° .

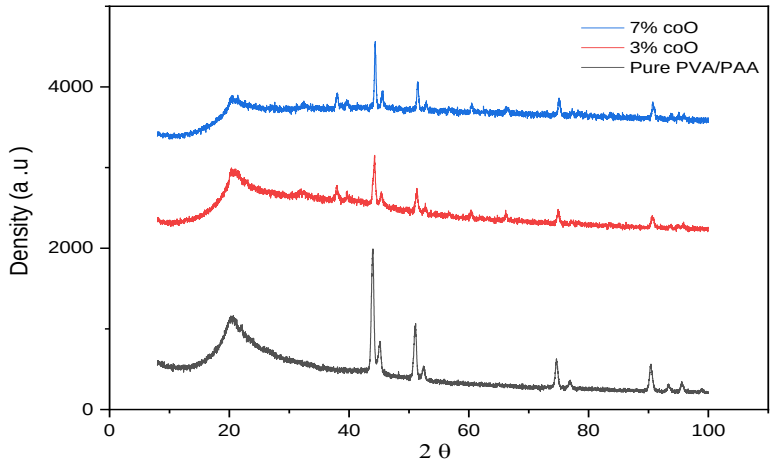


Figure 2: The XRD patterns obtained for pure (PVA/PAA) polymer films doped with CoO nanoparticles at weight ratios (3%, 7%).

3 RESULTS AND DISCUSSION.

3.1 Structural Analysis

3.1.1 X-Ray Diffraction (XRD)

The XRD test has been used to done to investigate the structure type (phase) and the crystalline size of prepared CoO NPs using the chemical precipitation method. Figure 1 presents the obtained XRD patterns of prepared CoO NPs.

After calcination of CoO nanoparticles at temperatures (800C°), several characteristic peaks were observed at (2θ = 19.001°, 31.27°, 36.84°, 44.81°, 59.35°, and 65.23°) of (111), (200), (311), (400), (511), and (440). The detected characteristic peaks confirmed the formation of cubic structure of CoO nanoparticles with stereogenic group (Fd3m No. 227) and lattice parameters (a = 8.1290 Aº, b = 8.1290 A°, c = 8.1290 A°) and (α = β = γ = 90°) which are consistent with the usual data (ICDD). (00-043-1003) [14].

Table 1: Calculated XRD parameter of synthesized CoO NPs 800Cº.

Sample	CoO NPs at 800 °C
2θ (°) Standard	36.83
2θ (°) Practical	36.53
FWHM (deg)	0.4149
D (nm)	20.16
(hkl)	(311)

XRD test was conducted for pure (PVA/PAA) polymer films doped with CoO nanoparticles at weight ratios (3%, 7%). It was found that the peaks of the doped (PVA/PAA) polymer films increased in intensity and became sharper with increasing doping ratios. which leads to improving the properties of these films. Figure 2 makes it evident that the peaks formed for the polymer films at the ratios (3%, 7%) match the peaks of the CoO NPs nanoparticles shown in Figure 1 at (2θ = 20.3°, 32.2°, 37.84°, 44.81°, 60.4°, and 66.4°). Figure 2 shows the XRD patterns obtained for pure (PVA/PAA) polymer films doped with CoO nanoparticles at weight ratios (3%, 7%).

3.1.2 FESEM Analysis

To know the surface nature and shape of the cobalt oxide (CoONPs) nanopowder prepared by chemical deposition method, The electron microscope examined the produced samples' surface morphology. microscope type (FEI INSPECT F50) using a voltage difference of (40 kV). Figure 3 show the microscopic images of the cobalt oxide (CoONPs) nanopowder calcined at a temperature of (800Cº). Calcined cobalt oxide nanopowder (CoONPs) were represented as dense aggregates of nanoparticles. We notice that the nanopowder is experiencing a change in nanostructure, as well as an increase in crystallinity and granular size. This is because to the elevated calcination the temperature that results in the continued crystalline growth of the granules and their combination, improving the size of the granules [22].

Also, The scanning electron microscope with field emission (FE-SEM) examination was performed On the ready (PVA /PAA) films before and after reinforcing with cobalt oxide (CoONPs)

nanopowder (3%, 7%) at a temperature of (800 C°), Figure 4 shows the microscopic images of the pure films. The images show that the pure (PVA/PAA) film is homogeneous, which is consistent with [23]. When adding cobalt oxide nanoparticles (CoONPs) at a rate of 3%, 7%) at a temperature (800C°), as shown in Figures 5 and 6, the images demonstrate that the polymeric membranes supported by nanoparticles (PVA/PAA: CoONPs) contain many agglomerates dispersed across the surface, since the findings show that the nanoparticles (CoONPs) incline to form dispersed aggregates in nanocomposite membranes [22], [24].

3.1.3 FT-IR Analysis

FTIR analysis of the prepared films was performed to identify the active aggregates by measuring the transmittance spectrum as a wavenumber function in the 500 - 4000 cm-1 range. Figure 7 shows the characteristic infrared spectrum of the (PVA / PAA) films prepared by the solution casting method before and after reinforcement with different weight ratios (pure,1, 3, 5,7 and 9) (wt%) with calcined Nanoparticles of cobalt oxide (CoONPs) when the temperature is 800C°.

Fourier transform infrared (FT-IR) spectra of the PVA/PAA:CoO nanocomposite and pure PVA/PAA composite are displayed in Figure 7. The absorption bands typical of pure (PVA/PAA) are visible in all spectra composite and (PVA/PAA:CoO) nanocomposite (645,838,1062,1242,1704,2923 and 3244) cm^{-1} . It is evident that these treatments result in major variations in the intensity of certain absorption bands as well as certain discernible changes in the samples' spectral characteristics. Defects brought on by the interaction of the nanoparticles and the polymer might be connected to the bands blend which causes the improvement of the polymer properties. All samples showed broad bands at around (3244 cm^{-1}) because of the polymer matrix chain's O-H groups, peaks at (2923 cm^{-1}) are connected to the lengthening of the current (C-H) bond, 1704 cm^{-1} Stretching (C-O) , 1242 cm^{-1} (C-N) Converging, and 1062 cm^{-1} (C-H) bending for PVA/PAA as shown in Figure 7. Moreover, the vibrational peaks in the array (1062-645) cm^{-1} can be credited to (CoO) [26]. Indicating that cobalt oxide nanoparticles are doped within the (PVA /PAA) composite. The results of the experiment shown in Figure 7 after adding (CoO) nanoparticles. Some polymer chains were broken, and other chains were created in its place; these outcomes are comparable to the findings of the researchers [25], [26].



Figure 3: CoONPs powder calcined at 800°C as seen in FE-SEM images.

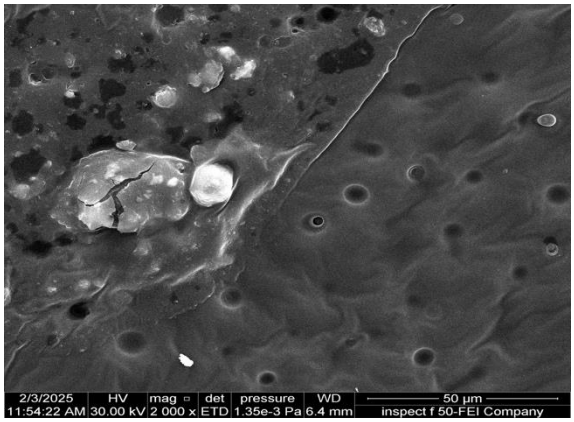


Figure 4: FE-SEM images of pure PVA/PAA films.

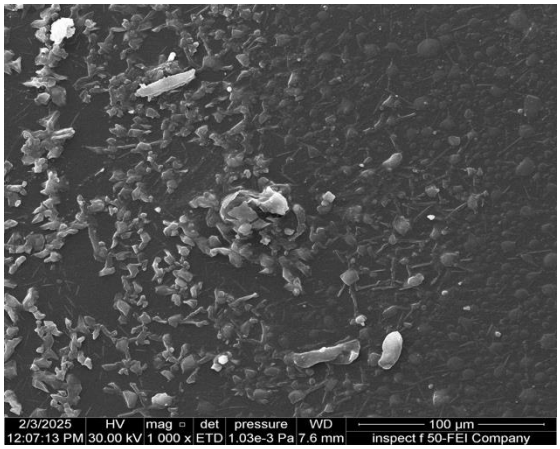


Figure 5: FE-SEM images of films supported by 3% (CoONPs).

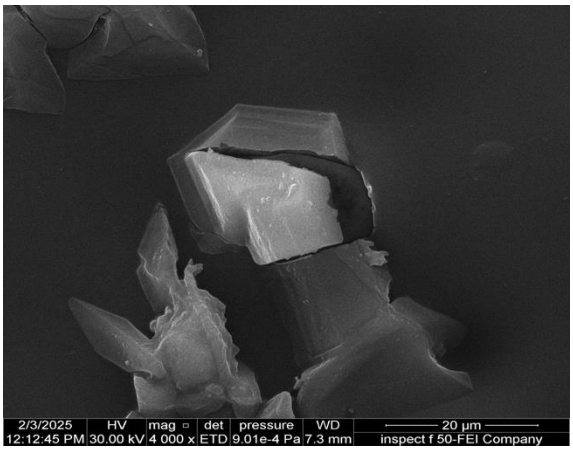


Figure 6 : FE-SEM images of films supported by 7% (CoONPs).

3.2 Optical Analysis

A study of transmittance and absorbance spectra helps in understanding and improving polymer structure and energy bands. Figures 8 and 9 demonstrate the transmittance and absorbance spectrum of the unadulterated mixture (PVA/PAA) and the mixture reinforced with varying weight percentages of burned Nanoparticles of cobalt oxide (CoO NPs) when the temperature is (800 C°). The application of CoO NPs (cobalt oxide nanoparticles) resulted in a decrease in the permeability of the PVA/PAA blend, where the decrease in permeability became more pronounced with the increase in the proportion of CoO NPs. The transmittance of the prepared samples increases with wavelength until it stabilizes at wavelengths above (650-800) nm. This is because increasing the weight ratios of nanoparticles leads to increased nanoparticle density, which in turn leads to increased dispersion and reduced transmittance, according to Reference [27].

On the other hand, The (PVA/PAA) mixture's absorbance rose after the addition of (CoO NPs). In addition, the absorbance decreased significantly with increasing wavelength and stabilized at a wavelength higher than (650-800) nm. This can be ascribed to incident energy being absorbed and dispersed light by (CoO NPs) within the (PVA/PAA) mixture, where part absorbs a portion of the incident light, while the remainder is scattered [28].

The α, the PVA/PAA films' absorption coefficient, was calculated using (2) according to Figure 10. It is demonstrated that the coefficient of absorption of the as-prepared samples is higher at greater photon energies and diminishes with increasing photon energy. The absorption coefficient increases as the content of CoO nanoparticles increases. It is explained by the rise in charge carriers, leading to a higher absorption coefficient of the nanocomposite films. In addition, α is an indicator of the kind of electrical transition found in the films that were developed, as the values of α for PVA/PAA: CoO are [29], [30].

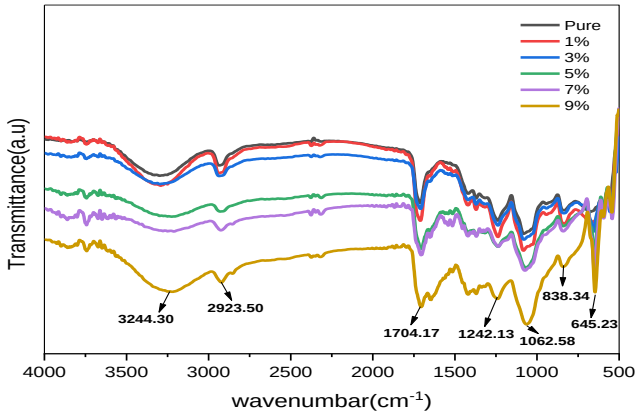


Figure 7: Infrared spectrum of cobalt oxide nanoparticles (CoONPs) calcined at 800 °C and pure (PVA/PAA) films supported with varying weight percentages (pure, 1, 3, 5, 7, and 9) (wt%).

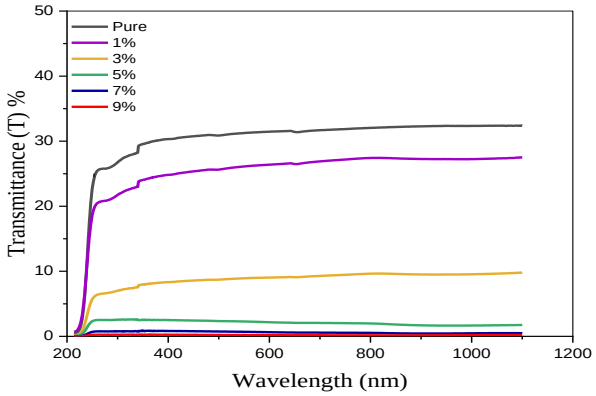


Figure 8: Transmittance spectrum of pure (PVA/PAA) films supported by various weight ratios (wt%) of cobalt oxide nanoparticles (CoONPs) (pure, 1, 3, 5, 7, and 9).

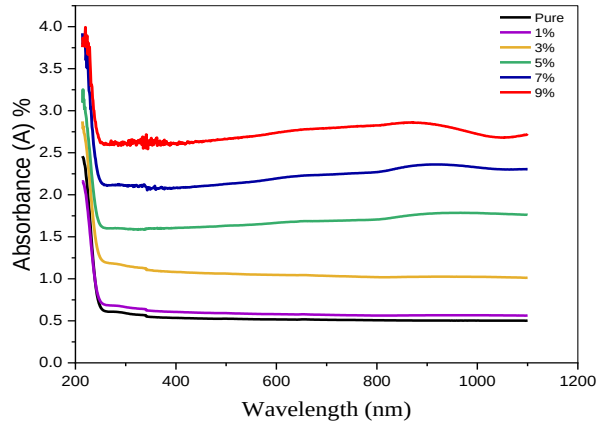


Figure 9: Absorption spectrum of pure (PVA/PAA) films supported by various weight ratios (wt%) of cobalt oxide nanoparticles (CoONPs) (pure, 1, 3, 5, 7, and 9).

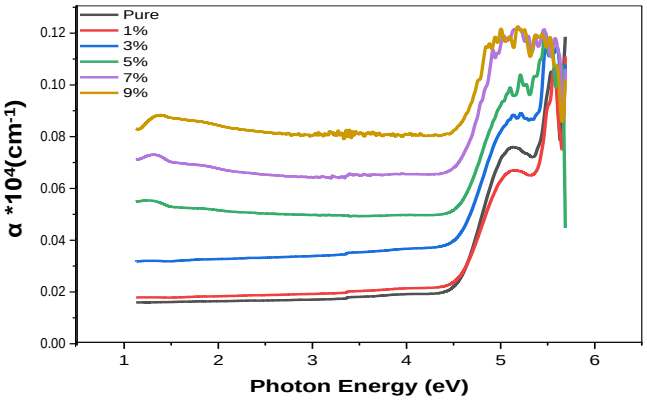


Figure 10: Absorption coefficient for pure (PVA/PAA) films supplemented with varying weight ratios (wt%) of cobalt oxide nanoparticles (CoONPs) (pure, 1, 3, 5, 7, and 9).

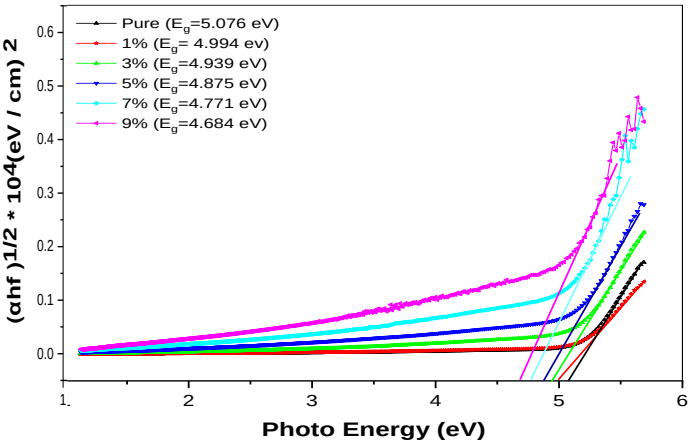


Figure 11: Energy gap for pure (PVA/PAA) films supplemented with varying weight ratios (wt%) of cobalt oxide nanoparticles (CoONPs) (pure, 1, 3, 5, 7, and 9).

As also shown in Figure 11, using pure (3), the energy gap (E_g) value of the PVA-PAA films' indirect electronic transition was determined at 5.076 eV. However, when the amount of cobalt oxide nanoparticles in the reinforced films increases, their E_g values decrease. Upon calcination at 800°C, the values become 4.99 eV, 4.93 eV, 4.87 eV, 4.77 eV, and 4.68 eV for strengthening ratios of (1, 3, 5, 7, and 9) %, respectively. As cobalt oxide (CoO) nanoparticle concentration rises, the defects in the films become more obvious, leading to a reduction in the energy gap measurement. A decrease in energy gap values occurs due to these defects, which generate specific regions of energy levels within them, and the optical properties of the components produced may change as caused by impurities and surface flaws on the nanoparticles that contribute new electrical states [31] .

The index of refraction (n_o) and indicator of extinction (k_o) values of the films PVA / PAA were estimated using (4) and (5). It was observed from Figure 12-13 that as the concentration of (CoO NPs) increased, both (n_o and k_o) increased. (n_o) showed an upward trend, the incident photon energy rises, resulting in an increase in (n_o) for the films that have been prepared. In addition, higher refractive index values with increasing enhancement are due to increased nanoparticle density, also known as packing density. The increase in loss shadow can explain the higher absorption coefficient (α), as there is a strong relationship between the two variables, as agreed by many researchers [27], [32] .

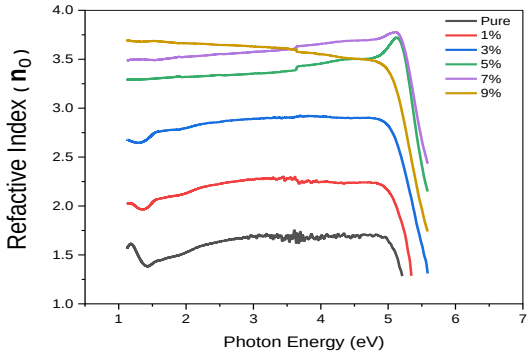


Figure 12: Index of refraction for pure (PVA/PAA) films supported with different wt% (pure,1, 3, 5,7, and 9) of cobalt oxide nanoparticles (CoONP).

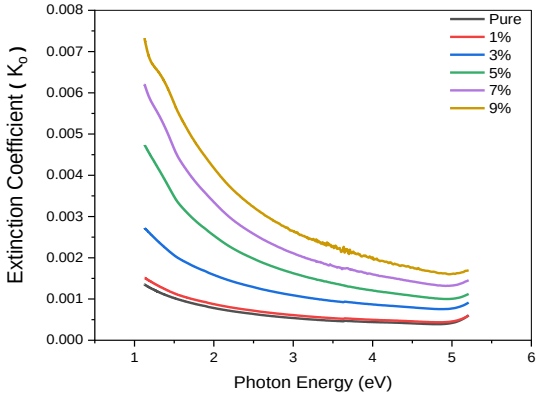


Figure 13: Extinction coefficient for pure (PVA/PAA) films supported with different wt% (pure,1, 3, 5,7, and 9) of cobalt oxide nanoparticles (CoONP).

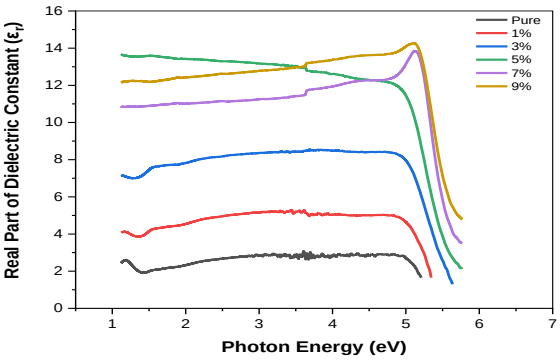


Figure 14: The actual part of pure (PVA/PAA) films supported with different wt% (pure,1,3,5,7and9) of (CoONPs) The temperature at which it was calcined was 800 Cº.

Figures 14-15, which illustrates the change in the real and imaginary parts of the dielectric constant of pure PVA/PAA films supported with varying weight percentages, was calculated using the real portion (ε_r) and the imaginary part (ε_i) of magnesium oxide nanoparticles (CoONPs) as a function of photon energy.

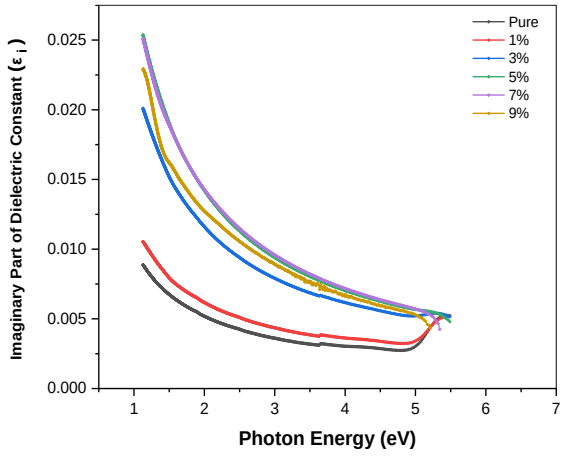


Figure 15: Imaginary part the dielectric constant of pure (PVA/PAA) films supported with different wt% (pure,1,3,5,7and9) of (CoONPs) The temperature at which it was calcined was 800 Cº.

The findings indicate that when calcined cobalt oxide nanoparticles are added to PVA/PAA films, the real and imaginary components of the dielectric constant rise (CoONPs) are added, which is associated with the rise in incoming light absorption and scattering brought on by an increase in nanoparticle proportions [33], [34]. Table 2 displays the energy gap, absorption coefficient, peak extinction coefficient, and peak refractive index values for pure PVA/PAA films that are supported by varying amounts of CoONPs.

Table 2: Values of (energy gap, absorption coefficient, peak refractive index, and peak extinction coefficient) for pure and reinforced (PVA-PAA) films.

Sample	Energy Gap (eV)	(α*10^4 cm^{-1}) Max	(n_o) Max	(k_o) Max
PVA/PAA	5.076	0.024	1.746	0.0013
PVA/PAA: CoONPs (1%)	4.994	0.026	2.306	0.0015
PVA/PAA: CoONPs (3%)	4.939	0.042	2.927	0.0027
PVA/PAA: CoONPs (5%)	4.875	0.054	3.692	0.0047
PVA/PAA: CoONPs (7%)	4.771	0.072	3.706	0.0061
PVA/PAA: CoONPs (9%)	4.684	0.088	3.780	0.0073

4 CONCLUSIONS

The chemical precipitation method has proven effective in Cobalt oxide nanoparticle synthesis, which are used in different weight ratios to enhance the polymer blend (PVA/PAA). The nanocomposite (PVA/PAA:CoONPs) was created using the solution casting method. X-ray diffraction data indicated the effect of calcination temperatures, accompanied by an improvement in crystallinity and crystallite size. FESEM results indicated uniformity, purity, and smoothness of the pure films, but the supported films showed bright spots with agglomerates with high nanoparticle concentration. Visual analysis of the samples indicated that increasing addition of reinforcement is associated with higher values of (A), (α), (n_o), and (ε_r) and (ε_i) components of the dielectric constant, accompanied by a decrease in the values of permeability and energy gap.

We will leverage this research to improve the physical properties of polymer membranes for use in several applications. Future applications: Polymer membranes are used in solar cells, as well as in transistors and medical applications.

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Synthesis and Structural Analysis of Molybdenum Disulfide Nanoparticles by Sol-Gel Technique

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Keywords: Molybdenum Disulfide, Sol-Gel, XRD, FESEM, FTIR.

Abstract: Sol-gel was used to make molybdenum disulfide nanoparticles (MoS2) for this work. X-ray diffraction (XRD), Field emission scanning electron microscopy (FESEM), and Fourier transform infrared spectroscopy (FTIR) methods were used to examine the results. The XRD test showed that the nanoparticles were hexagonal, and the Scherrer method was used to find out that the average crystallite size (Dav) of the nanoparticles that were produced was around 40 nm. According to FESEM images, the crystalline forms are heterogeneous in size and shape, and the MoS2 nanosheets stack together to form a huge block with varying internal thicknesses. The average size distribution of MoS2 is about 42 nm. These results are consistent with the XRD. FTIR measurements performed for MoS2 nanoparticles showed that the strong and weak absorption bands of the (S - S) bond are located between (543-809) cm^{-1}, while the bands located (1100-1630) cm^{-1} are due to the (Mo-S) bond, while the bands located at (2740-2357) cm^{-1} are due to the (O-H) bond.

1 INTRODUCTION

Scientists are very interested in transition metal dichalcogenides (TMDs) in two dimensions right now because they have some very interesting properties. The chemical formula for two-dimensional transition metal dichalcogenides is MX_2. Ti, chromium, manganese, zirconium, nickel, molybdenum, tectonic, hydrogen, and iron are all transition metals from Group IVB to Group VIIB, which includes M. As a chalcogen atom, X is composed of selenium, sulfur, and copper [1]. Figure 1 [2] illustrates that TMD materials can be constructed in both layered and non-layered fashions. A high surface-to-volume ratio, significant catalytic activity, a high degree of hydrophilicity, a large number of edge facets, a high chemical inertness, and a changeable band gap are some of these materials' physical and chemical characteristics. Developments in atomically thin two-dimensional transition metal chalcogenides have produced a number of exciting innovations in photonics, energy storage, nanoelectronics, sensing, and optoelectronics [3]. Molybdenum disulfide is used in solid lubricants, photovoltaics, and rechargeable batteries, among other things, because it has good optical, electrical, and mechanical properties. The crystal structure of molybdenum disulfide is hexagonal, with two sulfur layers in the middle of metal layers that are held together by weak van der Waals forces [4]. MoS_2 is a layered transition metal chalcogenide that has been studied a lot. A straight band gap of 1.8 eV is found in MoS2 when it is in a single layer [5]. It's good news that MoS_2 can mostly make up for gapless graphene's flaws, because this means that two-dimensional materials can be used in photonic and next-generation switching devices [6]. The sol-reaction method was used to make MoS2 nanoparticles for this study, and XRD and FESEM were used to look at their structural features.

2 EXPERIMENTAL DETAILS

MoS_2 nanoparticles were prepared by sol-gel method by following the following steps:

1) Sodium molybdate dihydrate $Na_2MoO_4.2H_2O$ with a 241.95 g/mol molecular weight and a density of (g/cm^3) was dissolved in deionized distilled water at a concentration of 0.1 M. Thiourea $SC(NH_2)_2$ with a purity of 99%, a molecular weight of 12.76 g/mol, and a concentration of 0.1 M, prepared by (Poole-England-General Purpose Reagent Limited), was also used. Citric acid

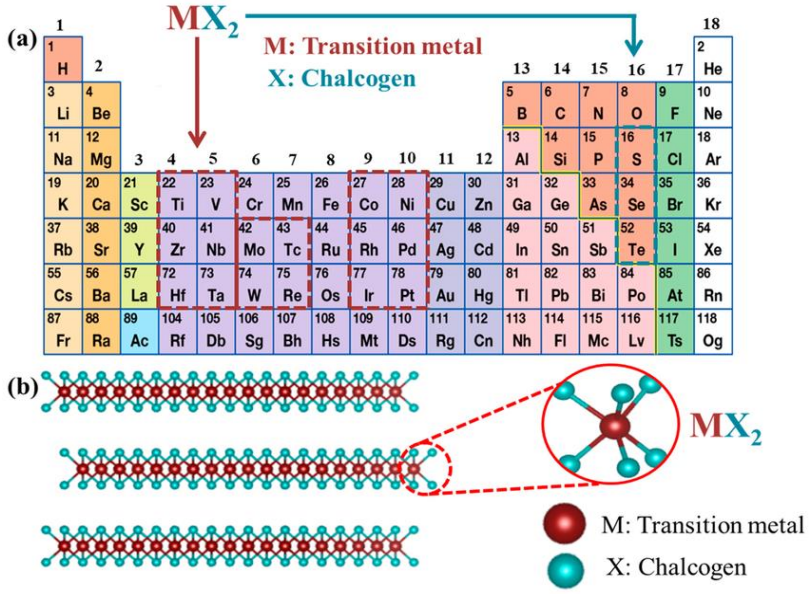


Figure 1: The components of TMDs are composed of three chalcogen atoms and sixteen transition metals [9].

with a molecular formula of C6H8O7, a concentration of 0.5 M, and a molecular weight of 192.123 g/mol was also added.

2) After completing the dissolution process, the acidity (pH) of the solution was measured and it was (pH=1.4). To make the solution neutral, ammonia solution (NH_4OH) was added at a concentration of (25%), prepared by SIGMA, in drops to the prepared solution at intermittent intervals, while the solution remained on the magnetic mixer until the solution became neutral (PH=7 ± 0.05), and the solution turned white.
3) Before the solution turned into gel, the magnetic mixer's temperature was raised until it reached 80°C. The temperature stayed the same, and the solution was left on the magnetic mixer for 40 to 50 minutes so that the liquid would slowly evaporate, as shown in Figure 2.
4) In order to eliminate all water molecules and other liquids, the resultant particles were annealed in an electric furnace set at 500°C for two hours. The particles were then left inside the furnace for two hours, and the particles were left inside the furnace for 24 hours until its temperature reached room temperature as shown in Figure 1, after that (MOS2) particles were obtained.



Figure 2. Steps for preparing MoS_2 nanoparticles by sol-gel method

3 RESULTS AND DISCUSSIONS

3.1. Results of XRD

The XRD of the produced MoS_2 nanoparticles is displayed in Figure 3. The diffraction peaks 2Θ~14.36°, 28.96°, 32.61°, 33.42°, 39.45°, 44.05°, 49.68°, 58.18°, 60.62°, 75.83° and 88.44° have appeared. It is referred to as Miller index favored directions (002), (004), (100), (101), (103), (006), (105), (110), (114), (116), and (118), respectively. The card number (01-075-1539) of MoS_2 from the Inorganic Crystal Structure Database (ICSD) is exactly what these values correspond [7]. Additionally, results indicated the hexagonal structure of the nanoparticles.

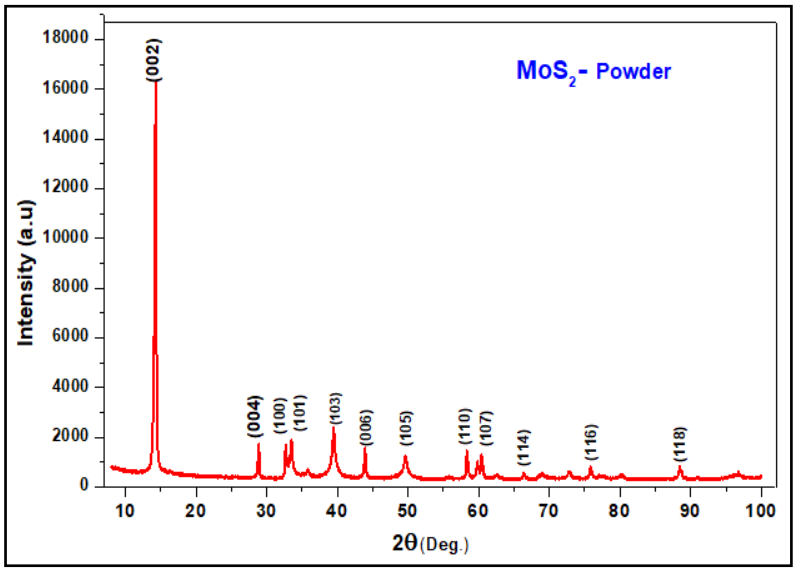


Figure 3: XRD of MoS_2 nanoparticles.

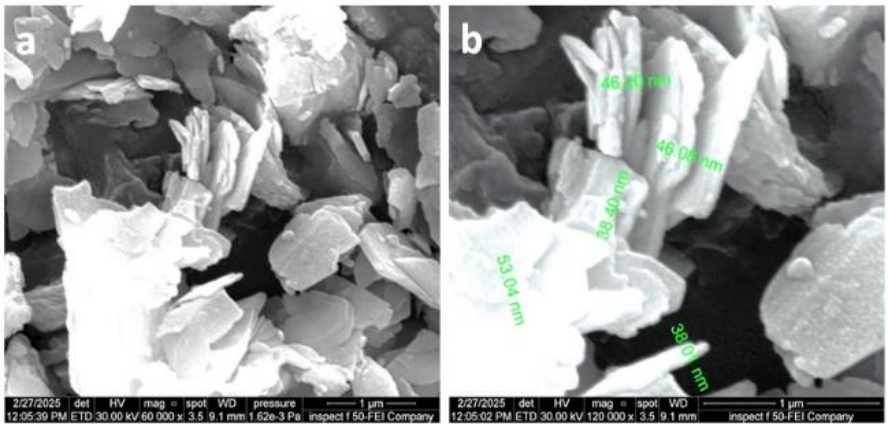


Figure 4: An FESEM image of MoS_2 nanoparticles a) and b).

Table 1: The MoS_2 nanoparticles' structural characteristics.

θ° (deg.)	d-spacing (Å)	FWHM (rad)	average crystallite size (D_{av}), (nm)	Micro Strain $*10^{-3}$	SSA ($m^2.g^{-1}$)
14.36	6.22457	0.2798	**29**	1.09295	41
28.96	3.09608	0.2058	**40**	0.34275	30
32.61	2.73764	0.2673	**31**	0.39945	38
33.42	2.67751	0.3577	**29**	0.8713	41
39.45	2.28262	0.5564	**41**	1.14817	29
44.05	2.06161	0.1965	**32**	0.2684	37
58.18	58.3337	0.1811	**50**	0.14795	59
60.62	1.53129	0.1895	**48**	0.14083	25
75.83	1.25372	0.2273	**44**	0.17428	27
88.44	1.10444	0.2442	**45**	0.13915	26

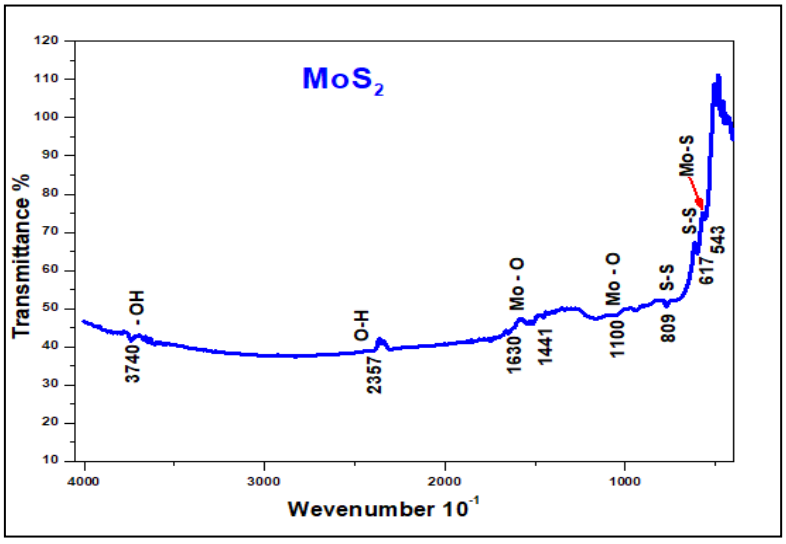


Figure 5: FTIR measurements for MoS_2 nanoparticles.

Using Bragg's law and the relation (1) [8], the distance between interplanar spacing (d_{hkl}) with the same Miller's coefficients (hkl) was computed [8] . when it was discovered that the value of (d_{hkl}) is close to and consistent with the MoS_2 standard card (ICSD card no. 75-1539) [7]

$$n\lambda = d_{hkl} \sin \Theta_B . \quad (1)$$

Where: d_{hkl} - the distance between interplanar spacing, Θ_B: Bragg angle, λ - wavelength, n - order of reflection.

For the produced MoS_2 nanoparticles, the crystal lattice constants ($a_o = b_o$, and c_o) were computed using the hexagonal structure relation, (2) [9]. Table 1 displays these values

$$\frac{1}{d^2{}_h} = \frac{4}{3}\left(\frac{h^2+hk+k^2}{a_o^2}\right) + \frac{l^2}{c_o^2} . \quad (2)$$

From planes 110 and 114, we found the lattice constants a_o and c_o. The numbers h, k, and l stand for Miller coefficients. It was found that the values of the lattice constants are the same as those on the MoS_2 international card number 75-1539. The information is shown in Table 1. We used the Scherrer method and (3) [10]–[12] to figure out the average crystallite size (Dav) of the nanoparticles that were made:

$$D_{av} = \kappa\lambda / (\beta \cos \theta^B). \quad (3)$$

The quantity of deficiencies in the crystal is measured by the dislocation density (δ). Good crystallization of MoS_2 nanoparticles made using the sol-gel approach was confirmed by the minimal dislocation density value found in this work (4) was used to compute the dislocation density [13] as shown in Table 1:

$$\delta = 1 / D_{av}^2. \quad (4)$$

According to one meaning, specific surface area (SSA) is the area measured in mass units (m^2/g). It determines the quality of materials and is a very significant attribute for nanomaterials. It also provides information regarding surface-level interactions [14]. The SSA values determined using the following (5) [15] are displayed in Table 1:

$$SSA = S_V / \rho . \quad (5)$$

When K_{SV} is set to 6, which is the number for a sphere, the surface density (SV) is displayed.and D_{av}, while ρ is the material density (ρ = 5.06 g/cm^3 for MoS_2 nanoparticles)., i.e., $SV = \frac{K_{sv}}{D_{av}}$. Rearranging (5) yields the following (6):

$$SSA = 6 \times 10^3 / (D_{av} \rho). \quad (6)$$

3.2 Analysis of FESEM

FESEM analysis was performed on the sol-gel-prepared MoS_2 nanoparticles. Figure 4a and 4b illustrates that the crystalline formations exhibit diverse, heterogeneous sizes and morphologies, and that the MoS_2 nano sheets stack together to form a large block with different internal thicknesses. These results are consistent with studies [16], [17], [18]. Figure 4b shows the average size distribution of MoS_2 approximately 42 nm. The X-ray diffraction results are in agreement with these results.

3.3 FTIR Measurements

FTIR analysis was performed on MoS_2 nanoparticles made using the sol-gel process in the 400–4000 cm^{-1} range. by computing the wavenumber dependency of the transmittance spectrum. The transmittance spectrum was measured as a function of wavenumber. Figure 5 shows relatively strong absorption bands at (543, 617, 806, 1100, 1441, 1630, 2357, and 3740) cm^{-1}. These results are consistent with studies [19], [20], [21], which showed that the strong and weak absorption bands of the (S - S) bond are located between (543-809) cm^{-1}, while the bands located (1100-1630) cm^{-1} are due to the (Mo-S) bond, while the bands located (2740-2357) cm^{-1} are due to the (O-H) bond.

4 CONCLUSIONS

Sol-gel was used to make molybdenum disulfide nanoparticles (MoS2) for this work. Transition metal dichalcogenides (MoS2) nanoparticles were prepared by sol-gel method using sodium molybdate dihydrate [Na2MoO4.2H2O] with thiourea (SC(NH2)2. The XRD examination yielded nanoparticles with an average size of 41 nm, which matched the particle size estimated from FESEM images, which was approximately 42 nm, therefore, MoS2 nanoparticles can be used in the field of batteries and catalysts. The average size distribution of MoS2 is about 42 nm. These results are consistent with the XRD. FTIR measurements performed for MoS2 nanoparticles showed that the strong and weak absorption bands of the (S - S) bond are located between (543-809) cm^{-1}, while the bands located (1100-1630) cm^{-1} are due to the (Mo-S) bond, while the bands located at (2740-2357) cm^{-1} are due to the (O-H) bond

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Effect of Lithium on Physical and Sensing Properties of Titanium Oxide Nanostructured Thin Films Prepared by Chemical Spray Pyrolysis

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Keywords: Li-Doped TiO2 Thin Films, Structural, Morphological Optical, Chemical Spray Pyrolysis.

Abstract: Li-doped titanium oxide thin films are grown through Spray Pyrolysis (SP) method. XRD peaks showed that samples were polycrystalline. The appropriate peak was at (121) equivalent to $2\theta = 30.70^{\circ}$, the Grain size (D) increased from 9.58 nm to 10.17 nm, whereas strain (ε) decreased from 36.17 to 34.08, whilst dislocation density (δ) decreased from 108.96 to 96.68. According to the AFM photo, surface roughness declined (8.08 - 3.67) nm when TiO_2 was increased to 4% Li. The average particle size values were 88.78, 85.62, and 60.89 nm for TiO2, TiO_2:2 % Li, and TiO_2:4% Li, respectively. The transmittance of TiO_2 and TiO_2: Li films reduced from 85 TiO_2 % to 75 % as Lithium content rise from 1 to 4 at%. Research indicates that the absorption coefficient reduces as the lithium content rises, whereas the bandgap energy, extinction coefficient, and refractive index decline as the lithium content rises. The TiO_2 gas sensor showed increased resistance at 200 ppm NH3, with 4% Li doping having the highest. Higher Li doping in TiO_2 decreases sensor sensitivity to NH3 gas, with a reduction at all concentrations.

1 INTRODUCTION

Due to its numerous advantageous attributes, titanium oxide (TiO_2) is an intriguing substance that has the potential to be employed in numerous applications. These properties are high transparency, non-toxicity, high refractive index, affordability, good optical and electrical properties, and chemical stability [1]. In addition, TiO_2 can be deposited on large areas and is inexpensive, making it an appropriate material for industrial use. Due to the preferred properties, TiO_2 is utilized in multiple uses, including optoelectronics devices, gas sensors, photocatalyst photoelectrochemical water splitting, and solar energy conversion. [2]-[4]. Numerous techniques are employed to deposit TiO_2 thin films, including e-beam evaporation [5], sol-gel [6], sputtering [7], precipitation [9], anodic oxidation [9], hydrothermal [10], PLD[11], CVD [12] and CSP [13]. However, the spray pyrolysis deposition method has appropriate advantages: affordable deposition equipment, the ability to coat large areas, and controlling the composition is easy [14]. The Chemical Spray Pyrolysis technique fabricated the Undoped TiO_2 and TiO_2: Li thin films to get inexpensive large-area films with good characteristics. The article discusses the alteration in physical characteristics of TiO_2 thin films as a result of Li doping.

2 EXPERIMENTAL

Thin films of TiO_2 and TiO_2: Li grown by CSP technique. The base was cleaned with chromic acid for 4 hours, rinsed with running water for 20 minutes, and in an ultrasonic bath filled with absolute ethanol

for 8 minutes. 0.1M of $TiCl_2.2H_2O$ was supplied from Sigma-Aldrich Chemicals, with 0.1M of $LiCl_2.4H_2O$ from Merck Chemicals. Various Li contents were used (0, 2, 4%). The following were the deposition conditions: the base temperature was 400 degrees Celsius, The separation between the substrate and the outlet was 28 cm, the spraying rate was 10s, stopping by 90 s to avoid cooling, spray rate was 5 ml per second, and N_2 is being used as the gas transport. Film thickness was measured employing weighing method, and it was discovered to be 300±30 nm. XRD investigated the structural properties. AFM is used to probe sample surface. A double beam spectrophotometer was utilizied to obtain transmittance of the entended samples. Gas sensitivity was measured by change in resistance.

3 RESULTS AND DISCUSSIONS

Figure 1 represents XRD peaks of all fabricated thin films of Undoped TiO_2, doped with Li content of 0.0, 2.0% and 4.0%. From Figure 1, the peaks of TiO_2 and TiO_2: Li thin film is located at 25.34°, 30.72°, 49.13° and 64.12° that belong to (111), (121), (132) and (203) planes respectively. A high peak at (121) is fit with ICDD card no 29-1360 [14], [15].

Grain size (D) was evaluated via (1) [16]:

$$D = \frac{k\,\lambda}{\beta cos\theta}. \quad (1)$$

Where k = 0.9, λ =1.54 Å, θ is Bragg's angle, and β is FWHM. Table 1 provides the obtained data. D rose from 9.58 nm to 10.17 nm, according to Lithium content, as presented in Table 1.
The δ was established by [17], [18]:

$$\delta = \frac{1}{D^2}. \quad (2)$$

Table 1. displays δ decreased from 10.96 to 96.68 with Lithium content.

Similarly, the lattice strain ε was determined according to [19]:

$$\varepsilon = \frac{\beta cos\theta}{4}. \quad (3)$$

It is seen that ε dropped from 36.17 to 34.08 with Lithium concentration (Table 1). Structural parameters (S_P) are seen in Figure 2.

AFM micrographs are shown in Figure 3. The domain showed tightly packed columnar crystals with sharp peaks. As TiO_2:4% Li increases, surface roughness (R_a) declined (8.08 - 3.67) nm. From Figure 3, the average Particle size (P_{Av}) and rms values were (88.78, 85.62 and 60.89) nm and 9.23, 8.12 and 3.89) nm for Undoped TiO_2, TiO_2: 2% Li and TiO_2: 4% Li respectively [20]-[23]. Table 2 displays AFM parameters P_{AFM}

Table 1: Structural parameters of D, Eg and SP of deposit films.

Specimen	2 θ (°)	(hkl) Plane	FWHM (°)	E_g eV	D nm	δ(× 10^{14}) lines/m^2	ε (× 10^{-4})
TiO_2 Undoped	30.72	121	0.85	3.28	9.58	108.96	36.17
TiO_2: 2% Li	30.70	121	0.84	3.22	9.81	103.91	35.34
TiO_2: 4% LI	30.65	121	0.81	3.17	10.17	96.68	34.08

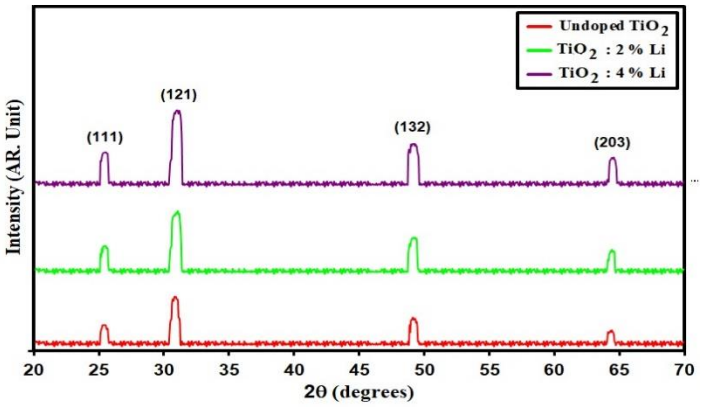


Figure 1: XRD styles of entended films.

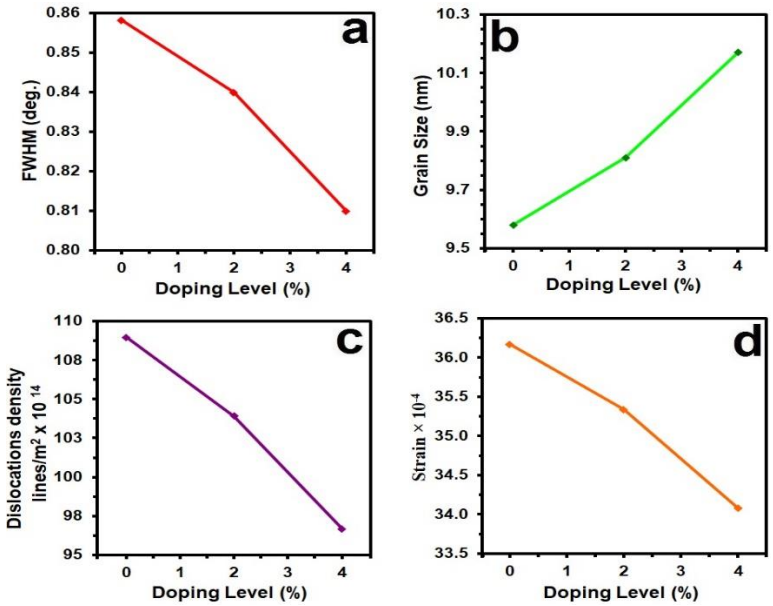


Figure 2: SP of the deposit films.

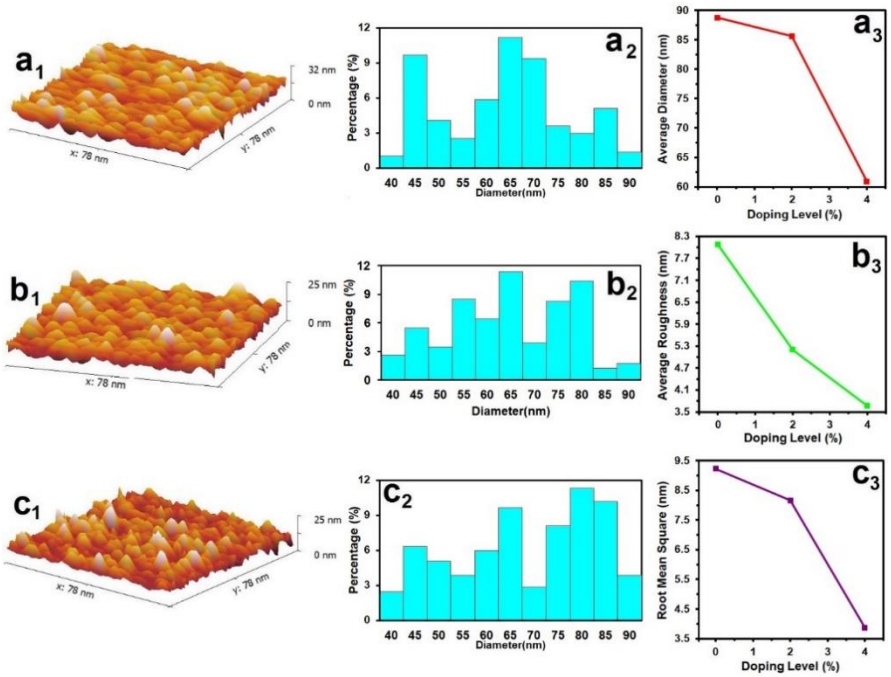


Figure 3: AFM images, granularly distributed and diversity of PAFM.

Table 2: PAFM of the deposit films.

Samples	P_{AV} nm	R_a nm	rms nm
Undoped TiO_2	88.78	8.08	9.23
TiO_2: 1% Sn	85.62	5.20	8.12
TiO_2: 3% Sn	60.89	3.67	3.89

The spectral distribution of transmittance (T) is offered in Figure 4. From Figure 4, it is possible to see declines in T as Lithium content increases [24]-[26].

The optical absorption coefficient (α) was evaluated via the following (4) [27]:

$$\alpha = \frac{2.303A}{t} . \quad (4)$$

The α was presented in Figure 5, and α values were rise by increasing Li content [28], [29].

Using Tauc's relation, bandgap E_g was found from (5) [30]:

$$(\alpha h\nu) = A\left(h\nu - E_g\right)^{\frac{1}{2}} . \quad (5)$$

From Figure 6 the bandgap is calculated from $(\alpha h\upsilon)^2$ versus hυ representing E_g declined from 3.28 eV to 3.17 eV with irising lithium content [31]-[33].

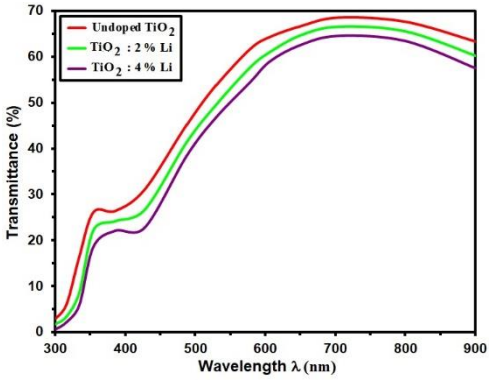


Figure 4: Transmittance (T) of the deposit films.

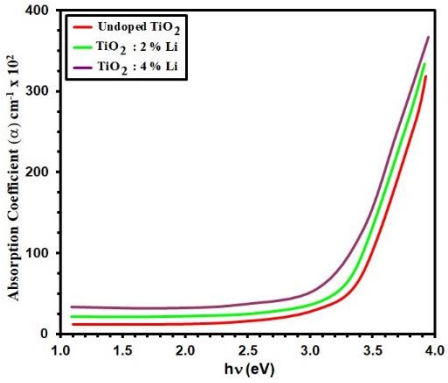


Figure 5: Absorption coefficient (α) of grown films.

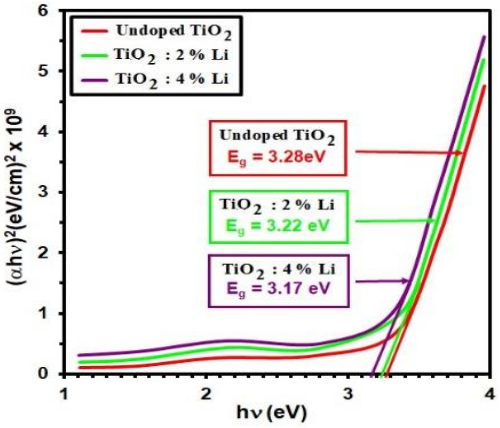


Figure 6: Plot of $(\alpha h\nu)^2$ versus hν for the TiO2 with different Li doping.

Extinction coefficient (k) is evaluated via (6) [34], [35]:

$$k = \frac{\alpha\lambda}{4\pi} . \quad (6)$$

The λ is the wavelength, Figure 7 offers k via λ , showing that k decreases with increasing Lithium concentration.

Refractive index (n) was evaluated as [36], [37]:

$$n = \left(\frac{1+R}{1-R}\right) + \sqrt{\frac{4R}{(1-R)^2} - k^2} . \quad (7)$$

R is reflectance Figure 8 offers the relationship between n and λ. From this Figure 8, it's clear that n decreases with the rise of Lithium content [38]-[40].

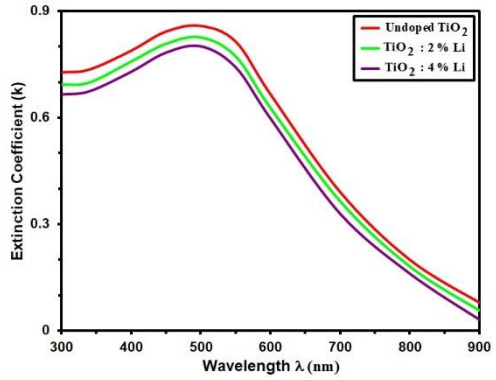


Figure 7: Extinction coefficient (k) of the deposit films.

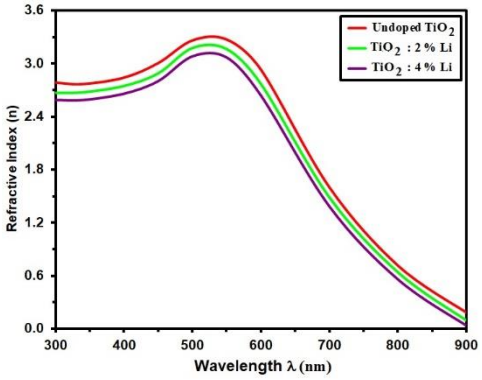


Figure 8: Refractive index (n) of entended films.

The sensitivity (s), is determined using the (8). below [41], [42]:

$$Sensitivity = \frac{\Delta R}{R_g} = \left|\frac{R_g - R_a}{R_g}\right| \times 100\ \% \qquad (8)$$

The gas sensor, was tested with 200 ppm NH_3. Figure 9 shows resistance-time data for TiO_2 and Li-doped TiO_2 at 125°C. NH_3 exposure causes oxidation, releasing electrons from O_2^+ ions to CB rising resistance and potential barrier [49]-[51]. The TiO_2 film with 4% Li showed the highest resistance, enhancing the material's sensing response [52]-[54].

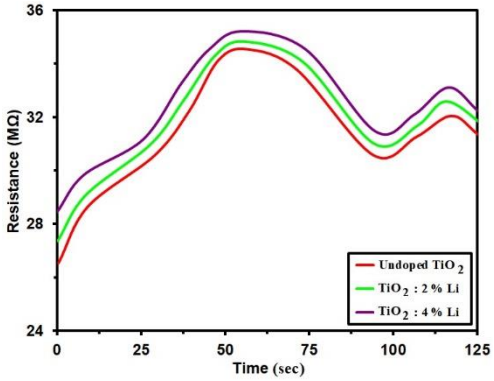


Figure 9: Resistance as a function of operating time for Undoped and TiO2: Li films.

Figure 10 illustrates that sensitivity to NH_3 decreases with increasing Li doping in TiO_2 films due to charge carrier recombination, with TiO_2: 4% Li showing the lowest sensitivity [55-58]. For different doping levelsundoped TiO_2, TiO_2: 2% Li, and TiO_2: 4% Li sensitivity decreased from 21,7 % to 4.1 % at 100 ppm, from 24.4 % to 7.9 % at 150 ppm, and from 28.7 % to 9.3 % at 200 ppm [59-60]. The reduction in sensitivity for undoped TiO_2, TiO_2: 2% Li, and TiO_2: 4% Li indicates that higher Li doping levels result in decreased sensor responsiveness to NH_3 gas [61], [62].

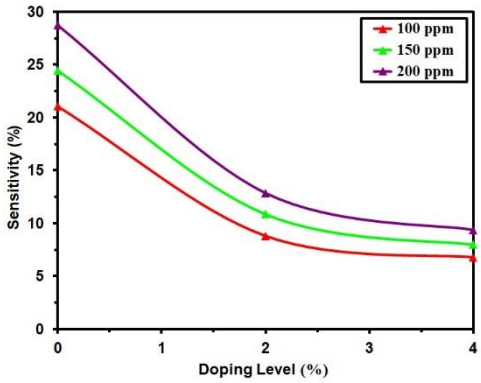


Figure 10: Sensitivity of Undoped and TiO2: Li films with different dopant.

4 CONCLUSIONS

The influence of two Lithium contents (2% and 4%) on Undoped Titanium Oxide films were studied. Lithium doped Titanium Oxide films were The entended films are grown by CSP. X-ray diffraction results confirmed the polycrystalline nature of the films, with the dominant peak observed at the (121) plane. As the Li content increased, the grain size increased slightly while both dislocation density and lattice strain decreased. AFM analysis revealed that surface roughness and average particle size decreased with higher Li doping, suggesting a smoother, more compact surface. Optically, increased Li content led to a reduction in transmittance, extinction coefficient, refractive index, and bandgap energy (from 3.28 eV to 3.17 eV), while the absorption coefficient increased. In terms of gas sensing behavior, all samples demonstrated a p-type response to NH_3 gas at 125°C, with the undoped TiO_2 showing the highest sensitivity. However, as the Li concentration increased, the sensitivity to NH_3 consistently decreased. These results suggest that while Li doping enhances structural and optical properties, it adversely affects gas sensing performance, indicating a trade-off between optical and sensing functionalities in doped TiO_2 films.

ACKNOWLEDGEMENTS

The authors appreciate the support of Mustansiriyah University.

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The Role of Sodium Halide in Improving the Plasma Properties of Mercury-Sodium Mixture under Atmospheric Pressure

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Keywords: Arc Discharge, Current Density, Cathode Temperature, Electron Temperature, Energy Flux Density.

Abstract: The model used in this study includes theoretical calculations and numerical simulation data to determine the impact of cathode surface temperature (Tw) on the current densities in plasma (electrons, ions, and emitted electrons) in arc discharge plasma. The goal of the study is to improve lamp lighting, increase the brightness ratio, and enhance overall performance. The model employed in this study is computational and numerical, utilizing simulations to predict the effects of cathode surface temperature on various plasma parameters. The study assumes thermal equilibrium in the plasma. The temperature change of the electrons was studied according to the change in temperature of the cathode surface inside the plasma. A mixture of mercury and sodium (0.1, 0.01 and 0.001 mol.) was used in varying proportions, and the effect of changing the concentration and choosing the optimal temperature to increase lighting efficiency and brightness was examined. Plasma physics, especially arc discharge, forms a strong foundation for lighting technology in gas discharge lamps. The effect of ionization and excitation processes near the cathode surface was studied, and it was observed that as the temperature increases As a consequence, the current densities (j_i, j_e, and j_{em}) also increase, leading to a rise in the plasma current density (j). By using varying concentrations of sodium (Na) with mercury gas (Hg), a noticeable increase in ionization processes and electron emission is observed as the halide concentration in the mixture increases. Choosing 10 V and 40 V as a method to study the effects of both low and high voltages on plasma properties. When applying different voltages, 10 volts and 40 volts, it is noted that reducing the applied voltage to 10 volts reduces the electric field and the energy supplied to the electron to release it, which affects the rates of ionization, collision, and thermal emission in the plasma.

1 INTRODUCTION

In physics, plasma is a state of matter that contains an equal number of positively and negatively charged particles. Often referred to as the fourth state of matter, plasma consists of charged particles, typically produced by heating a gas to the point where electrons are separated from the atoms or molecules that make it up. Plasma is an ionized gas containing both electrons and ions. It is formed as a result of heating the gas[1].The phenomenon is called an electric arc, which Leads to a reduction in the breakdown voltage. During discharge, the arc is formed, and the electrons emitted from the cathode generate a thermal field [2]. Electric arcs typically require a high current, greater than that of glow discharge. The voltage in short arcs usually exceeds 20-30 V, although in some cases, it can drop to just a few volts [3]. Arc discharge technology has diverse applications across various fields, including optical fiber technology, welding, and material processing [4]. The electric arc discharge is capable of generating active particles and high temperatures, making it useful in a wide range of industrial processes [5] . Peter et al. studied the scattering of light to measure the speed and size of particles in order to understand the behavior of arc discharge in material processing [6].

M. Auger et al. developed advanced experimental setups using arc discharges. similar to those used in neutrino physics, as an alternative to photon detection systems in large particle detectors [7] . R. Abbas Ali et al. conducted a comprehensive theoretical investigation of He-Ne gas mixture. And its effect on electronic transactions in plasma. In low varying electric field and thermodynamic equilibrium. The diffusion coefficients and total collision frequency were studied by solving the Boltzmann equation using the binomial approximation [8] .The aim of research improving the lighting of the lamp by adding sodium halide, which improves the lighting and

extends the life of the lamp. In this work, the cathode radius (0.001m) and the distance between the electrodes (0.01m) were determined at room temperature, and the work was conducted using (NCPL and Thermo cad) programs. It explores the theoretical aspects of the work, with the use of the programs NCPL and THERMOCAT. These modeling programs include tools for calculating various plasma parameters based on the temperature of the cathode and the plasma layers adjacent to the cathode. [9] [10]. The code calculates all of the following parameters Flux density of plasma at the cathode contact, $[q(T_w, U)]$ Electric current density as it passes through the plasma and reaches the cathode contact. $[j(T_w, U)]$. Temperature of the electron layer approaching the cathode, $[T_e(T_w, U)]$. Plasma pressure exerted on the cathode interface. $[P_{pl}(T_w, U)]$ .The database includes gases that produce plasma, such as mercury (Hg), cesium (Cs), xenon (Xe), copper (Cu), argon (Ar), sodium (Na), helium (He), air, and mixtures of cesium-mercury (Cs-Hg) and sodium-mercury (Na-Hg), as well as metal halides [11]. At heated cathodes in a high-pressure arc discharge, the imbalanced layer coefficients are evaluated as functions of the interface temperature T_w and the low voltage applied to the cathode surface. U is essential for accurate modeling of the high-pressure arc's behavior. The NCPL symbol is used for this evaluation [12]. When working with a pure monatomic gas producing plasma, we simply enter the chemical symbol for the gas in the "produced gas" field (e.g., He). It is important to pay attention to the case of the letters (uppercase and lowercase). The code calculates the plasma layer near the cathode using atomic parameters and other fixed data. Ionization of atoms occurs through electron collisions, and the momentum transfer cross-section is determined during elastic collisions (ion-atom interactions). The gas data is retrieved from the internal database, which includes elements such as Cu, He, Ar, Ne, Na, Hg, Xe, Cs, and Kr. Provided. The formatter will need to create these components, incorporating the applicable criteria that follow.

2 THEORETICAL PART

There are various forms of electric arcs, each with distinct characteristics arising from direct external connections involving alternating current and electric fields [12] . An arc discharge occurs in a gas-saturated medium between two conducting terminals the cathode and the anode at extremely high temperatures, which can vaporize or destroy most materials. An electric arc is characterized by an instantaneous discharge of electricity [13].

An electric arc is a continuous discharge, whereas an electric spark is an instantaneous discharge. Electrical discharge can occur with either direct or alternating current. An electric arc differs from a glow discharge in that it has a relatively high current density and a small voltage drop during arcing. At the cathode, the current density can reach up to one million amperes per cubic centimeter [14].

The two key equations for plasma and gas behavior are as Maxwell Distribution (Velocity Distribution) this describes the velocity distribution of particles in gases or plasmas, which is governed by the temperature and mass of the particles. The Maxwell distribution function is given by:

$$f(v_x) = N\left(\frac{T}{2\pi m}\right)^{1/2} \exp\left(-\frac{mv_x^2}{2T}\right). \quad (1)$$

Where *v* is the particle velocity, mmm is the particle mass, Tis the temperature, and n is the number density of particles. Saha Equation (Ionization Equilibrium) This equation relates the number densities of electrons Ne, ions Ni and atoms Na in a plasma under ionization equilibrium. The (2) is:

$$\frac{N_e N_i}{N_a} = \frac{g_e g_i}{g_a} = \left(\frac{m_e T_e}{2\pi\hbar^2}\right)^{3/2} exp\left(-\frac{J}{T_e}\right). \quad (2)$$

Where g_e - the electron statistical weights. g_i: Is the ion statistical weights. g_a: Is the atom statistical weights. m_e is the mass of electron, T_e: the temperature of the electron, J Is the ionization potential of an atom.

The Boltzmann distribution describes the spatial distribution function of atomic particles when equilibrium is established under the influence of a weak external environment. It is expressed as:

$$N(r) = N_0 \exp\left[-\frac{U(r)}{T}\right]. \quad (3)$$

Where N_0 - the particle number density at the position, $U = 0$, $U(r)$ - the particle interaction potential with an external field, *T* is the temperature.

In a high-pressure arc discharge, both the voltage drop (U) and the cathode surface temperature (T_x) influence the alternating current. This study focuses on examining the relationship between the cathode surface temperature (T_x) and voltage (U) with respect to the plasma layer near the cathode, specifically analyzing the current flow rate and current density (j) [15] .

$j = j(T_w, U)$, specifying for all ($T_w \geq$ Tc). (4)

Where T_W - the cathode surface temperature, changes with changes in current density and applied voltage

Current flux density is described by the formula $q = q(T_w, u)$ all define is

$$(T_w \geq Tc). \quad (5)$$

Where q - Energy Flux Density, distribution of temperatures cathode structure determined by figuring out the heat conduction formula [16] .

$$\nabla(K\nabla T) = 0\,. \quad (6)$$

Where ∇T - the cathode material's thermal conductivity K - a shift in temperature.

There is a boundary situation .The cathode body's surface temperature distribution can be determined by using the cathode body's thermal conductivity and it is represented by the equation in $q(T_w, u)$ function

$$k\frac{\partial T}{\partial n} = q\,(T_w, U\,). \quad (7)$$

Where U - the low combined voltage. , T_W - cathode surface temperature, q: Energy flux density.

The density of the plasma's energy flux can be expressed by the following[17] .

$$q_p = q_i + q_e - q_{em}. \quad (8)$$

Where, q_p - plasma's energy flux density. q_e - plasma electrons' energy flux density q_{em} - plasma energy flux density of electrons emitted from the cathode surface. The following form can be used to express the energy flux of ions, elections and energy flux elections Emitted

$$q_i = \mathrm{j_i}\,[\,\mathrm{Z_eU_D} + \mathrm{E} - \mathrm{ZA_{eff}} + \mathrm{k(2T_h} + -2\,T_W), \quad (9)$$

$$q_e = J_e\big(2\mathrm{K}T_e + A_{eff}\big), \quad (10)$$

$$q_{em} = J_{em}\big(2\mathrm{k}T_W + A_{eff}\big)\,. \quad (11)$$

Where, $j_i -$ ions current density, T_h - heavy particle temperature (ions, neutral particles), T_e- temperature electrons. Z - rate of ion charge accumulation, E - average ionization energy of the layer near the cathode , U_D - low voltage within space charge envelop, A_{eff} - effective work function (Schottky correction), $\mathrm{J_e} = \mathrm{e\,J_e}$ - is the quantity of ions that provide a voltage to the cathode's electrical surface, J_{em} - the current density of electrons emitted from the cathode; $J_{em} = e\,J_{em}$, $J_i = Z_e\,j_i$ quantity ions that provide an electrical flow across the cathode's surface.

3 RESULTS AND DISCUSSION

3.1 Effect of Hg-Na Concentration on Plasma Properties

Different concentrations (0.001, 0.01, and 0.1, mol.) of Hg-Na, were chosen to determine the effect of changing the ratios in the mixture on the plasma properties. As the concentrations (0.001, 0.01, 0.1, mol.) increase, a proportional increase in emitted electrons is observed, indicating the impact of higher temperatures on the density of liberated electrons due to collisions with molecules, which in turn reduces the voltage barrier. In the case of applying higher voltage (U=40V), stronger electricity is generated with a shorter voltage barrier and increased electron emission due to the higher electric field. Additionally, increasing the concentrations (0.1 and 0.5 mol.) leads to a decrease in the voltage barrier due to increased ionization processes, which enhances collisions and increases electron emission .Hg pure has a lower ion concentration, and its plasma exhibits a less pronounced response to the increase in voltage, resulting in a smaller effect on ion energy flux density compared to (Hg-Na) max. With higher concentrations. The results indicate that pure mercury exhibits superior electrical and thermal properties compared to the mercury-sodium mixture, as it can withstand higher temperatures due to its lower melting point. Sodium, being an alkaline element, impacts plasma efficiency at elevated temperatures by influencing ionization processes and forming compounds that reduce performance. In contrast, pure mercury remains more stable, improving plasma density and efficiency at high temperatures. The results indicate that pure mercury exhibits superior electrical and thermal properties compared to the mercury-sodium mixture, as it can withstand higher temperatures due to its lower melting point. Sodium, being an alkaline element, impacts plasma efficiency at elevated temperatures by influencing ionization processes and forming compounds that reduce performance. In contrast, pure mercury remains more stable, improving plasma density and efficiency at high temperatures.

3.2 Electron Temperature

Figure 1 illustrates the relationship between electron temperature and cathode temperature. A balance and relative stability exist between the two. The emission of electrons increases with the surface temperature of the cathode due to the thermal effect. However, the temperature of the electrons rises more significantly

than that of the cathode, often reaching thousands of degrees Celsius, while the cathode surface temperature remains much lower. This occurs because the energy gained by the electrons in the gas discharge increases their Velocity, which in turn raises their temperature.

Figure 1a and 1b which using a voltage barrier of 10 and 40 volts. In the case of pure mercury and for the mixture Hg-Na with different concentrations of Na (0.001, 0.005, 0.01, 0.05, 0.1and 0.5 mol.). We observe that the temperature effect becomes more pronounced at higher sodium concentrations. This is because sodium, being an alkali metal, increases the number of atoms interacting with mercury, which leads to a higher number of electrons being released into the plasma. These electrons absorb more energy from the cathode surface, causing the plasma temperature to rise [1].

The voltage barrier also plays a role in this effect. Increasing the voltage from 10 V to 40 V strengthens the relationship between cathode temperature and electron temperature. As the applied electrical voltage increases, the electrons gain more energy upon acceleration, raising their temperature. This, in turn, enhances the efficiency of the arc discharge [18].

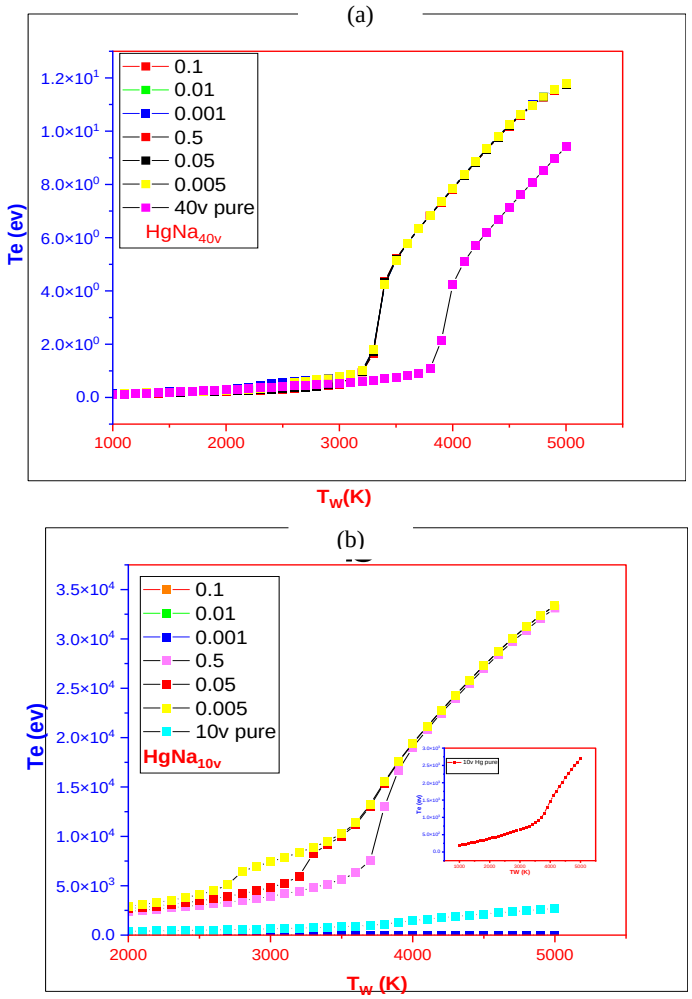


Figure 1: Electron temperature Te from the vs. cathode surface temperature TW for all concentrations a) U =40 V, b) U =10 V.

4 CURRENT DENSITY OF CONCENTRATION

Figure 2a and 2b, Figure 3a and 3b and Figure 4a and 4b shows the relationship between the current density (j, ji , je and jem) and the cathode surface temperature(TW) when using different voltages of 10 and 40 volts for pure mercury and for different concentrations of the mercury and sodium mixture (0.001,0.01 and 0.1mol) as the voltage barrier increases, the effect of TW on (*j*, *ji* , *je* and *jem*) increases. Increasing the current density of the electrons increases the degree of ionization of the gas .Increasing ji leads to plasma stability and reduced oscillations the density of the plasma affects the distribution of heat within the system The density of the electrons emitted from the cathode increases because the acceleration of the electrons in the cathode leads to secondary emissions, so the density of electrons in the plasma increases [19].

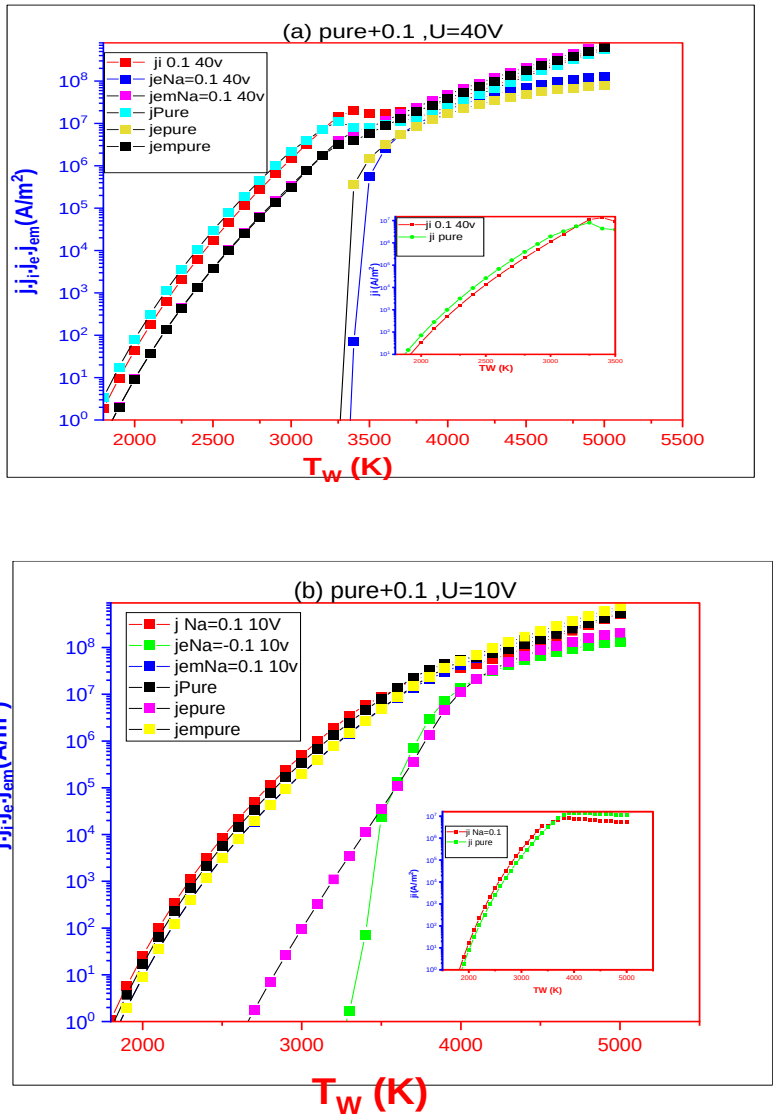


Figure 2: The current density (j, ji , je and jem) from the vs. cathode surface temperature (TW) at the concentration (0.1mol.): a) U=40V, b) U=10V.

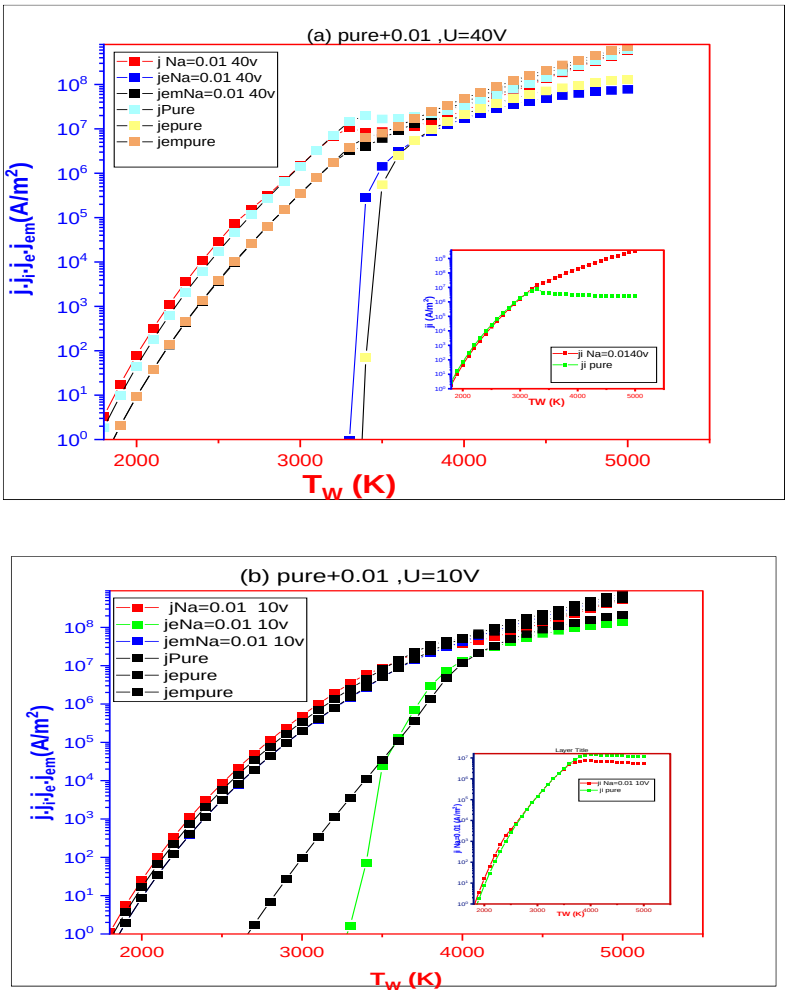


Figure 3: The current density (j, ji, je and jem) from the vs. cathode surface temperature TW at the concentration (0.01mol.): a) U=40V, b) U=10V.

When comparing the results obtained with a previous study (Tapark Abdlraheem Saber et al.) [20], which used a mixture of xenon and sodium, it is observed that both studies highlight the importance of cathode temperature and voltage in influencing plasma behavior and efficiency. However, our study places a greater emphasis on how varying sodium concentrations and cathode temperature directly affect plasma density, while the other study focuses on the role of the electric field in lowering the voltage barrier and enhancing ionization. The numerical simulations this Study are beneficial for optimizing arc discharge to improve lighting efficiency, while the thermodynamic insights from second Study are essential for understanding how electric fields and temperature variations interact to influence electron emission and plasma stability. As the cathode surface temperature (Tw) increases, the emitted electron current increases sharply, while the ion current decreases, especially at high voltages. Furthermore, high concentrations and voltages enhance current and power densities due to increased ion heating and higher electron temperatures this is consistent with the results obtained by H F Jassam [12].

The results indicate that pure mercury exhibits superior electrical and thermal properties compared to the mercury-sodium mixture, as it can withstand higher temperatures due to its lower melting point. Sodium, being an alkaline element, impacts plasma efficiency at elevated temperatures by influencing ionization processes and forming compounds that reduce performance. In contrast, pure mercury remains more stable, improving plasma density and efficiency at high temperatures.

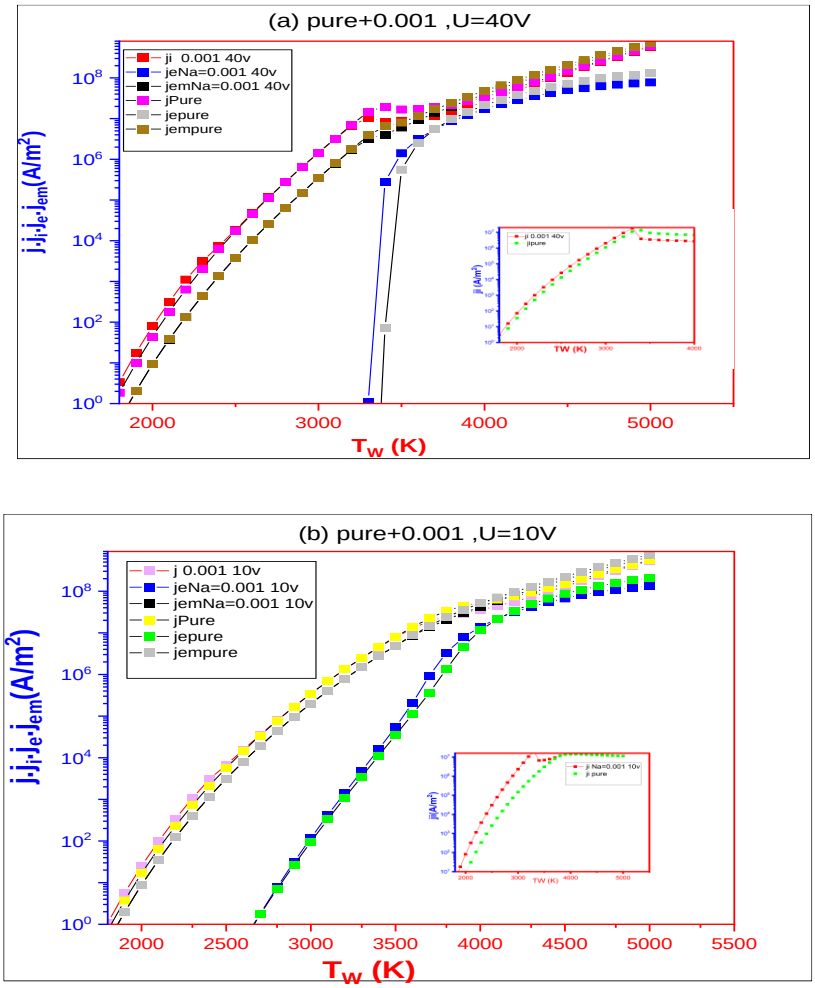


Figure 4: The current density (j, ji, je and jem) from the vs. cathode surface temperature TW at the concentration (0.001mol): a) U=40V, b) U=10V.

5 CONCLUSIONS

This paper provides computational analysis of the effect of sodium halide concentrations on the plasma characteristics of mercury-based arc discharges under atmospheric pressure. Simulations present that increasing the cathode surface temperature leads to a important improvement in current density and energy flux across all sodium concentrations and for pure mercury, highlighting the strong thermal coupling between cathode heating and plasma behavior. From the results, it is evident that as the surface temperature of the cathode increases, the density of the energy flux electrons and ions increases for all concentrations of sodium (Na), as well as for pure mercury. The plasma density also increases in general. Different concentrations of sodium affect the thermal behavior of the arc discharge system, which contributes to improving the lighting and efficiency of the generated plasma. Additionally, the effect of varying the voltage barrier from 10 V to 40 V enhances the influence of the cathode surface temperature on elastic and inelastic collisions, ionization processes, and the release of high-energy electrons. This leads to a significant increase in electron temperature compared to lower voltage conditions.

ACKNOWLEDGMENTS

Authors would like to thank Mustansiriyah University Baghdad-Iraq for its support in the present work.

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Thermoplasmonic Generation of Gold Nanorods Based on Size and Structure

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Keywords: Gold Nanorods, Thermoplasmonic.

Abstract: Thermoplasmonic phenomena have emerged as a key area in plasmonic research because of their ability to utilise metallic nanostructures for generating nanoscale heat. Gold nanorods (GNRs) are particularly promising in this field due to their unique geometry, which enables dual plasmonic resonance modes – longitudinal and transverse. These modes allow efficient absorption of light at different wavelengths, which enhances their thermoplasmonic capabilities. GNRs serve as efficient nanoscale heat sources by converting absorbed light into heat through electron–phonon and phonon–phonon interactions. The versatility of plasmonic GNRs has led to widespread applications in biomedical fields, such as photothermal cancer therapy, drug and gene delivery and photoacoustic imaging. However, challenges persists in optimising the control of heat distribution and minimising dependence on costly photothermal agents and devices. Recent developments in photosensitising materials and laser technologies, including diode lasers, have considerably expanded the potential of thermoplasmonic applications in medicine. This study focuses on the impact of size, structure and the surrounding medium on the plasmonic and thermoplasmonic performance of GNRs. The aim is to enhance the efficiency of GNRs in therapeutic and diagnostic applications.

1 INTRODUCTION

Thermoplasmonics has attracted wide interest within the nano-photonics and plasmonics research community due to its immense technological potential. This field utilises metallic nanostructures to generate heat at the nanoscale [1]. Thermoplasmonics combines two fields: nano-thermodynamics and nano-optics. Thermoplasmonics also focuses on controlling heat generation in nanoparticles through light absorption. This absorption is improved and confined close to the nanoparticle surface, which then heats up and acts as a nanoscale heat source [2]-[4]. In contrast to bulk metallic materials, noble metal nanoparticles can control and confine light within nanoscale volumes due to surface plasmin resonance (SPR).

When plasmatic nanoparticles (Au) are exposed to laser light, they oscillate with their resonant surface plasmon oscillation; this oscillation enables them to strongly absorb the light and rapidly convert it into heat through multiple photophysical processes [5]. We have chosen to work with nanorods due to their double absorption bands, which are exhibited by surface plasmons associated with oscillations along the two main axes of nanomaterials [6]. The two characteristic lengths of the nanorod's geometry are intrinsically linked to the excitation of surface plasmons at two different wavelengths. The presence of the two modes provides an advantage over other nanoparticle shapes: one is the longitudinal SPR, and the other is the transverse SPR. Throughout this process, oscillating electrons convey their kinetic energy to the particle lattice via electron-phonon interactions, subsequently followed by phonon-phonon interactions with the surrounding medium [7], [8].

Gaining a predictive understanding of how nanoparticles distribute incident optical energy has been a longstanding challenge, which is difficult to address experimentally with a high degree of certainty [9]. The use of metallic nanoparticles as essential building blocks for sophisticated electronic systems and gadgets is growing

Gold nanorods (GNRs) have extensive biomedical applications due to their unique surface chemistry and electronic and optical properties; these properties depend on the size and shape of the object and the type of surrounding medium [10].

An important step forward is the implementation of selective photothermal analysis in medicine [11]. Over the past decade, efforts have been made to develop novel approaches for cancer treatment. Hyperthermia provides adequate external energy to generate heat in target tissues. Nowadays, hyperthermia is used to destroy tumours [12]. Thermoplasmonics has resulted in significant applications, particularly in biology and medicine, including photothermal cancer therapy, photothermal imaging, drug and gene delivery, and photoacoustic imaging [13].

In recent years, numerous studies have focused on using metallic nanostructures to delivery heat in photothermal applications. Tissue temperature control has been widely used for therapeutic applications in various medical fields, such as oncology, physiotherapy, urology, cardiology, ophthalmology and photothermal therapy [14].

The photothermal effect is attracting great interest due to developments in new photosensitising materials and improved light sources. However, studies are often limited by the need to utilise exogenous photothermal agents and costly irradiation devices [15]. Therefore, a nanoscale heat source that works under laser illumination by generating heat is needed in medical treatments. For example, a diode laser is a monolithic semiconductor device that directly converts electrical energy into laser light. It offers unique levels of power and wavelength scalability, which support a wide range of medical applications. Different semiconductor compositions enable selected wavelengths; for example, GNRs can have output wavelengths in the blue, green, red or near and mid infrared ranges [15]-[18].

Diode lasers for medical applications underscores their exceptional role in revolutionising medical treatments. These lasers offer a compact, efficient and scalable light source suitable for numerous biomedical applications, such as thermoplasmonic therapies. Their ability to deliver precise wavelength outputs tailored for GNRs optimises light absorption, which improves heat generation at the nanoscale. Moreover, the integration of diode lasers with advanced nanoparticle systems such as GNRs opens new possibilities for minimally invasive and highly targeted treatments, particularly in oncology and physiotherapy [19]. Thermoplasmonic applications, specifically those using GNRs, have transformative potential across various fields. By optimising the size, shape and environmental parameters of these nanoparticles, they can enhance the efficiency of targeted hyperthermia, improve drug delivery mechanisms and enable highly precise photoacoustic imaging. Recent research focuses on integrating GNRs with other materials, such as high refractive index coatings (e.g. SiO_2) to further increase their absorption and heat generation efficiency. Developments in laser technology will also play a crucial role in expanding the applications of GNRs in medicine, particularly in oncology and minimally invasive therapies [20].

In this investigation, the finite element approach with COMSOL Multiphysics is used. It is based on the Mie theory and heat transport models. The investigation focuses on the thermoplasmonic generation of GNRs with different lengths in air and water environments. This analysis is conducted under the illumination of an 810 nm laser diode.

2 MANUSCRIPT PREPARATION

The cylindrical symmetry structure represented GNRs of different lengths (D) and a constant radius (d) immersed in air, followed by homogeneous deionised water at room temperature. The GNRs were irradiated using an 810 nm laser.

Optical part: COMSOL Multiphysics 6.1 software is used to design the correct settings for GNRs of different lengths, as shown in Figure 1. The electromagnetic wave frequency domain model is employed to examine the numerical computation of the absorption cross section (ACS) for the GNRs in various mediums, as calculated numerically and displayed
in (1) [21]:

$$\delta_{abs} = k\,\mathrm{Im}(\alpha) - k^4 / 6\pi\,|\alpha|^2. \qquad (1)$$

where k denotes the wave number and α is the polarizability.

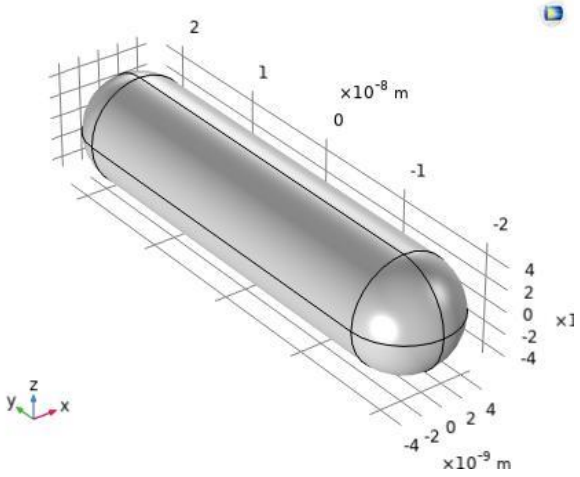


Figure 1: Diagram showing nanorod structure.

Table 1. Physical properties of materials used in this study.

Parameters	Au	Water	Air	SiO_2	Unit
Thermal Conductivity K	317	0.6	0.02	1.38	W/(m.k)
Specific heat Capacity c	128.5	4187	1000	703	J/(Kg.K)
Mass Density ρ	19320	1000	2.9	2203	Kg/m^3

High quality can be achieved by using λ/6 as the mesh in this particular model. All materials' real and imaginary components of their refractive indices of all materials are interpolated.

Thermal analysis: The photothermal response of GNRs, which arise from the absorption of incident light energy, is investigated using (2) [22]:

$$\Delta T = \frac{\delta_{abs} I}{4\pi R_{eq} \beta K_m} . \quad (2)$$

Where I is the light irradiance, E is the electric field amplitude of the incoming light, n is the refractive index, K_m is the thermal conductivity of the surrounding medium and R_{eq} is the equivalent radius:

$$R_{eq} = \left[(3D - d)\ \frac{d^2}{16}\right]^{\frac{1}{3}}. \quad (3)$$

Here, *D* and *d* represent the length and diameter of the gold nanorods (GNRs), respectively, and a corrective factor is applied to account for deviations from idealized geometries or assumptions in the analytical model:

$$\beta \simeq 1 + 0.096587\ ln^2\left(\frac{D}{d}\right). \quad (4)$$

The heat transfer model is used with the same boundary conditions that were utilized to ascertain the optical properties are applied to the heat transfer model. Table 1 lists the characteristics of the materials employed in this investigation.

In (1): Au [23], Water [24], Air [25], SiO_2 [26].

3 RESULTS AND DISCUSSION

To gain deeper insights into the thermoplasmonic behaviour of GNRs, exploring the interplay among their geometric parameters, optical properties and the surrounding medium is necessary. The results demonstrate a clear correlation between the length of GNRs and the red shift in SPR [19].

Figure 2 displays the ACS for several GNRs as a function of wavelength (400–1,400 nm). For different GNR lengths and constant diameters in air and water.

In air, the SPR characteristics show a red shift and an increase in the ACS magnitude as the length of GNRs extends. In water, the SPR characteristics exhibit a larger red shift and a decrease in the ACS magnitude compared with those in air. The shift is due to that higher refractive index of water (1.33) causes more light to bend and scatter, which decreases the light available for absorption by the GNRs [27].

The proposed GNR is considered to lie in a horizontal direction, which makes an angle ($\Theta = 90°$) with respect to the normal incident of the plain background field (and laser illumination) to achieve maximum absorption, as discussed in [28].

The orientation of GNRs is pivotal in optimising their thermal efficiency. As demonstrated, aligning the nanorods at an angle of 90° to the incident light improves the excitation of longitudinal plasmon resonance modes, which results in maximum heat generation. This characteristic is particularly advantageous for targeted therapies. Precise control of heat localisation is crucial to minimising damage to surrounding tissues.

Figure 3 presents the absorption cross section (ACS) as a function of wavelength (500–800 nm) for various orientation angles of the gold nanorods (GNRs), specifically at 0°, 30°, 60°, and 90°. When $\Theta = 0°$, the incident light is aligned with the nanorod axis, effectively exciting longitudinal oscillations of the free electrons along the rod's length. As the orientation angle increases, the alignment between the incident electric field and the nanorod axis diminishes, resulting in a progressive decrease in the ACS. This demonstrates that maximum absorption occurs when the incident field is parallel to the nanorod axis, and it gradually declines as the angle deviates from this alignment [29].

The temperature elevation of GNRs corresponding to laser irradiation is shown in Figure 4. The proposed laser source is a diode laser

with a power of 1 mW/μm² and a wavelength of 810 nm, with normal incidence.

Figure 5 illustrates the temperature elevation of gold nanorods (GNRs) under laser irradiation. The proposed laser source is a diode laser operating at a wavelength of 810 nm, with a power density of 1 mW/μm² and normal incidence. This configuration is designed to effectively excite the longitudinal plasmon resonance of the GNRs, leading to localized photothermal heating.

In an air environment, the maximum opto-heat generation of GNRs occurs at length of 70 nm due to its higher ACS magnitude, followed by 60 and 80 nm, as indicated by (2). In a water environment, the maximum opto-heat generation of GNRs occurs at length of 50 nm due to its higher ACS magnitude, followed by 45 nm, as explained previously. This result is due to the decrease in the collective refractive index.

In certain experimental conditions, the lengths of gold nanorods (GNRs) may be constrained to values shorter than 60 nm. Under such conditions, the absorption cross section (ACS) is significantly reduced, resulting in minimal temperature elevation. To enhance the photothermal efficiency of these short nanorods, one effective strategy is to coat them with a material possessing a high refractive index, such as silicon dioxide (SiO_2). This coating enhances the local electromagnetic field around the nanorods, thereby increasing their light absorption and subsequent heat generation.

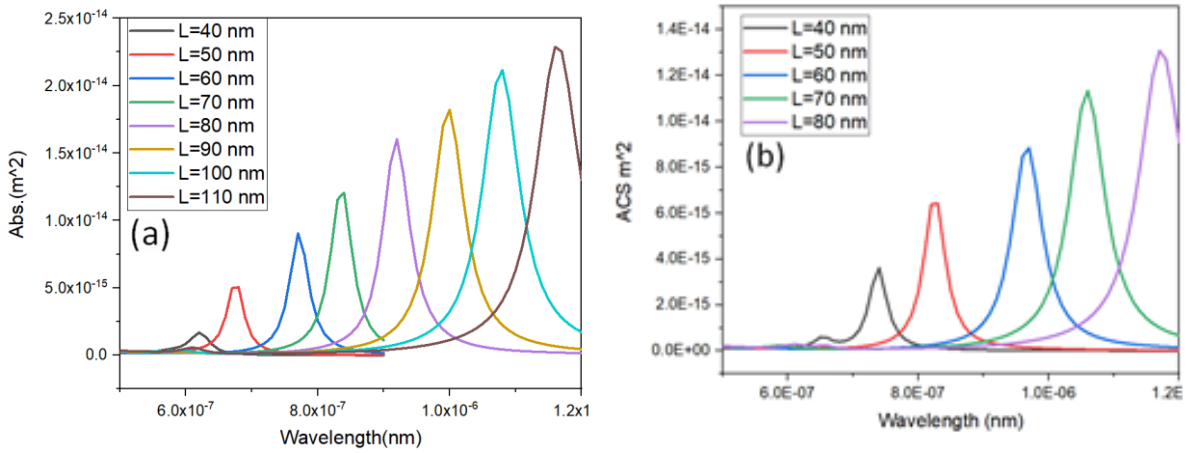


Figure 2: ACS of different nanorod lengths in a) air and b) water.

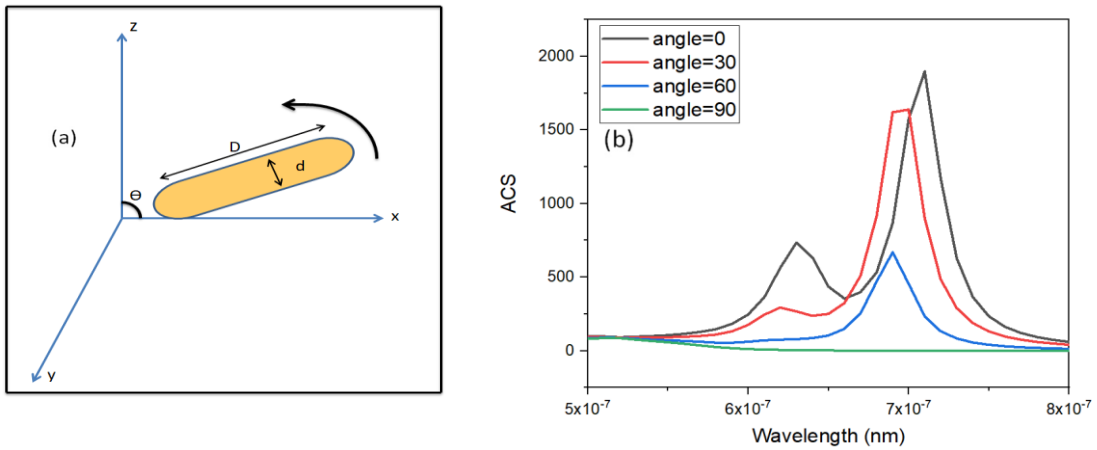


Figure 3: Schematic of GNR orientation a) and b) absorbance of GNRs at different angles

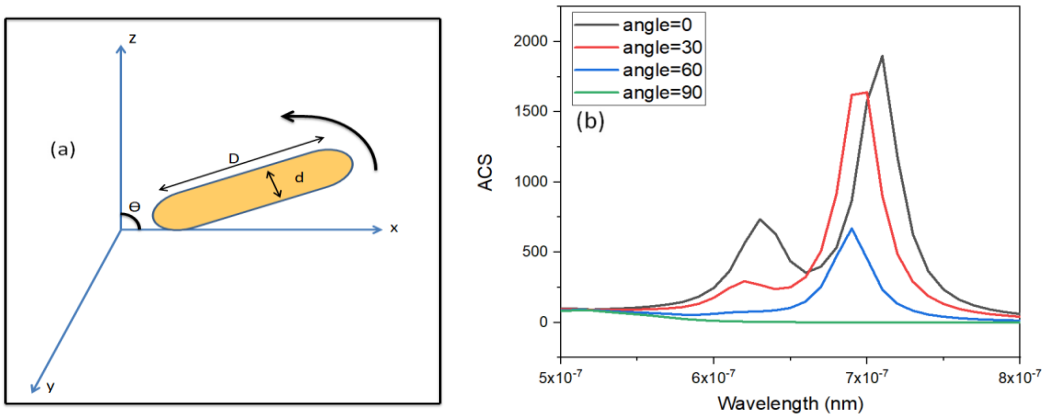


Figure 4: Heat elevation of GNRs in a) air media and b) water media.

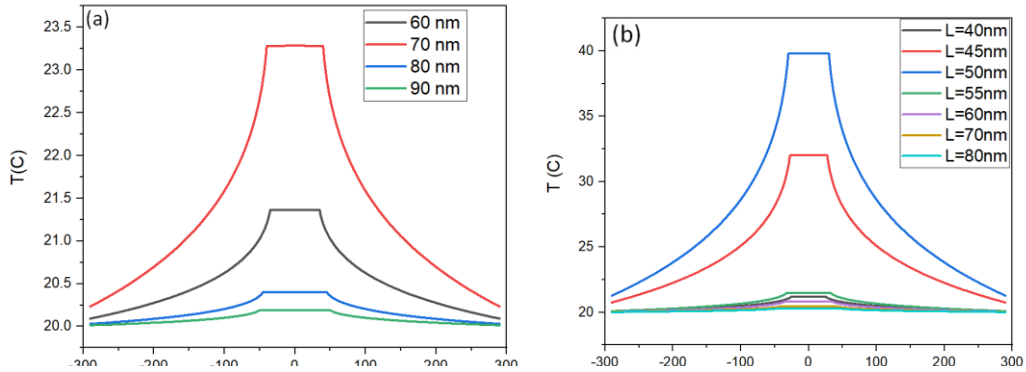


Figure 5: Heat elevation of GNRs in a) air media and b) water media.

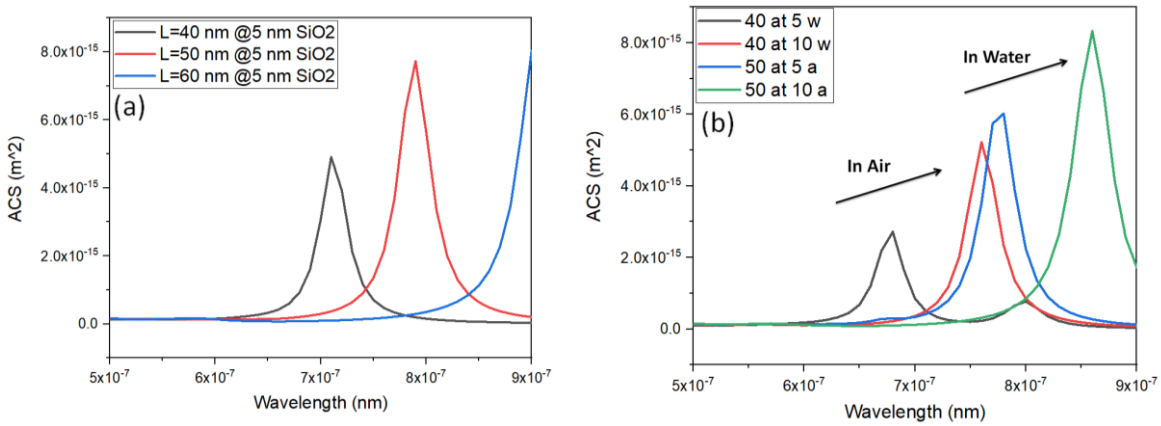


Figure 6: Absorption cross section (ACS) of gold nanorods (GNRs): a) for varying GNR lengths at a fixed SiO_2 shell thickness, and b) for a fixed GNR length with varying SiO_2 shell thicknesses, in both air and water environments.

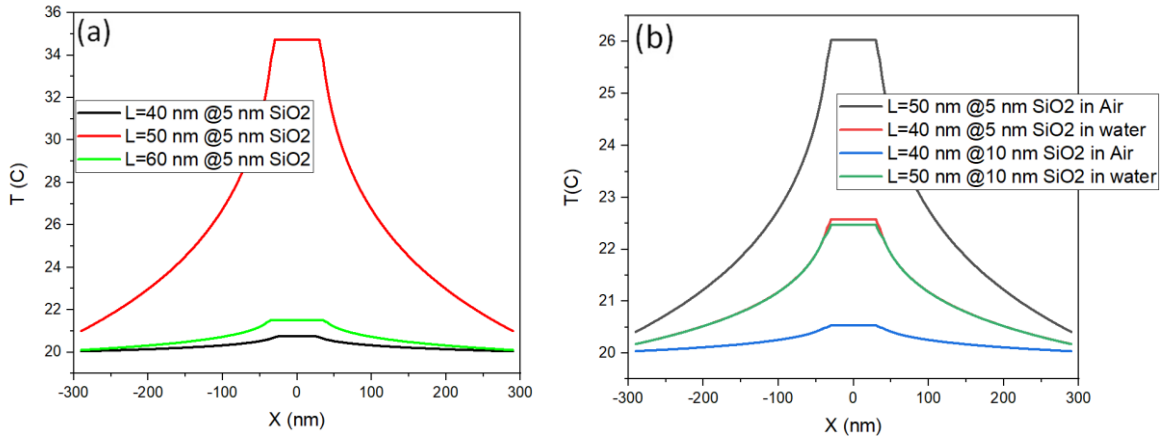


Figure: 7: Heat elevation of GNRs for (a) different GNR lengths @ fixed SiO_2 shell thickness and (b) different GNR lengths @ different SiO_2 shell thicknesses.

Figure 6 show the ACS for various GNR as a function of wavelength (500–900 nm) for two scenarios: varying gold nanorod (GNR) lengths at a fixed SiO_2 shell thickness, and a fixed GNR length with varying SiO_2 shell thicknesses.

Utilizing high-refractive-index materials such as silicon dioxide (SiO_2) as coatings is crucial for enhancing the optical and thermal performance of gold nanorods (GNRs). The application of a SiO_2 shell not only improves the structural stability of the nanorods but also significantly increases their ability to absorb and retain incident light, as evidenced by the elevated absorption cross section (ACS) and enhanced thermal response. These dielectric coatings enable precise tuning of the surface plasmon resonance (SPR) characteristics, thereby optimizing the photothermal conversion efficiency. As illustrated in Figure 6, variations in the SiO_2 shell thickness directly influence heat generation across different wavelengths. This tunability renders SiO_2-coated GNRs highly suitable for advanced applications such as controlled hyperthermia and high-resolution optical imaging [30].

A red shift in the surface plasmon resonance (SPR) wavelength is observed with increasing gold nanorod (GNR) length, primarily due to the enhanced sensitivity of the plasmonic response to the refractive index of the surrounding medium [31].

A red shift is also observed when the gold nanorod (GNR) length is held constant and the thickness of the SiO_2 shell is varied. As the SiO_2 shell thickness increases, the absorption cross section (ACS) correspondingly increases, indicating enhanced light absorption and plasmonic coupling due to the higher effective refractive index surrounding the GNRs [28]. (Fig. 7). Heat elevation profiles of gold nanorods (GNRs) as a function of distance (*x*, in nanometres): (a) for varying GNR lengths at a fixed SiO_2 shell Figure 7a shows that the maximum heat elevation occurs for gold nanorods (GNRs) with a length of 50 nm and a SiO_2 shell thickness of 5 nm, which corresponds to the highest absorption cross section (ACS) observed in Figure 6a. Concurrently, Figure 7b illustrates the influence of increasing shell thickness on opto-thermal heat generation, highlighting the tunability of the ACS through dielectric coating. This observation is consistent with prior studies that demonstrate enhanced photothermal performance resulting from modifications in the surrounding refractive index [32].

4 CONCLUSIONS

This paper proves that the thermoplasmonic performance of gold nanorods (GNRs) can be expressively improved by regulating their length and orientation, and the adjacent medium. Longer GNRs aligned at 90° to the incident light proved enhanced longitudinal surface plasmon resonance and better heat generation. The adjacent medium likewise plays an important role. GNRs in water exhibit a red shift in absorption but reduced heating compared to those in air. The findings demonstrate that engineering GNR properties, including length, orientation, surrounding media and coating thickness, provides exceptional flexibility in customising their thermoplasmonic performance. These results pave the way for integrating GNRs into advanced biomedical devices for cancer therapy, drug delivery and high-resolution imaging. Future research should concentrate on optimising the interaction between

GNRs and their environment to unlock their full potential in real-world applications, such as industrial heat management and minimally invasive medical treatments.

ACKNOWLEDGMENTS

We would like to express our sincere gratitude to the Head of the Department of Physics, Faculty of Science, University of Kufa, and to Assistant Professor Dr. Faeq Al-Temimei for their continuous support and encouragement throughout this research. We are also deeply thankful to Assistant Professor Dr. Ali Kadhim for his invaluable help and unwavering support in completing this study. Additionally, we extend our heartfelt appreciation to our fellow postgraduate colleague, Ahmed Jamil, whose collaboration and assistance have been truly invaluable during this academic journey.

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SECTION 4

INNOVATIONS IN APPLIED CHEMISTRY AND MATERIALS SCIENCE

Proceedings of the 13th International Conference on Applied Innovations in IT (ICAIIT)

JUNE 2025

Determination of Amlodipine in Pharmaceutical Formulation by Charge Transfer Complex Method

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Keywords: Amlodipine, Schiff Base, Charge-Transfer Complex, Spectrophotometry, Pharmaceutical Formulation.

Abstract: Charge transfer complexes were attended by the interaction of Schiff base (amlodipine + Picolin aldehyde) with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to create a Compound that produced the maximum absorption at a wavelength of 436 nm by selecting the optimal conditions for the interaction between the two compounds which were adopted in subsequent experiments. The study covers the development of a straightforward and precise spectrophotometry for the measurement of amlodipine (AML) in pharmaceutical formulations and pure forms. The molar absorption rate was 1.4556×10^4 $l.mol^{-1}.cm^{-1}$, the correlation coefficient was 0.9981, the sandal sensitivity was $0.0281.\mu g.cm^{-2}$, And the Beer's law limit was 5–40 $\mu g.ml^{1}$. When this technique was used to estimate the amount of AML in pharmaceuticals, the composition of the resultant compound revealed that the detection limit (0.2596 $\mu g.ml^{-1}$). The quantitative limit (0.7865$\mu g.ml^{-1}$), and the Rec value (%103.1124-96.8260) were all within the range of 0.2257-0.6258. Additionally, AML measurement approach has proven effectively. Characterized complexes by U.V, FTIR and Spectrum of Mass.

1 INTRODUCTION

Spectrophotometric determination is a cornerstone technique in both quantitative analysis and quality assurance [1,2]. High blood pressure is a chronic illness that also increases the risk of cardiovascular disorders. Common all over the world. It is fairly significant to assess antihypertensive medications for medication safety because hypertension patients frequently need long-term medication to control their problems, such as amlodipine and other blood pressure-lowering medications [3]. Amlodipine (AML) is a dihydropyridine with special properties that set it apart from other medications in this family [4]. It belongs to the class of calcium channel blocking medicines[5,6]. It decreases the amount of calcium that enters cells by binding exclusively to voltage-gated L-type calcium. The coronary and peripheral blood arteries enlarge as a result of the relaxation of their smooth muscles. Its primary application is in the management of hypertension [7]. And angina [8]. Edema, palpitations, and redness are among the adverse effects of AML that are most frequently experienced at dosages more than 10 mg [4]. The chemical structure of AML is depicted in Figure 1 [9]. AML pharmacological characteristics are displayed in Table 1 [10,11,12].

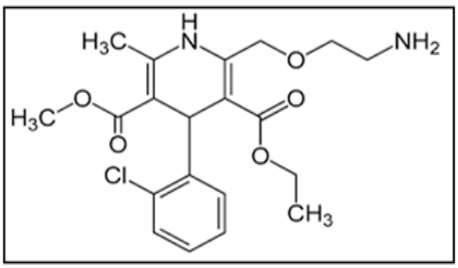


Figure 1: The chemical structure of AML.

Table 1: Some properties of AML.

Subject	Properties
Chemical Name (IUPAC)	3 - Ethyl - 5- methyl (±) - 2 - [(2-aminoethoxy) methyl]-4- (o-chlorophenyl) 1,4-dihydro-6 - methyl-3,5-pyridinedicarboxylate
Molecular Formula	$C_{20}H_{25}ClN_2O_5$
Molar mass	408.88 g / mol
Color	White

2 EXPERIMENTAL

Weights were determined using the four-ranked electrical scale, since the model is 220/C/1. Ultraviolet - visible spectroscopy technology was used in the Department of Chemistry, College of Education for Girls, Anbar University, Iraq model (Jasco V-650 double beam spectrophotometer) Japanese made with 1 cm plastic cells to measure the spectrum and absorbance.

The prepared samples were diagnosed at Samarra University, using an infrared technology model (Shimadzu FTIR-8400 S) Japanese-made, and mass spectrometry technology was also used model (Shimadzu - GCMS - QP 2010 Plus) Japanese-made.

2.1 Materials and Chemical Reagents Used

All of the synthetic materials utilized are of the best grade available.

2.2 Preparation of Schiff Base

Aldehyde condensation was used to create Schiff base (Picolin aldehyde). Reflux was used to dilute (0.2g, 0.002mol) of aldehyde with pure primary amine (AML) in ten milliliters of pure ethanol. After that, the resultant solution was put in a circular flask, and (0.8g, 0.002mol) AML, the main amine, was dissolved in 10 milliliters of 100% ethanol before being added.

Two to three Glacial acetic acid droplets were added as a stimulant to speed up the reaction. For three hours, the mixture was kept at 70^0 C for the reaction escalation procedure.

After cooling the reaction mixture to room temperature, yellow crystals formed. For a full day, the stuff was kept in a baker's. Wash with cold distilled water to purify the precipitate and filter after the solvent has evaporated as much as possible. After that, the sediment was removed, dried, and stored [13].

2.3 Preparation of Solutions

2.3.1 Solution of Schiff Base (100 µg.ml-1)

A specific amount of Schiff Base (0.01 g) was dissolved in a predetermined volume of ethanol, and a volumetric flask containing ethanol was then filled with the solution until it reached 100 ml.

2.3.2 Solution of 8-Hydroxyquinoline (100 µg.ml-1)

After a specific amount of 8-Hydroxyquinoline (0.01 g) was dissolved in a predetermined volume of ethanol, the solution was made up to 100 ml in a volumetric flask.

2.3.3 Solution of 2,3-Dichloro-5,6-Dicyano-1,4-Benzoquinone (DDQ) (100 µg.ml-1)

In a 100 ml volumetric vial, 0.01 g of 2,3-dichloro-5,6 -dicyano- 1,4- benzoquinone was dissolved in ethanol to create the solution. The volume was then increased by the same solvent concentrically 100 µg / ml.

2.3.4 Solution of Chloranil (100 µg.ml-1)

In order that create the solution, 0.01 g of chloranil was dissolved in a 100 ml volumetric vial of ethanol. The same solvent was then used to fill the capacity to the brim, yielding a solution concentration of 100 µg/ml.

2.3.5 Solution of Hydrochloric Acid (0.5 M)

It was created by extracting 4.3 milliliters of HCl, a concentrated acid with an 11.64 M concentration. The acid was then progressively diluted by adding water, and the volume was adjusted using 100 ml of distilled water in a volumetric bottle.

2.3.6 Solution of Sodium Hydroxide (0.5 M)

In a 100 ml volumetric vial, 2 g of the pure material was dissolved in 100 ml of distilled water to create it.

2.4 Preparation of Pharmaceutical Preparation for Schiff Base

The preparation of the medication, amlong Indian made (Brand- Micro) comes in pill form. Five mg of AML are contained in each tablet, and the solutions were made as follows: Ten tablets were thoroughly pulverized after being weighed individually. One tablet has an average weight of 0.1906 g and contains 5 mg of AML. The active ingredient, as well as 0.2 g (0.002 mol) of less concentrated picoline aldehyde in ten ml of absolute ethanol, was then added to 0.8 g of pharmaceutical preparation and the powdered meal in 30 ml of absolute ethanol, and added to the solution.

Additionally, add to the reaction 2 to 3 drops of olive acid as a supporting element. After allowing the mixture to reflux for three hours at 70 degrees

Celsius, allow the interaction mix to cool to room temperature. After obtaining yellow crystals. The material was put in a Baker for a full day in order to evaporate the most solvent. The precipitate was then purified and the filter paper was named using cold distilled water. To remove things that have not disintegrated. After that, the precipitate was collected, dried, and preserved. 0.01 g of the precipitate and the least amount of ethanol were taken out, put in a 100 ml bottle, and the size was adjusted to the solvent's mark to reach a concentration of 100 µg/ml. as a practical remedy [13].

2.5 The Method of Work

This method depends on the formation of the ion-pair complex between the reagent and drug [14].Using 1 ml of the drug's base lipids at a 100 µg/ml concentration and 1 ml of 2,3-dichloro -5,6-dicyano-1,4-benzoquinone at a concentration of 100 µg/ml, the shipping transfer complex was created. Using distilled water and a wiping for wavelengths between 190 and 800 nm, add 0.5 ml of sodium hydroxide in a 10-milliliter container at a concentration of 0.5 M. The resultant complex is yellow and exhibits maximum absorption at 436 nm, which was utilized in subsequent experiments.

3 RESULTS AND DISCUSSION

3.1 The Choice of Type Reagent

To determine which of the following compounds would make the best reagent, a study was undertaken. 2,3 – dichloro - 5,6 – dicyano - 1,4 -benzoquinone, Chloanil and 8-Hydroxyquinoline and the reagent that provides the highest absorption value in comparison to other reagents was selected. Following the addition of 1 ml of the aforementioned reagents to a series of volumetric of 10 ml containing 1 ml of the sealing base at a 100 µg/ml concentration, followed by the addition of 0.5 ml of sodium hydroxide with a concentration of 0.5 M and finishing with distilled water, it was discovered that 2,3-dichloro-5,6-dicyano-1,4-benzoquinone with Schiff base provided the highest absorption in comparison to the other reagents that were examined. The results are displayed in Table 2.

Table 2: Effect of different reagents.

Reagent	max (nm) λ	Absorbance
2,3 - dichloro-5,6 – dicyano-1,4 - benzoquinone (DDQ)	436	0.467
8-Hydroxyquinoline	358	0.225
Chloranil	371	0.192

3.2 Effect of Reagent Volume

The increasing sizes have been added 0.5-4 ml of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone with a concentration of 100 µg/ml in a sequence of volumetric of 10 ml that contains a fixed size 1 ml of the base of the concentration of 100 µg/ml to determine the optimal size for the reagent 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, which gives the resulting complex the highest absorption. Next, 0.5 ml of sodium hydroxide solution with a concentration of 0.5 M is added, and finally, the size is completed using purified water to the point of the mark. Next, as indicated in Table 3, we noted the absorption values for the component that was generated in comparison to the Blanc solution.

Table 3: Effect of DDQ concentration.

V(ml) of 2,3_dichloro_5,6_dicyano-1,4-benzoquinone (DDQ) 100 µg/ml	Absorbance
0.5	0.387
1	0.467
1.5	0.526
2	0.558
2.5	0.577
3	0.589
3.5	0.563
4	0.549

According to the findings of the study to determine the ideal reagent size, which is displayed in the above table, the best reagent size from which the resultant complex is obtained is 3 ml of a solution containing 100 µg/ml of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone. Because it had the highest absorption, it was chosen as the optimal reagent size.

3.3 Effect of Acid

A study of the effect of acid was conducted in which hydrochloric acid was used at a concentration of 0.5 molar. Different volumes of it, ranging from 0.5 - 1.5 ml, were added to the solution containing 1 ml of Schiff base with a concentration of 100 µg/ml and 3 ml of the reagent solution 2,3-dichloro-5,6-dicyano-1,4-benzoquinone with a concentration of 100. µg/ml and the absorbance values of the complex formed were recorded at each of these values as shown in the Table 4.

Table 4: Effect of acid volume on the complex.

V (ml) of 0.5 M hydrochloric acid	Absorbance
Without acid	0.589
0.5	0.577
1	0.525
1.5	0.442

Subsequent investigations avoided the use of acid since the findings of a study on its effect on the formation of a chelating complex for the medication AML indicated that adding acid decreased the absorption of the resultant complex.

3.4 Base Volume's Effect

A series of 10 ml volumetric bottles containing 1 ml of Schiff base for AML were filled with increasing volumes of sodium hydroxide at a particular concentration of 0.5 molar and in various volumes ranging from 0.5-2 ml with a concentration of 100 µg/ml, and 3 ml of the 2,3-dichloro-5,6-dicyano-1,4-benzoquinone reagent solution. The concentration was 100 µg/ml, and the Table 5 indicates that the absorption was measured at a wavelength of 436 nm.

Table 5: Effect of base volume on the complex.

V(ml) of Sodium Hydroxide 0.5 M	Absorbance
0.5	0.570
1	0.627
1.5	0.543
2	0.535

A study that looked at the effect of sodium hydroxide on the charge transfer complex formation reaction for the drug AML revealed that adding the basal medium in a volume of 1 ml gave the highest absorption value, which was utilized in subsequent studies.

3.5 Order of Additions

Several laboratory experiments were carried out using varying addition sequences to determine the best arrangement for the complex formed. Schiff base solution in one milliliter with 100 µg/ml and 3 ml of the 2,3-dichloro and 5,6-reagent solution were added. Table 6 displays the absorption values of dicyano-1,4-benzoquinone in different sequences, contingent on the optimal reaction conditions, and 100 µg/ml and 1 ml of NaOH solution at a concentration of 100 µg/ml 0.5 M.

Table 6: Impact of the addition sequence on the charge transfer complex's absorption.

Reaction component	Order number	Absorbance
D+R+B	I	0.627
D+B+R	II	0.615
B+R+D	III	0.597

Table 6 shows that the arrangement D+R+B, where (D) is the drug's Schiff base and (R) is 2,3-dichloro-5,6-dicyano-1,4-benzoquinone and (B) is base. has the best absorption of the final product. As a result, this arrangement was used in further studies.

3.6 Temperature's Effect

One of the crucial factors in the reactions that needs to be investigated is temperature. To determine which temperature is ideal for the resulting compound to exhibit the maximum absorption, 1 ml of Schiff base solution with a concentration of 100 µg/ml and 3 ml of the reagent solution 2,3-dichloro-5,6-dicyano-1,4-benzoquinone with a concentration of 100 µg/ml and 1 ml of NaOH solution with a concentration were added.

Table 7: Temperature's impact on the charge transfer system.

Temperature (C^0)	Absorbance
15	0.525
20	0.572
25	0.627
30	0.664
35	0.708
40	0.739
45	0.731
50	0.724

0.5 M solution was prepared, and distilled water was added to bring the volume to the desired level. Then, the absorbance measurement of the resultant

complex during the reaction was performed within a temperature range of 15–50 °C, as shown in Table 7.

The maximum absorption happens at 40° C. This temperature was therefore selected for use in subsequent experiments.

3.7 Time's Effect

A study was carried out to ascertain whether the compound created was stable between the Schiff base of the drug AML and the reagent 2,3-dichloro and 5,6-dicyano-1,4-benzoquinone by choosing the optimal time at which the complex formed gives the highest absorption.

A solution of sodium hydroxide with a concentration of 0.5 molar was added after 1 ml of the Schiff base with a concentration of 100 µg/ml was taken and 3 ml of the reagent solution with a concentration of 100 µg/ml was added with a volume of 1 ml, and complete the volume up to the mark with distilled water and heating at a temperature of 40 C°.

Then the absorption of the complex was measured at different times, ranging from the beginning of the preparation of the complex to 20 minutes and Table 8 shows this.

Table 8: Time's impact on the charge transfer complex.

Time (min)	Absorbance
Moment of reaction	0.739
5	0.795
10	0.833
15	0.829
20	0.814

After 10 minutes, as shown in Table 8, the complex exhibits its maximum absorption, providing ample time for additional testing.

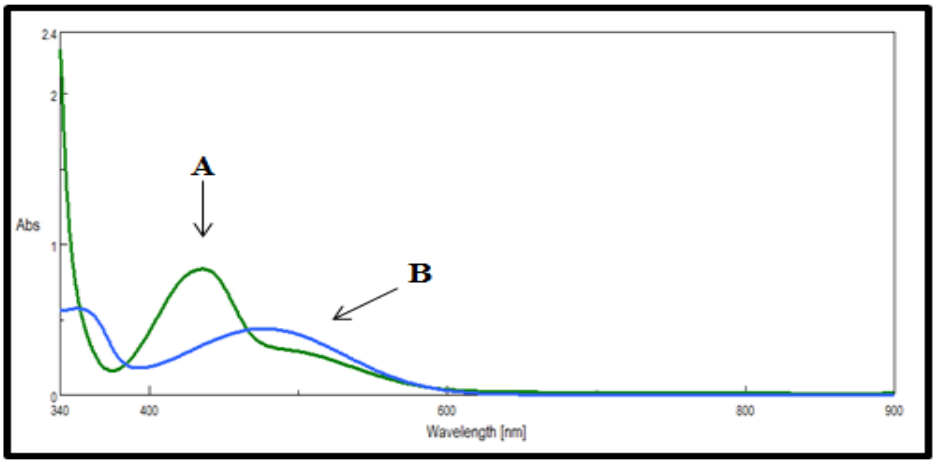


Figure 2: (A) Complex versus blank, (B) Reagent vs. ethanol.

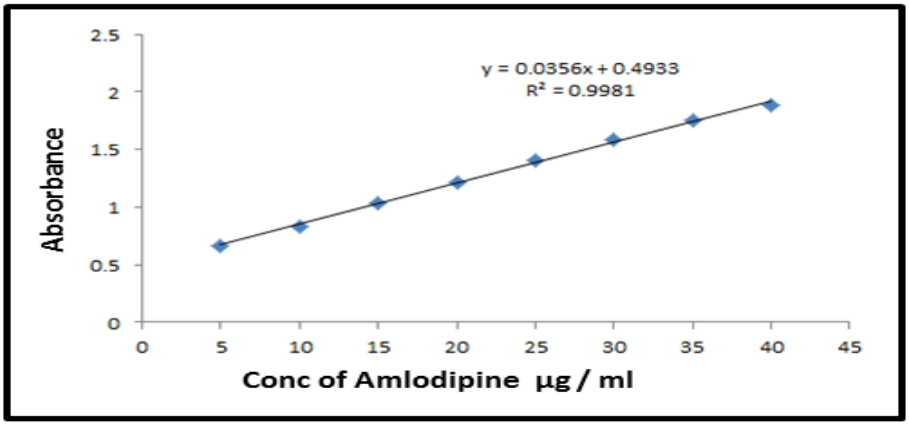


Figure 3: AML calibration curve of the proposed method.

3.8 Final Absorption Spectrum

One milliliter of Schiff base at a concentration of 100 µg/ml was used to record the reaction's absorption spectra. and 3 ml of the reagent solution of 2,3-dichloro5,6-dicyano-1,4-benzoquinone with a concentration of 100 µg/ml, then adding 1 ml of sodium hydroxide solution with a concentration of 0.5 M, fixing the necessary temperature for the reaction at 40° C.

Then diluting with distilled water in a bottle. In a volumetric volume of 10 ml, the absorption spectra at wavelength 436 nm was recorded against the corresponding blank solution after the complex was allowed for 10 minutes to allow the reaction to finish, as shown in Figure 2.

3.9 Calibration Graph

A calibration curve, as shown in the figure, was created after the ideal conditions for the reaction were fixed. It demonstrates that the Beer-Lambert law was used to estimate AML for concentrations between 5 - 40 µg.mL-1 with a final volume of 10 mL and a correlation coefficient (r = 0.9981). At higher concentrations, however, a deviation occurs. After calculation, the molar absorptivity came out to be 1.4556 × 104 l.mol-1.cm-1. Figure 3 shows that Sandal's index was 0.0281 µg.cm-2.

3.10 Accuracy and Precision

For the calibration curve, three different concentration levels ranging from 10 to 40 $\mu g.ml^{-1}$ were chosen. To calculate the approach's precision (RSD%) and recovery (REC%), as shown in Table 9.

Table 9: The approach precision and accuracy in determining AML.

Conc. AML Taken µg/ml	Abs.*	Conc. AML found µg/ml	Rec%	RSD%
10	0.838	9.6826	96.8260	0.6258
25	1.411	25.7781	103.1124	0.2699
40	1.880	38.9523	97.3808	0.2257

3.11 Detection Limit and Quantitative Limit

The lowest analyte concentration in the test sample that can be consistently identified from zero is known as the limit of detection (LOD). The boundary of The lowest concentration of a material that can be determined quantitatively is known as the limit of quantification (LOQ) [15].

Because the blank is colorless, as shown in Table 10, the detection limit and the quantitative limit have been established at the calibration curve's optimum misfortune (5 $\mu g.ml^{-1}$) [16].

Table 10: Quantitative Limit and Detection Limit.

Concentration µg/ml	Slope	s	LOD µg/ml	LOQ µg/ml
5	0.0356	0.0028	0.2596	0.7865

3.12 Application for Analysis

The suggested method for examining amlong 5 mg Indian made (Brand – Micro). A positive result was obtained after implementing the recommended strategy, as shown in Table 11.

Table 11: The analytical application, of the proposed approach.

Conc of AML µg/ml pharmaceu-tical preparation	Abs.*	Conc. of AML found µg/ml	Rec%	RSD%
10	0.841	9.7669	97.6690	0.1880
25	1.409	25.7219	102.887	0.1587
40	1.889	38.2051	98.0128	0.1543

3.13 Characterization of Compound by IR and Mass

The infrared (IR) spectrum of the Schiff base is shown in Figure 5. This spectrum is compared with both the spectrum of the primary amine (AML) shown in Figure 4, In the infrared (IR) spectrum of the Schiff base, no absorption band appears at (3288) cm^{-1}, which belongs to the ν bond (NH_2), compared to the spectrum observed in the primary amine spectrum. The spectrum also does not show any absorption around the frequency (1713) cm^{-1}[17]. which is assigned to the bond ν(C=O) of the aldehyde The spectrum of the Schiff compound also indicates the appearance of a new band at (1635) cm^{-1} belonging to C=N, which indicates the preparation of the new compound [18].The mass spectrum showed a peak at m/z 498, which represents the molecular weight of the Schiff base formed for AML, as show in Figure 6. Preparation of the Schiff base is shown in Figure 7.

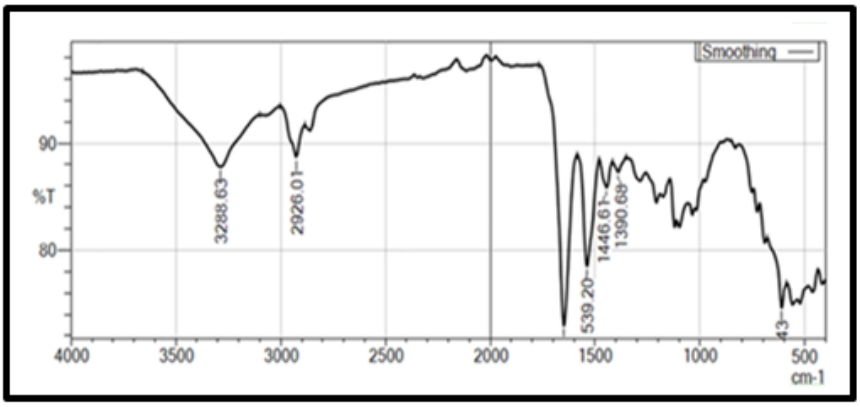


Figure 4: IR spectrum of AML.

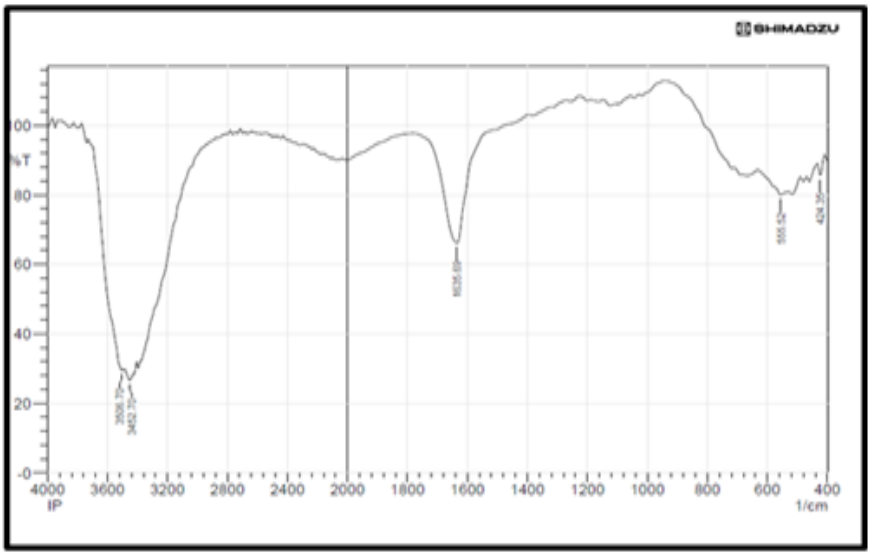


Figure 5: IR spectrum of Schiff base.

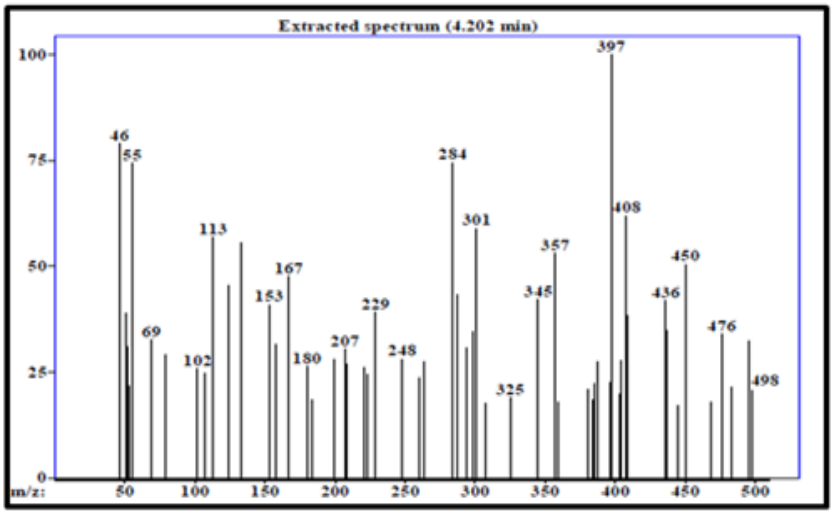


Figure 6: The mass spectrum of Schiff base.

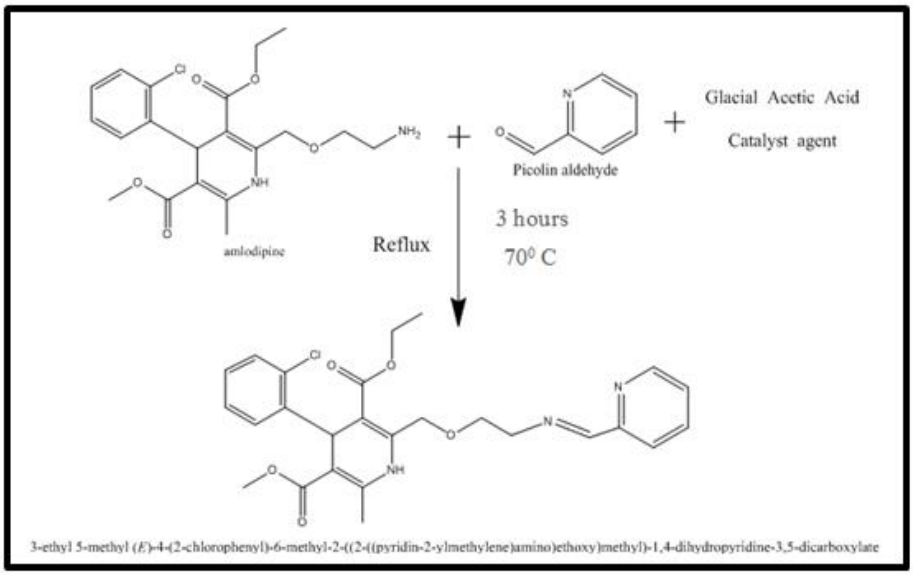


Figure 7: Schiff base preparation scheme.

4 CONCLUSIONS

In this study, a straightforward and reliable spectrophotometric method was successfully developed for the determination of Amlodipine (AML) in pharmaceutical formulations. The method is based on forming a yellow charge-transfer complex between a synthesized Schiff base of AML and DDQ reagent under basic conditions. A good and simple spectroscopic method has been developed to estimate AML through a complex charge transfer reaction with the reagent DDQ in the basic medium. The method is based on adding the reagent DDQ to a Schiff base and then adding sodium hydroxide and the temperature necessary to conduct the reaction 400 C, then the reaction's ultimate result, which is yellow in color, is created once the additions are finished, after which the absorption is measured at 436 nm. It follows Beer's law in the concentration range of 5-40 µg/ml.The method was successfully applied to AML in the pharmaceutical preparation Amlong 5 mg. The method demonstrated strong linearity across a concentration range of 5–40 µg/ml, with a high correlation coefficient (R^2 = 0.9981), excellent sensitivity, and low limits of detection (LOD = 0.2596 µg/ml) and quantification (LOQ = 0.7865 µg/ml). It also showed high precision and recovery rates, validating its accuracy for pharmaceutical analysis.

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Assessing Health Risks of Heavy Metals in Juices and Soft Drinks in Baghdad, Iraq, and their Impact on Human Health

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Keywords: Heavy Metals, Juices and Soft Drinks, Health Risk Assessment, Hazard Quotient.

Abstract: The current study assessed the levels of health risks resulting from exposure to concentrations of some heavy metals (Ag, Al, As, Cd, Co, Cr, Cu) found in juices and soft drinks that are consumed daily and available in local markets in Baghdad/Iraq. 45 samples of these products were collected and luminiu using by ICP-OES device. The results showed that most of the concentrations in these measured samples were within the permissible limits according to World Health Organization standards, while the concentrations of some elements recorded slightly high levels. Such as silver (Ag), which reached (0.54) parts per million, and the concentration of luminium (Al) in Fresh powder juice reached (0.46) parts per million in Rani juice, and the average concentration and standard deviation reached For silver (Ag) (0.2746 ± 0.15857), which is higher than the permissible limit set by the World Health Organization (0.1), and the P-Value (0.0916) was greater than 0.05, unlike the other elements, the values were less than the permissible limit previously. The World Health Organization (WHO) also calculated the daily intake dose (DID) and hazard index (HQ) for the subjects taken and studied. The results of the study showed that some of the permissible reference doses for some elements, such as silver and aluminium, were exceeded, which indicates the presence of potential health risks. The current study also recommended the importance of following up on these food products and strengthening control over them, especially for juices and soft drinks, in order to avoid potential health risks that could occur. To which consumers are exposed.

1 INTRODUCTION

Juices and soft drinks are considered refreshing, non-alcoholic drinks. Many people consume them daily, regardless of age, gender, or social and economic status. They are characterized by their sweet and refreshing taste. The reason for the great demand for soft drinks is due to the ability of these drinks to give a feeling of refreshment and happiness, in addition to It is easy to obtain and relatively low cost [1]. Although these juices and soft drinks may be refreshing and add a kind of pleasure to daily life, consuming them excessively may lead to major health risks, such as weight gain, high blood pressure, tooth decay, heart disease, and other health problems [2], and recent studies have indicated that consuming juices and soft drinks may lead to negative effects on bone health, as they increase the risk of fractures [3]. Some of these drinks may contain high levels of heavy metals that may exceed permissible limits [4]. Heavy metals pose a major threat to a person's general health as a result of their accumulation in his body, as their presence in juices and soft drinks represents a major risk in the long term, including the risk of cancer, and also leads to organ damage over time [5]. Some studies have also shown the presence of heavy metals in some beverages that may be toxic, including silver, luminium, copper, cobalt, cadmium, and arsenic, which causes real risks to consumers [6]. Exposure to luminium may lead to health problems related to the nervous system. Including memory loss or Alzheimer's disease, it also affects the bones and reduces their mineral density, causing osteoporosis, and luminium also causes various problems for the body as a result of its accumulation in various tissues [7]. Arsenic is also considered one of the most dangerous toxic elements in the body, and exposure to it through drinks contaminated with this element may lead to the risk of developing blood pressure, diabetes, as well as other heart diseases, in addition

to damage to the blood vessels and atherosclerosis [8]. As for cadmium, it is a metal. It is toxic and may cause major health problems such as kidney failure and osteoporosis. Continued exposure to cadmium increases the risk of bladder cancer as well as lung cancer, and small amounts of it are harmful in the long term. Long-term accumulation in the body may lead to potentially toxic effects on vital organs in the body, including the kidneys and bones [9]. Although copper is considered essential for the human body, exposure to it in large quantities may cause damage to the liver and kidneys in the long term. It also causes problems in the digestive system such as nausea, vomiting, and diarrhea, and this confirms the danger of copper accumulation on the health of vital organs [10]. Necessary preventive measures must be taken, such as monitoring the quality of products and limiting the consumption of juices and soft drinks that may contain unsafe levels of heavy metals.

2 MATERIALS AND METHODS

2.1 Sample Collection

A total of 45 samples of juices and soft drinks were collected from different areas in the capital, Baghdad/Iraq, based on the presence of major commercial markets and retail stores in residential neighborhoods. This approach was taken to ensure a good representation of the types of juices and soft drinks available in local markets. The sampling included about 15 types of products and was conducted during a period of 30 days, from November 3, 2024 to January 6, 2025. The sampling process included products from local and imported brands to ensure product diversity and obtain accurate results. The samples were carefully selected, then placed in clean and sterile containers to preserve their safety from any external contamination, and then transported to the laboratory to conduct the necessary analyzes and determine the concentrations of heavy metals.

2.2 Chemical Analysis

A one milliliter sample was taken and put into glass container to be analyzed later. Following this, 1 milliliter of a 69% sulfuric acid solution was introduced to the container, alongside 1 milliliter of 35% hydrogen peroxide and 4 milliliters of nitric acid. Subsequently, this solution underwent microwaving at an intensity that allowed the temperature to rise from 25° C to 180° C over a fifteen minute duration. This was then followed by 30 minutes of cooking the solution at a constant temperature of 180° C in order to fully complete the digestion. After waiting for the digestion as well as the rest of the mixture to sufficiently cool down, special filters were used to separate the desired sample from the rest of the mixture. This filtered sample was then diluted volumetrically to 30 milliliters using distilled water. In the end, the heavy metals concentration found in liquid samples were measured using an Agilent ICP-OES device located at the atomic energy authority [11].

2.3 Calculating the Health Risks of Heavy Metals

The health risks of heavy metals in juices and soft drinks were calculated by calculating the Daily Intake Dose (DID), the Hazard Quotient (HQ) for each element, and the Hazard Index (HI) for multiple exposures to the elements.

2.3.1 Calculating the Daily Intake Dose (DID)

The dose of daily handling of each element was calculated based on the average concentration of the element in the sample, where the daily consumption rate of soft drinks and juices is 0.5 liters per person, and a weight of 70 kilograms for adults [12].

$$DID = (Concentration \times Intake\ Rate)/(body\ weight)$$

2.3.2 Reference Doses (RfD)

Reference doses for assessing health risks from exposure to heavy metals in food and beverages have been adopted by the US Environmental Protection Agency (EPA).

2.3.3 Calculating the Hazard Quotient (HQ)

The hazard quotient (risk index) for each element was calculated to assess whether the daily intake dose exceeds the safe reference dose. It is calculated by [13]:

$$HQ = DID/RfD.$$

2.3.4 Calculating the Overall Hazard Index (HI)

The overall hazard index was calculated by summing the hazard quotients for all elements [14]:

$$HI=HQ_1+HQ_2+HQ_3+\cdots+HQ_n.$$

2.4 Statistical Analysis

Statistical analyzes of the data obtained by the SPSS (version 22), to ensure reliable and accurate results. Where the averages and standard deviation were calculated. Anova testing was also conducted to find out whether there are statistically significant moral differences between different groups of samples.

3 RESULTS AND DISCUSSION

3.1 Results

The results of Table 1 showed that the highest concentration of silver (Ag) was in Rani juice, reaching 0.54 ppm, while the lowest concentration was recorded in Al-Shater juice at 0.12 ppm (see Fig. 1). For aluminum (Al), the highest concentration was in Fresh Powder juice, measuring 0.46 ppm, while the lowest concentration was in Fayrouz Pomegranate juice, where it reached 0.00 ppm. Arsenic (As) recorded the highest concentration of 0.1 ppm in Limonza Powder juice, while it was not detected (ND) in the rest of the samples. Cadmium (Cd) and chromium (Cr) were not detected (ND) in any of the samples. The highest cobalt (Co) concentration was in Dalia Orange and Mom Orange juices, reaching 0.05 ppm, while it was absent in other samples. Copper (Cu) showed low concentrations, with the highest concentration recorded in Al-Shater juice at 0.02 ppm, and the lowest in Coca-Cola metal can juice at 0.00 ppm.

Table 1: Concentrations of heavy metals (mg/L) in various juices and soft drinks in Iraq.

Juices and soft drinks	concentration						
	Ag	Al	As	Cd	Co	Cr	Cu
Coca-Cola metal can	0.26	0	0	0	0	0	0
Cola Ugarit juice	0.25	0.13	0	0	0	0	0
Al-Shater juice	0.12	0.05	0	0	0	0	0.02
Jaffa orange juice	0.17	0.04	0	0	0	0	0
Caspian powder juice	0.27	0	0	0	0	0	0
Fresh powder juice	0.13	0.46	0	0	0	0	0
Limonza powder juice	0.35	0	0.1	0	0	0	0
Daily orange juice	0.43	0.03	0	0	0	0	0
Rani juice	0.54	0.04	0	0	0	0	0
Fayrouz pomegranate juice	0.37	0	0	0	0	0	0
Ugarit Cola Iraq	0.38	0.01	0	0	0	0	0
Rand orange juice	0.47	0.04	0	0	0	0	0
Cola Tamim	0.38	0.01	0	0	0	0	0
Dalia orange juice	0	0.09	0	0	0.05	0	0
Mom's orange juice	0	0.16	0	0	0.05	0	0

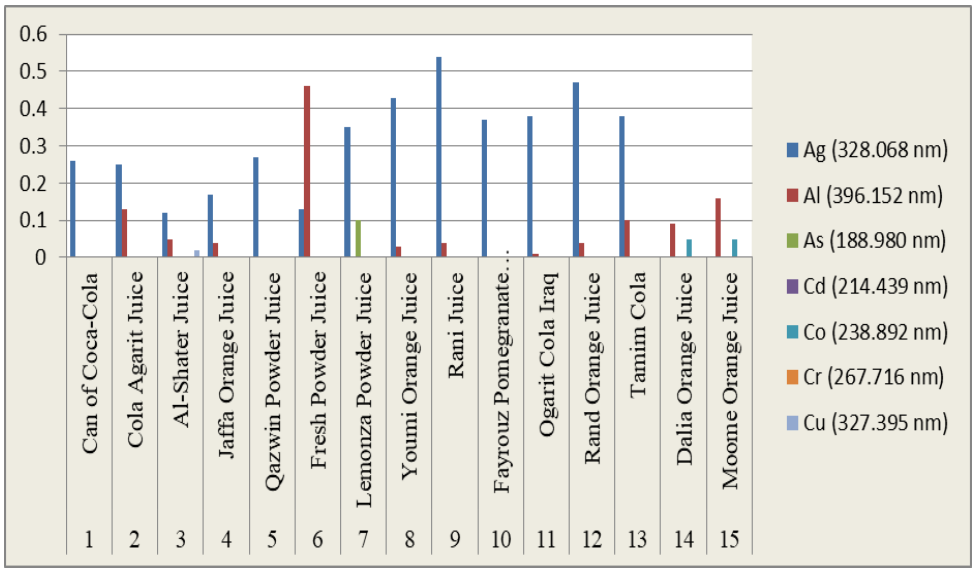


Figure 1: Concentration of heavy metals (mg/L) in different beverage samples.

The chart illustrates the concentrations of heavy metals for each beverage sample, where the colored bars within the chart represent the specific color for each element, while their lengths indicate the concentration levels of each element in the sample.

The results of Table 2 showed that the mean concentrations and standard deviations for all samples of silver (Ag) were (0.2746 ± 0.15857), which is higher than the permissible limit set by the World Health Organization (0.1). The P-value (0.0916) was greater than 0.05. As for aluminum (Al), the mean concentration was (0.08214 ± 0.11323), which is lower than the WHO limit of (0.2), and the P-value (0.1561) was greater than 0.05. Regarding arsenic (As), it reached (0.00666 ± 0.02494), which is below the permissible limit set by WHO (0.01), and the P-value was (0.0942), greater than 0.05. For cadmium (Cd) and chromium (Cr), the mean concentration and standard deviation were (0.00000 ± 0.00000), indicating that these elements were not detected in the samples. On the other hand, cobalt (Co) recorded (0.00666 ± 0.01699), which is lower than the permissible limit set by WHO (0.1), and the P-value was (0.0437), less than 0.05. Copper (Cu) was (0.00133 ± 0.00498), which is lower than the WHO limit of (0.2), and the P-value for these elements was less than 0.05.

The results of Table 3, showed that the daily doses consumed of silver and aluminum amounted to (0.00784) and (0.00234), which is higher than the reference dose specified by health authorities for silver, which amounted to (0.005), and for aluminum (0.001). It also showed the risk index for silver amounted to (1.569143), and the aluminum index reached (2.346857), which is higher than 1. As for the daily doses consumed for arsenic and cadmium, they reached (0.00019) and (0.00000), which are less than the reference dose (0.0003) and (0.0001), and the risk index for arsenic and cadmium reached (0.634286) and (0.000000), which is less than 1, While the daily dose of cobalt was (0.00019), which is less than the reference dose (0.0003), and the risk index for cobalt was (0.634286), . It is less than 1, and the daily dose of chromium was (0.00000), which is less than the reference dose (0.003), and the risk index for it was (0.00000). It is less than 1, while the daily dose of copper was (0.000038).) which is much less than the reference dose (0.04), and the risk index was (0.000950), which is less than 1. Figure 2 shows the hazard index (HQ) for each of the different heavy elements (Ag, Al, As, Cd, Co, Cr, Cu) in juices and soft drinks. The dashed red line also indicates the overall Hazard index (HI) which reached 5.1855.

Table 2: Calculating average concentrations of heavy metals, standard deviation, and P-value, and comparing them to the permissible limits according to World Health Organization standards.

Elements	Mean±Std	WHO Limite	P-Value
Ag (PPM)	0.2746 ±0.15857	0.1	0.0916
Al (PPM)	0.08214±0.11323	0.2	0.1561
As (PPM)	0.00666±0.02494	0.01	0.0942
Cd (PPM)	0.0000±0.00000	0.003	(N/A)
Co (PPM)	0.00666±0.01699	0.1	0.0437
Cr (PPM)	0.00000±0.00000	0.05	(N/A)
Cu (PPM)	0.00133±0.00498	2.0	0.0192

Table 3: Daily dose taken, reference dose, and hazard index (HQ) for some heavy metals.

Elements	Daily Intake Dose (DID) (mg/kg/day)	Reference dose (RfD) (mg/kg/day)	Danger indicator (HQ
Ag (PPM)	0.00784	0.005	1.569143
Al (PPM)	0.00234	0.001	2.346857
As (PPM)	0.00019	0.0003	0.634286
Cd (PPM)	0.00000	0.0001	0.000000
Co (PPM)	0.00019	0.0003	0.634286
Cr (PPM)	0.00000	0.003	0.000000
Cu (PPM)	0.000038	0.04	0.000950
Overall Hazard Index (HI)			5.1855

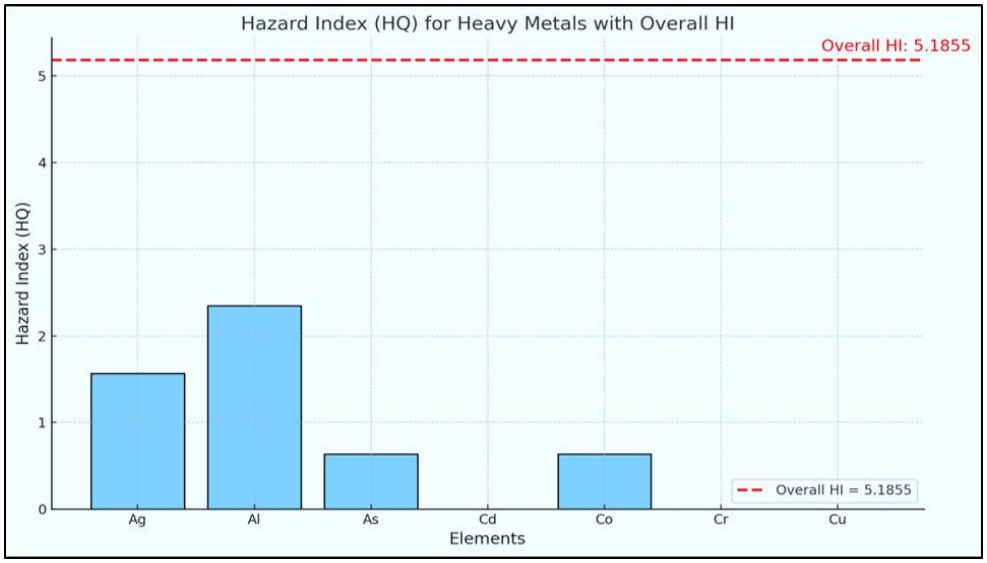


Figure 2: Hazard index of heavy metal concentrations in juices and soft drinks.

3.2 Discussion

The results of this study showed that the average concentration of silver (Ag) in the analyzed beverages exceeded the permissible limit set by the World Health Organization (WHO), with a statistically significant difference indicated by a P value of less than 0.05. This finding raises potential health concerns, especially given the cumulative toxicity of silver in humans. The results of a similar study conducted in European coastal waters, carried out by a research team led by Tapin and others in Europe in 2010, which analyzed silver concentrations in beverages, showed that the results

showed that the average concentration of silver in the samples exceeded the permissible limit according to the standards of the World Health Organization (WHO). This indicates potential health risks associated with chronic exposure [15]. Another different study was conducted by a group of researchers led by "Athanasiadis, V." And others in 2023. The study focused on analyzing caffeine and mineral elements in different types of drinks. The study indicated that the levels of mineral elements were within permissible limits, which indicates that these drinks are safe for consumption in terms of mineral content [16], which requires treatment of these Problems require more stringent measures. Take regulatory measures and strengthen quality control in beverage production. The study also showed that the average concentration of aluminum (Al) was less than the permissible limits set by the World Health Organization, indicating minimal health risks associated with exposure to aluminum from beverages sampled, and these differ with the results of Nicholas and Ekoha (2013) in the study he conducted on canned foods and beverages sold in Nigerian markets. The results showed that some products had high concentrations of aluminum, indicating the possibility of aluminum leaking from the containers into the content [17]. In a study conducted by Abercrombie and Fowler in 1997 on the potential content of aluminum in canned beverages, the results showed the presence of varying amounts of aluminum in samples of canned beverages, and the average aluminum concentration was less than the permissible limit. This is due to the fact that proper protective coatings and appropriate storage reduce the leakage of aluminum into the Drinks[18]. The concentrations of arsenic (As), cadmium (Cd), and chromium (Cr) also showed no presence and were within permissible limits, which reflects compliance with safety standards in most samples. In a study conducted by Leconte et al. (2021) in France showed that chronic exposure to cadmium represents a health risk, even at low levels. [19] They also focused on lowering the guideline values for cadmium to ensure better protection of public health, as shown by the study conducted by Lazović et al. (2023) in Serbia, which focused on measuring levels Cadmium, lead, mercury, and arsenic in vegetables and fresh vegetable products during the period from 2015 to 2017, as some samples that exceeded permissible limits were detected. This confirms the need to improve agricultural practices and monitor the water sources used [20]. Cobalt (Co) levels were within permissible limits, but the Hazard Quotient (HQ) indicates the possibility of long-term health effects if exposure continues. Although copper (Cu) is essential to human health in trace amounts, it has been found in concentrations well below permissible limits, indicating no immediate health risks. These results are consistent with the study of Katsina State in Nigeria, which showed that copper levels were few within the permissible level, and the reason is due to the lack of industrial activity in those areas [21]. Another study conducted in northeastern Ethiopia to determine the levels of some Heavy elements in herbal preparations, including cadmium and copper, as results for copper (Cu) showed less than permissible limits, according to international safety standards, as well as results for Cadmium (Cd) and arsenic (As) in drinks did not exceed the permissible limits [22]. as the hazard index (HQ) for these two elements was less than 1. On the contrary, the results of a study conducted by Edirisinghe & Jinadasa (2020) showed that the concentrations of cadmium The arsenic in Sri Lankan rice exceeded the permissible limit, increasing potential health risks, especially for the most vulnerable people [23], while cadmium was not detected. (Cd), in the juices and soft drinks in the current study, and this indicates that these juices and soft drinks are not affected by industrial activities. On the contrary, the study conducted by Du and others in China (2020) showed that industrial activities, such as mining and smelting, contributed to an increase in the concentration of cadmium in beverages and foods, which clearly leads to increased environmental and health risks [24].

4 CONCLUSIONS

The study revealed that most of the juices and soft drinks available in local markets in Baghdad contain levels of heavy metals that fall within acceptable limits according to World Health Organization standards. However, recording excesses in the concentrations of some elements such as silver (Ag), chromium (Cr), and aluminum (Al) in some samples indicates the need to be careful, especially with repeated use of these products. The results also showed the importance of strengthening control over food products, whether imported or locally manufactured, in order to adhere to health safety standards. The study also stressed the importance of conducting additional research to understand the sources of pollution and determine appropriate ways to reduce risks. It is worth noting that consumer

awareness plays an important role in reducing health risks. Therefore, it is recommended to moderate the consumption of these drinks and choose healthy alternatives when possible.

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Green Synthesis of Copper Manganese Oxide Nanoparticles from Ginkgo Biloba Extract and Study of their Activity on PC3 Cancer Cell Line

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Keywords: Green Synthesis, Copper Manganese Oxide with Composition, Nanoparticles, Ginkgo Biloba, Flutamide PC3 Cell.

Abstract: This study presents a novel, cost-effective method for synthesizing $Cu_{1.4}Mn_{1.6}O_4$ nanoparticles utilizing Ginkgo Biloba leaf extract. The approach integrates green chemistry, co-precipitation, and ultrasound techniques into a single methodology for nanoparticle preparation. The prepared nanoparticles underwent characterization through various techniques, including XRD, FT-IR, EDX, SEM, and DLS. While the average size noted in the XRD was 38.12 nm, the average particle size observed in the SEM was 103.60 nm. The average particle size measured by DLS was 196.3 nm. The efficacy of the synthesized $Cu_{1.4}Mn_{1.6}O_4$ nanoparticles was evaluated against the pharmaceutical Flutamide, utilized in Iraq for the treatment of prostate cancer, on the PC3 cell line. The results demonstrated the exceptional efficacy and superiority of the produced nanoparticles compared to the administered medication. Their characterization included reduced cytotoxicity relative to the drug's toxicity on red blood cells during the toxicity screening test, where $Cu_{1.4}Mn_{1.6}O_4$ exhibited cell killing rates of 37.68%, 47.75%, 63.38%, 87.07%, and 98.00%. results in 24 hours showed an IC50 value = 38.46; In contrast, Flutamide demonstrated cell-killing rates of 6.51%, 10.45%, 27.69%, 32.48%, and 52.53%and results showed an IC50 value of 296.8 at 24 hours too.

1 INTRODUCTION

Prostate cancer is a prevalent malignant neoplasm in males, primarily impacting prostate tissue. Crucially important for the male reproductive system, the prostate gland generates semen. Prostate cancer generally exhibits gradual growth, and its initial symptoms are ambiguous. It may induce symptoms including frequent urination, an urgent compulsion to urinate, and discomfort. As the illness advances, symptoms, including ostealgia, lethargy, and weight reduction, may manifest. Preventive strategies for prostate cancer encompass sustaining a healthy lifestyle, undergoing frequent examinations, and avoiding exposure to detrimental substances [1]. Numerous studies have shown that the development of prostate cancer is mostly dependent on growth factors, which either directly raise steroid hormone levels or, using an active feedback process, increase enzyme activity. Radiation therapy, surgery, and chemotherapy are the present choices for prostate cancer treatment. Treatment with conventional chemotherapy, which is often used to treat androgen-independent prostate cancer, can lead to resistance and progression, further complicating the situation given the lack of available treatment options [2]. Although it is the most successful cancer treatment, these chemotherapy drugs have numerous side effects due to their cytotoxic properties [3]. Furthermore, reducing the chance of cancer death and recurrence is the goal of radiation treatment. Usually, it entails radiation exposure to surrounding organs, which raises the risk of heart and lung illness. Particularly when used with some kinds of adjuvant chemotherapy [4], these therapies could raise leukemia risk. Wild plants and animals have long been the main source of natural remedies that have significantly improved human health. However, creating medications from these materials has grown more difficult due to continuous changes and human exploitation of wild ecosystems. Consequently, scientists are currently investigating the large ocean of alternative medicine sources [5].

For thousands of years, ginkgo biloba (Ginkgo Biloba L.) has been used as a Chinese herb to cure bronchitis and asthma [6]. In recent pharmacological studies, Ginkgo Biloba leaf extract (EGB) has shown anti-inflammatory, antioxidant, neuroprotective, anti-cardiovascular, and peripheral vascular properties in addition to its anti-platelet aggregation activities [9]. Terpene ticlatones and flavanol glycosides are their primary bioactive constituents [10]. Oncology nanotechnology can aid in updating cancer detection and treatment. Patients with breast cancer now have new hope thanks to the development of novel targeted technologies at the nanoscale made possible by advances in materials science and protein engineering [11]-[13]. Nanoparticles (NPs), sometimes called drug carriers, offer a novel way to deliver medications to cancer cells with great specificity for the targeted cancer cells by precisely penetrating tumours [14]. A novel class of nanomaterials called bimetallic nanoparticles (BNPs) comprises two distinct metallic components [15]. Their structure resembles that of monometallic counterparts in certain aspects; however, the synergistic interaction between the two components enables the manifestation of diverse novel properties and applications. BNPs have garnered considerable attention from researchers owing to their distinctive catalytic, electronic, optical, and magnetic properties [16]. Materials made of two metals that have unique properties because of their synergistic effects are called bimetallic nanoparticles. Compared to monometallic nanoparticles, bimetallic nanoparticles (nanoalloys) offer a greater potential for use [17]. Unlike chemical and physical methods, biosynthesis of bimetallic nanoparticles is a safe, reasonably priced, clean, ecologically benign technique [18]. Investigated and proven to be more effective than their monometallic counterparts are the anticancer capabilities of some bimetallic nanoparticles, including silver and gold, silver and copper, and zinc and silver [19]. A colon adenocarcinoma cell line (HT-29) was used in prior work to assess the anticancer effects of copper-manganese bimetallic nanoparticles (CMBNPs), which were produced from pumpkin seed extract [20]. Numerous studies have shown impressive anticancer efficacy against various cancer cell types using copper and manganese bimetallic nanoparticles. For instance, it has been documented that copper nanoparticles use oxidative stress and DNA damage to cause apoptosis and cell death and prevent the growth of cancer cells. Manganese nanoparticles have also shown promise in cancer treatment. They boost the formation of reactive oxygen species (ROS) in cancer cells, inducing death and cell cycle arrest [21]. Moreover, these nanoparticles' distinct physicochemical characteristics, including their size, shape, and surface modifications, can be altered to maximize their effectiveness against cancer cells and reduce their negative effects on healthy tissue [22]. These results highlight the potential of manganese and copper bimetallic nanoparticles as effective targets for anticancer treatment. Bimetallic copper and manganese nanoparticles have shown exceptional antibacterial efficacy against various pathogenic microbes in addition to their anticancer qualities. For instance, copper nanoparticles demonstrated strong antibacterial action by rupturing bacterial cell membranes and interfering with essential biological functions [23]. Manganese nanoparticles demonstrate significant efficacy as antimicrobial agents, especially against drug-resistant bacterial strains [24].

2 MATERIALS, TECHNIQUES AND METHODS

2.1 Items of Research

Cu (NO3)2, Molecular Weight=187.57 from Sigma-Aldrich Purity=98%, Mn(NO3)2, Molecular Weight=178.95 from Sigma-Aldrich Purity=99%, Deionized water from Iraq Babylon Purity=99%, Na2CO3, Molecular Weight=106.00 from Sigma Purity=98%, NaBH4 Molecular Weight=37.83 from Sigma-Aldrich Purity=99%, CH3CH2OH Molecular Weight=46. 07 from England (BDH), Purity=99%, Ginkgo biloba from Iraq Market, Flutamide tablets 20mg from ALIUD PHARMA(Germany).

2.2 Extraction of Ginkgo Biloba Leaf Extract

Combine 50g of Ginkgo biloba leaves with 500 ml of deionized water in a 10:1 ratio, stirring the mixture magnetically for one hour at 50°C. Subsequently, filter the mixture and store the filtrate in a cool location [25].

2.3 Synthesis of $Cu_{1.4}Mn_{1.6}O_4$ Nanoparticles by Green Chemical Methods

To create one molar, 3.75 grams of Cu (NO3)2 salts should be dissolved in 50 millilitres of ginkgo biloba

leaf extract. 3.57 grams should be dissolved in 50 millilitres of the extract to create one molar of Mn(NO3)2. Following a half-hour of mixing and 350 cycles at 40°C, the two solutions were put on the magnetic stirrer. Following ten minutes in an ultrasonic device, the mixture was placed on a magnetic stirrer, the pH was adjusted to 7, and the Na2CO3 solution was gradually added at a concentration of two molar. After that, NaBH4 was gradually added to it at a one-molar concentration. The precipitate was filtered and cleaned twice with ethanol and three times with deionized water. At 180°C, the residue was dried. The residue was then burnt for four hours at 650°C.

2.4 Approaches for Characterization

The $Cu_{1.4}Mn_{1.6}O_4$ Nanoparticles were analyzed using various methods. The nanoparticles' crystallite size was ascertained by XRD (Shimadzu, Kyoto, Japan). Shimadzu (Tokyo, Japan) was used to obtain the FTIR spectra of the materials. A 200 kV Zeiss SEM (Germany) was used for the SEM analysis. Anton Paar Litesizer DLS 100.

2.5 MTT Test for $Cu_{1.4}Mn_{1.6}O_4$ Nanoparticles

Using 10 mg/ml of 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide as the MTT dye, $Cu_{1.4}Mn_{1.6}O_4$ nanoparticles showed. The $Cu_{1.4}Mn_{1.6}O_4$ nanoparticle samples were dissolved in 0.2% DMSO to create concentration gradients quantified in ppm at 20, 40, 80, 160, and 320. A 200 µl sample of suspended cells (1 × 10^4 cells/well) in RPMI media was distributed. The cells were cultured in 5% CO_2 for 24 hours at 37°C. The cell cultures were cultured for a further twenty-four hours under the same circumstances following treatment with 20 µl of $Cu_{1.4}Mn_{1.6}O_4$ NPs. Each sample was then incubated for five hours at 37°C with 10 µl of MTT reagent added. At 570 nm, the absorbance was measured [26].

2.4 Assay for Hemolysis Using $Cu_{1.4}Mn_{1.6}O_4$ Nanoparticles

The hemolysis assay evaluated $Cu_{1.4}Mn_{1.6}O_4$ at different concentrations (50, 250, and 500 ppm) to determine the presence of hazardous or non-toxic substances. The blood sample was obtained from the laboratory, placed in an EDTA tube, observed under a microscope at a magnification of 100, and subsequently analyzed on a slide. Following the separation of blood cells and plasma in an EDTA tube, the mixture underwent centrifugation for 10 minutes. After removing the plasma layer from the cells, the cells were subjected to a ten-minute cycle of centrifugation, during which they were continuously washed with PBS and supplemented with 1 mL of PBS. The cells were extracted from the PBS after two minutes. After multiple rounds of washing, 1 mL and 9 mL of PBS were amalgamated to create the blood cell suspension. Each tube has a volume of 1200 µL for the antagonist, administered at varying concentrations. Three hundred microliters of the cell suspension are added to the last volume (1.5 ml). Each tube is incubated for two hours before being spun apart for five minutes at 1000 cycles per minute. Then, the difference in hemolysis was quantified using control conditions (test tubes containing blood and PBS, test tubes containing blood, and deionized water only). After centrifugation, the (+) option indicates the compound's toxicity when combined with blood components. The drug was safe, as indicated by the (-) option, which shows that the blood components were not mixed after centrifugation [27].

3 RESULTS AND DISCUSSION

3.1 Characterization of $Cu_{1.4}Mn_{1.6}O_4$

Nanoparticles by FTIR: Functional groups are examined by FTIR characterization. The FTIR spectrum was utilized to analyze the chemical bonding of $Cu_{1.4}Mn_{1.6}O_4$ nanoparticles, with a wavenumber range of 400 to 4000 cm-1. Absorption peaks are seen in Figure 1, including one at a wavenumber of 442.92 cm-1, corresponding to the metal's vibrational mode within a tetrahedral structure (Cu-O) [28]. The octahedral vibration mode (Mn-O) was seen with a wave number of 524.6 cm-1 [29].

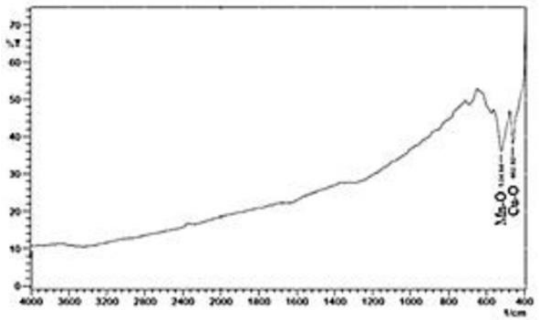


Figure 1: $Cu_{1.4}Mn_{1.6}O_4$ nanoparticles FTIR spectra.

3.2 Characterization of $Cu_{1.4}Mn_{1.6}O_4$ Nanoparticles by Dynamic Light Scattering (DLS)

The granular size of $Cu_{1.4}Mn_{1.6}O_4$ was assessed to evaluate the stability of the nanoparticles in colloidal solutions and their response to the solvent. Ionic water served as a solvent, and the particle size of $Cu_{1.4}Mn_{1.6}O_4$ was measured at 196.3 nm, as illustrated in Figure 2.

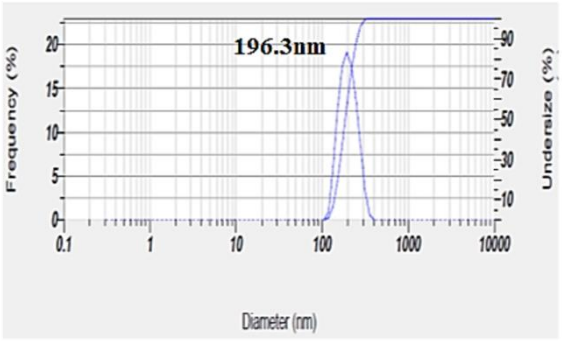


Figure 2: $Cu_{1.4}Mn_{1.6}O_4$ nanoparticles by dynamic light scattering (DLS).

3.3 Characterization of $Cu_{1.4}Mn_{1.6}O_4$ Nanoparticles by (XRD) and (EDX)

XRD was utilized to measure $Cu_{1.4}Mn_{1.6}O_4$ nanoparticles. The XRD measurements are presented in Figure 3. Among the several identified peaks at 2θ, ten are principal, with seven exhibiting sharpness and intensity, signifying a high level of $Cu_{1.4}Mn_{1.6}O_4$ crystallinity. The values of the peaks are 35.45, 37.74, 39.75, 48.34, 62.62, 65.62, 67.65, 69.82, and 77.97. The relative intensity data of the peaks on the XRD diagram aligns favourably with the parameters of $Cu_{1.4}Mn_{1.6}O_4$, as per ICCD card

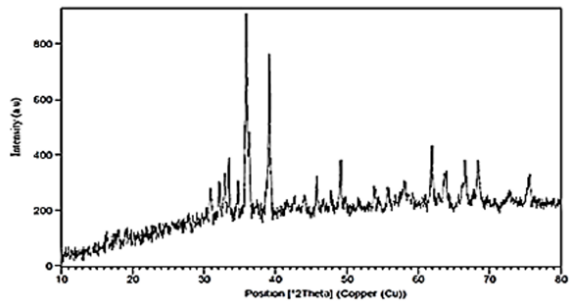


Figure 3: X-ray diffraction spectrum of $Cu_{1.4}Mn_{1.6}O_4$ nanoparticles.

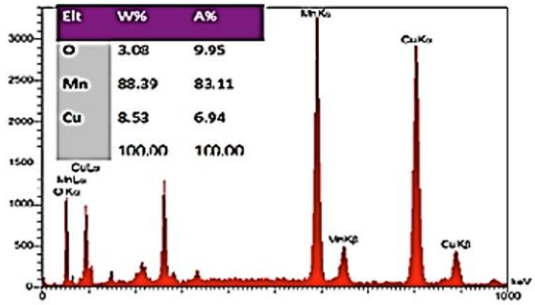


Figure 4: Energy dispersive X-rays (EDX) of $Cu_{1.4}Mn_{1.6}O_4$ nanoparticles.

standard No. (96-153-3678) and the cubic data of the Crystal system [30]. The average crystalline size was calculated to be 38.12 nm using the Debye-Scherrer equation. Energy-dispersive X-ray spectroscopy (EDX) was used to quantify $Cu_{1.4}Mn_{1.6}O_4$ synthesized utilizing green chemistry Figure 4. The results indicated the presence of copper at 36.9%, oxygen at 26.6%, and manganese at 36.5%, signifying a high purity level.

3.4 Characterization of $Cu_{1.4}Mn_{1.6}O_4$

Nanoparticles by SEM The morphological and structural compositions of $Cu_{1.4}Mn_{1.6}O_4$ nanoparticles synthesized through green chemistry were analyzed utilizing a scanning electron microscope (SEM). Figure 5 shows that the particles were prepared in the nanometer range. The SEM images demonstrated that most of the nanoparticles were adequately separated, although a portion was observed in an agglomerated state. The observed agglomeration results from electrostatic effects, with the particle diameter measuring approximately 103.60 nm [33].

3.5 Inhibition of $Cu_{1.4}Mn_{1.6}O_4$ Nanoparticles for PC3

The impact of green chemistry-prepared $Cu_{1.4}Mn_{1.6}O_4$ nanoparticles on the vitality of PC3 cell lines was investigated, and the drug Flutamide was compared. Following a 24-hour incubation period, the concentrations of the nanoparticles at 20, 40, 80, 160, and 320 ppm (the percentage of killing cancer cells, respectively) were 37.68%, 47.75%, and 63.38%. Figure 6 illustrates how $Cu_{1.4}Mn_{1.6}O_4$'s killing efficacy on cancer cells increases with concentration, reaching 87.07% and 98.00%. The results in 24 hours showed an IC50 value = 38.46, as in Figure 7.

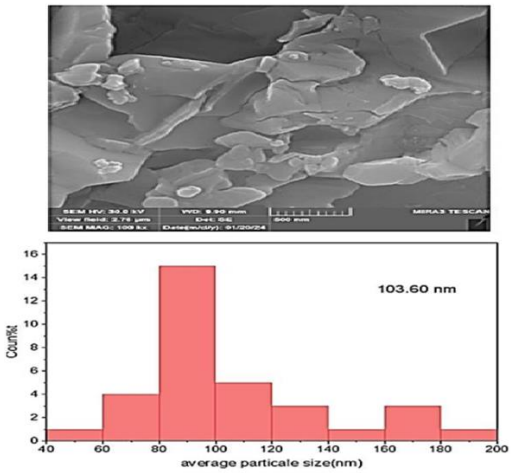


Figure 5: SEM of $Cu_{1.4}Mn_{1.6}O_4$ nanoparticles.

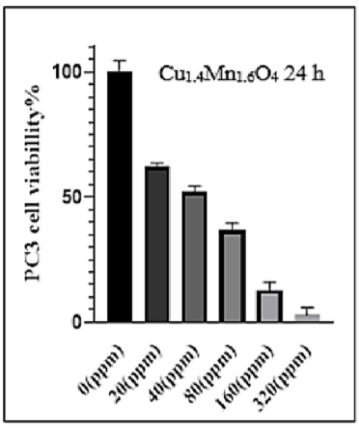


Figure 6: Inhibition of $Cu_{1.4}Mn_{1.6}O_4$ nanoparticles for PC3 in 24 h.

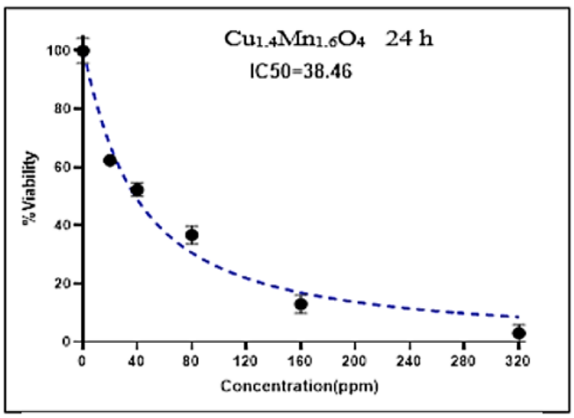


Figure 7: IC50 of $Cu_{1.4}Mn_{1.6}O_4$ nanoparticles for PC3 in 24 h.

The results of Flutamide after a 24-hour incubation at concentrations of 20, 40, 80, 160, and 320 ppm were as follows: the percentage of cancer cell mortality was 6.51%, 10.45%, 27.96%, 32.48%, and 52.53%, respectively. The lethality of $Cu_{1.4}Mn_{1.6}O_4$ nanoparticles surpasses that of Flutamide under the same doses and circumstances, as illustrated in Figure 8.

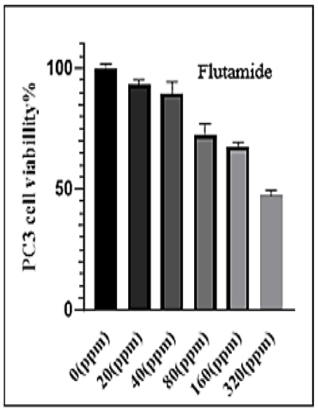


Figure 8: Inhibition of Flutamide for PC3 in 24 h.

The results showed an IC50 value of 296.8, as shown in Figure 9.

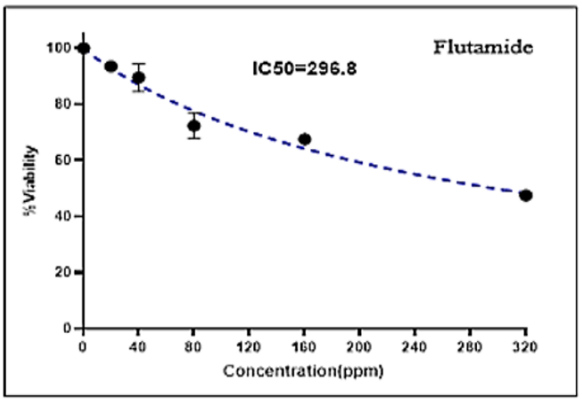


Figure 9: IC50 of Flutamide for PC3 in 24 h.

3.6. Toxicity Test of $Cu_{1.4}Mn_{1.6}O_4$ Nanoparticles on Blood Cells

Blood cells were used to investigate the cytotoxicity of $Cu_{1.4}Mn_{1.6}O_4$ Nanoparticles at 500, 250, and 50 ppm. The outcomes were compared to those of the medication Flutamide. As seen in Figure 10, the results indicated that $Cu_{1.4}Mn_{1.6}O_4$ Nanoparticles were not hazardous. However, as Figure 11 illustrates, the drug's toxicity was noted at the same concentrations as the control group.

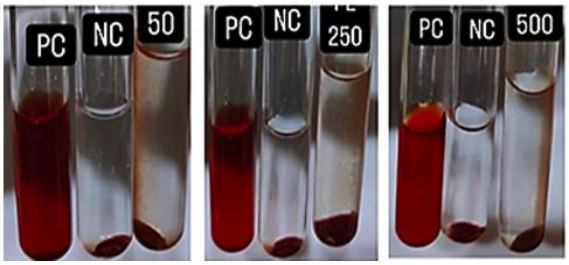


Figure 10: Hemolysis test for $Cu_{1.4}Mn_{1.6}O_4$ nanoparticle.

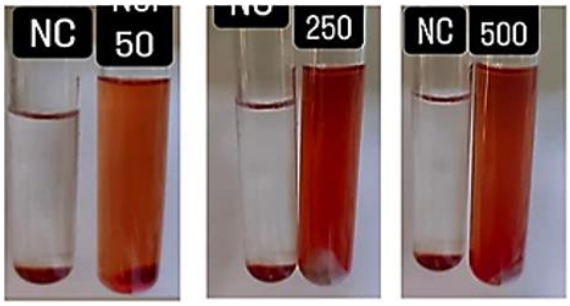


Figure 11: Hemolysis test for Flutamide nanoparticle.

Numerous studies have indicated that cellular absorption efficiency, concentrations, exposure length, and nanoparticle size may all influence the extent of NP-mediated cell death [34]. Recent data demonstrate that nanoparticles can eliminate tumour cells with minimal to no effect on the mortality of normal cells [35], [36]. Assessment of Apoptotic Evaluations Mitochondria serve as critical signalling nodes in the apoptotic pathway; thus, various apoptosis regulators may inhibit or inflict damage on mitochondria during apoptosis. Anti-apoptotic proteins, including Bcl-2 and Bcl-2 family proteins Bak and Bax, are particularly important for mitochondrial-mediated death. Additionally, extensive research has investigated the capacity of nanoparticles (NPs) to induce oxidative stress and subsequently generate reactive oxygen species (ROS) that can eliminate tumour cells [37], [38].

4 CONCLUSIONS

This work offers a fresh, reasonably priced approach for the synthesis of $Cu_{1.4}Mn_{1.6}O_4$ nanoparticles from Ginkgo biloba leaf extract. The method combines co-precipitation, green chemistry, and ultrasonic methods into one process for manufacturing nanoparticles. Using XRD, FT-IR, EDX, SEM, and DLS, among other methods, the produced nanoparticles were characterized. Although the average particle size shown in the SEM was 103.60 nm, the average size detected in the XRD was 38.12 nm. Measuring by DLS, the average particle size was 196.3 nm. On the PC3 cell line, the efficacy of the synthetic $Cu_{1.4}Mn_{1.6}O_4$ nanoparticles was assessed against the pharmacological Flutamide, used in Iraq for the therapy of prostate cancer. The findings showed the remarkable efficiency and quality of the created nanoparticles as compared to the given medicine. $Cu_{1.4}Mn_{1.6}O_4$NPs showed positive cytotoxicity against cancerous cells in the prostate. Apoptotic pathways can be triggered by treated cells. Moreover, the tumor suppressor gene p53's mRNA expression was increased by $Cu_{1.4}Mn_{1.6}O_4$NPs. The recently generated $Cu_{1.4}Mn_{1.6}O_4$NPs could be a major springboard for additional cancer treatment research. In contrast to chemotherapy, the present study's findings offer promise for stopping the spread of prostate cancer.

ACKNOWLEDGMENTS

The authors are grateful to the department for their valuable support during this study.

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Preparation of CdO Nanoparticles Using Two Different Salts and Investigation of their Antibacterial Activity

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Keywords: CdO Nanoparticles, Bacterial Resistance, Eco-Friendly Synthesis, Bioactive.

Abstract: This study reports synthesizing two types of CdO NPs using CdSO4 and CdCl2 salts as precursor materials and Petroselinum extracts as a reducing agent. The nanoparticles were characterized by XRD, TEM, FE-SEM, EDX, and DLS techniques. The results showed that the properties of the prepared CdO nanoparticles varied depending on the type of salt used as a precursor. The XRD confirmed that the two prepared CdO nanoparticles have particle diameters of 58.35 CdSO4 and for CdCl2 is 44.02 nm. FE-SEM micrographs confirmed that the obtained particles have different shapes and sizes, and the average particle diameters were 77.16 and 109.57 nm for CdO nanoparticles from CdSO4 and CdCl2, respectively. The CdO nanoparticles that obtained from CdSO4 have a narrow particle size distribution was 265.6, in contrast, in the case of CdCl2, the distribution of the particles was 356.3 nm with a broad peak. Anti-bacterial evaluation of the prepared CdO exhibits revealed a significant inhibition against four types of bacteria Staphylococcus aureus, Streptococcus pneumonia, Klebsiella pneumonia, and Escherichia coli.

1 INTRODUCTION

Nanotechnology is a branch that is applied in many fields, such as biology, physics, chemistry, and engineering, due to their special physicochemical properties [1, 2]. The metallic nanoparticles have applications in many areas, specifically cosmetics, coating, electronics, packaging, and biotechnology, and enormous potential for their use in humans [3, 4]. The antibacterial agents were used extensively, which generates pressure to escalate rates of antibiotic resistance [5]. However, new resistant bacterial strains to current antibiotics have become a serious issue for public health, which has awakened the need to find new bactericidal agents [6]. Oxide nanoparticles with heavy metals such as Cd can eliminate cancer cells at a low concentration and are non-toxic to mammalian cells [7], [8] . Nanoparticles are being studied more deeply, and several therapeutic modulations of nanoformulations have been created as a new brand perspective for cancer treatment [9].

However, the synthesis of new compounds like nanoparticles with anti-microbial properties is essential, with potential applications which can help fight antibiotic resistance towards pathogenic bacteria [10]. Nanoparticles can be used in small amount of cadmium oxide as medicines with tiny sizes [11], [12], [13], [14], [15]. Scine, the synthesis of NPs has been a matter of concern because chemical methods are toxic. On the contrary, biological methods are considered eco-friendly. Whoever, many reports have confirmed that plant extracts can be used as reducing and capping agents in forming NPs since they contain various phytocompounds: phenols, alkaloids, and terpenoids. So, the aim of this study is to use two types of salts the CdSO4 and CdCl2 as precursor materials to synthesize the cadmium oxide nanoparticles (CdO NPs) with using leaf extract petroselinum crispum as a reducing agent and investigate their effect on the selected pathogenic bacteria (Staphylococcus aureus, Streptococcus pneumonia, Klebsiella pneumonia and Escherichia coli).

2 MATERIALS AND METHODS

Petroselinum crispum leaves were collected from the local gardens at Diyala governorate in Iraq. All chemicals involved in the experiments are reagent grade. Cadmium sulfate (CdSO4), Cadmium chloride (CdCl2), and Sodium hydroxide were purchased from Sigma Aldrich and BDH. Deionized water was used for the experiment. The anti-bacterial test includes Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, and Klebsiella pneumonia, obtained from Baqubah General Hospital, Diyala, Iraq.

2.1 Preparation of the Plant Extract

The green reducing and stabilizing agent was prepared from the petroselinum crispum leaves which were collected from the Diyala governorate gardens in Iraq. The leaves were washed using deionized water to remove impurities, and then cut into small pieces. 25 g of cut leaves were put in 100 mL of deionized water and set the temperature at 80 °C. After some time, the water turned greenish, indicating leaf extract formation in the water. The filtered extract was transferred into the burette to be used as a reducing agent for cadmium oxide nanoparticle synthesis, Figure 1.



Figure 1: Synthesis of leaves extract petroselinum crispum.

2.2 Synthesis of Cadmium Oxide Nanoparticles

Cadmium sulfate (CdSO4) and cadmium chloride (CdCl2) salt solutions were prepared using deionized water. Leaves extract petroselinum crispum was added dropwise, and sodium hydroxide solution was introduced slowly to the solution under a stirrer until the pH=12. After 2 hours, the obtained precipitates were filtered using a Buckner funnel, washed with distilled water several times, and dried in an oven for 2 hours. Then, calcination was carried out in the furnace at 600 ºC for 6 hours to remove any contamination [16]. As shown in Figure 2.

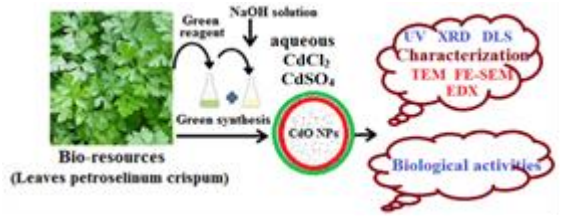


Figure 2: Synthesis of CdO-NPs using CdSO4 and CdCl2 salts as a sources of cadmium metal by petroselinum crispum leaves extract [17].

2.3 Characterization

The characterization of CdO NPs was done using several techniques, including Fourier transform infrared (FTIR) spectroscopy in the range of 400–4000 cm-1 for functional groups. Field Emission Scanning Electron Microscopes (FE-SEM) and Transmission Electron Microscopes (TEM) were utilized to examine the CdO nanoparticles morphology. Energy-dispersive X-ray spectroscopy (EDX) was used to study the FE-SEM micrographs and for elemental mapping. X-ray diffraction (XRD) was utilized to calculate the average crystalline size, and DLS technique was used to examine the surface area, pore size, and distribution of CdO NPs, respectively.

3 RESULTS AND DISCUSSION

3.1 XRD Analysis

Figure 3 exhibits the XRD patterns for the prepared CdO, from which the intensity and quality of XRD peaks vary depending on the salt that used and the variation of the precursor; the size and morphology of NPs also vary. The observed diffraction patterns showed strong reflections from the (111), (200), (220), (311), and (222) planes, which correspond to the face-centered-cubic (FCC) consistent with Monteponite structures of CdO NPs (from CdSO4 salt) and CdO NPs (from CdCl2 salt), with the purity of CdO NPs (from CdCl2 salt) which very well as compare to CdO NPs (from CdSO4 salt). The prepared cubic phase of the polycrystalline Monteponite CdO (JCPDS card number 050640) is agreed with previous literatures [18]. The crystallite size (D) at the highest reflection plane (111) for the prepared CdO nanostructures is calculated following Debye–Scherrer's equation:

$$D = \frac{\kappa * \lambda}{\beta \cos\theta} . \quad (1)$$

Where λ is x-ray wavelength, K corresponds to the shape constant (0.9), θ is Bragg angle, and β the full width height maximum (FWHM) of the diffracted peak.

The average size for the CdO nanoparticles was 55.36 nm and 45.69 nm for CdO NPs (from CdSO4 salt) and CdO NPs (from CdCl2 salt), respectively. The lattice parameters are listed in Table 1. Lattice parameter (a) was calculated using the relation:

$$a = d_{hkl}\sqrt{h2 + k2 + l2}\,, \qquad (2)$$

d_{hkl} is the interplanar spacing, and h, k, and l are the miller indices.

Table 1. The indexing XRD of two types CdO nanoparticles.

Nanomaterials	Plane	d_{111}(A°)	a (A°)	D, nm
CdO from $CdSO_4$ salt	(111)	2.6937	4.6656	58.35
CdO NPs from $CdCl_2$ salt	(111)	2.6903	4.6598	44.02

3.2 FE-SEM Micrographs

The surface morphology for the synthesized nanoparticles was observed using a scanning electron microscope. Figure 4 (a and b) shows the surface morphology of the CdO (from $CdSO_4$ salt) and CdO (from $CdCl_2$ salt) nanoparticles respectively, in which the nanoparticles are found to be cylindrical in shape with the average size of nanoparticles at 77.16 nm, see Figure 5a. Figure 5b exhibits CdO NPs (from $CdCl_2$ salt) as spherical shape nanoparticles with the average size of nanoparticles at 109.57 nm. The results confirm the effect of the anion associated with cadmium metal is very clear on the size and shape of the CdO nanoparticles since the different anions gave agglomeration of the nanoparticles and could be related to van der Waals forces among.

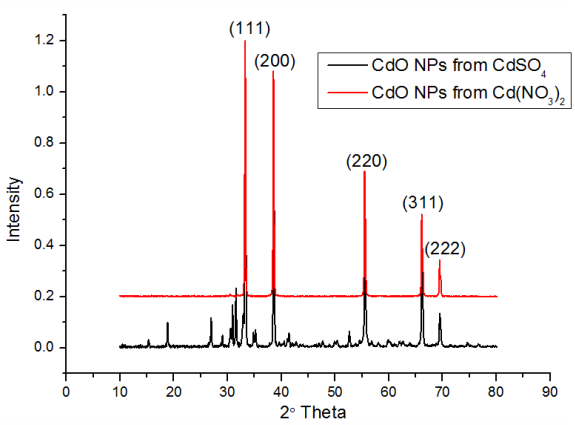
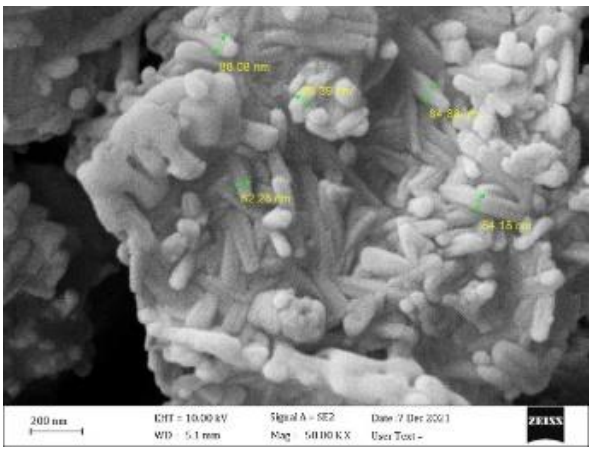


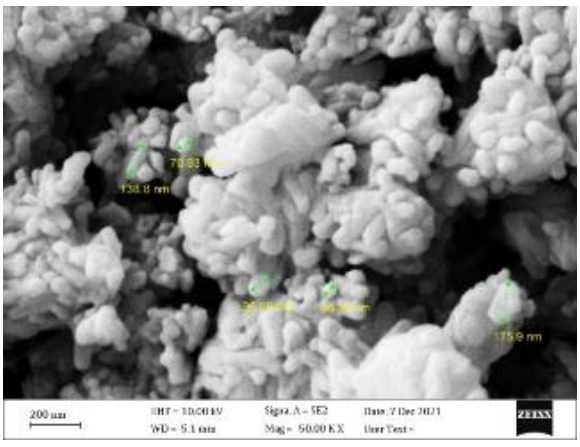
Figure 3: XRD patterns of CdO NPs from CdCl2 salt and CdO NPs from CdSO4 salt.

3.3 EDX Analysis

The Energy-dispersive X-ray (EDX) spectra show the components in the prepared samples, in which the fabricated samples are CdO nanoparticles. The EDS result in Figure 5 (a and b) shows the presence of Cd and O (Cd: 90% and O: 10%) for both samples.



(a)



(b)

Figure 4: FE-SEM images of CdO nanoparticles, a) CdO NPs from CdSO4 salt, and b) CdO NPs from CdCl2 salt.

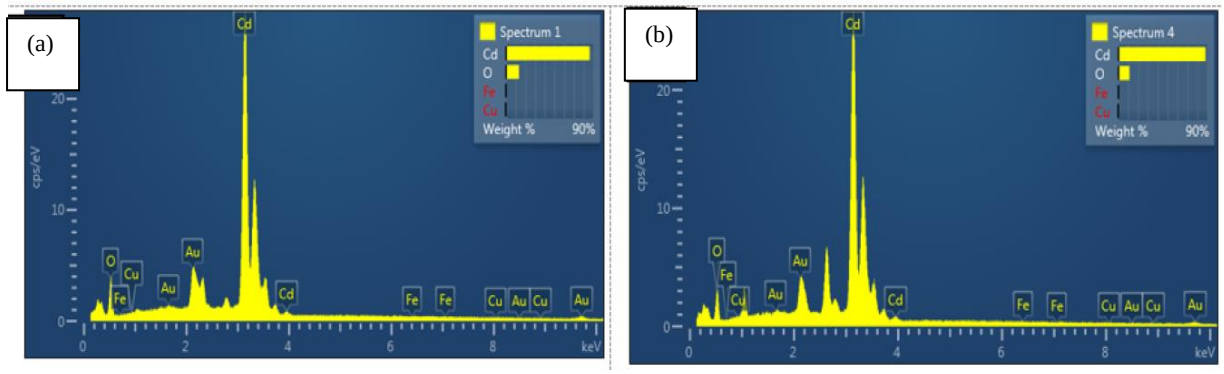


Figure 5: EDS images of CdO nanoparticles, a) CdO NPs from CdSO4 salt, and b) CdO NPs from CdCl2 salt.

3.4 TEM Image

The images of TEM for CdO nanoparticles are illustrated in Figures 6 (a and b). The formation of CdO nanoparticles was prepared by the Eco-Friendly method. The diameter was confirmed using TEM micrographs (28.83 nm to 37.2) nm for CdO NPs from CdSO4 salt and (27 nm to 36 nm) for CdO NPs from CdCl2 salt.

3.5 Dynamic Light Scattering (DLS)

The distribution function analysis of the suspension system was done using the Rayleigh-Debye model. The particle size distribution curves of CdO (from CdSO4 salt) and (from CdCl2 salt) nanoparticles are shown in Figure 7 (a and b). In Figure 7a, the dynamic light scattering (DLS) data for CdO NPs (from CdSO4 salt) gave one narrow peak with a particles distribution 265.6 nm [19]. In this case, CdO NPs (from CdCl2 salt) has one broad peak with a higher particles distribution 356.3 nm, Figure 7b. Increasing the average particle size is due to the anion-type effect, in which all particles have different sizes. The mean particle size increase can also be attributed to the agglomeration of the smaller particles.

Table 2: Anti-bacterial activity towards Staphylococcus Aureus, Staphylococcus Pneumonia, Escherichia Coli, and Klebsiella Pneumonia (50 and 100 mg/mL), Demeter (mm).

Sample	*S. Aureus*		*S.Pneumonia*		*E. coli*		*K.Pneumonia*	
Concentration (mg/mL)	50	100	50	100	50	100	50	100
CdONPs from $CdSO_4$ salt	18	23	22	24	21	23	20	22
CdONPs from $CdCl_2$ salt	20	23	21	24	18	20	19	21
Amoxicillin	20	21	R	R	R	10	R	11
DMSO	0	0	0	0	0	0	0	0

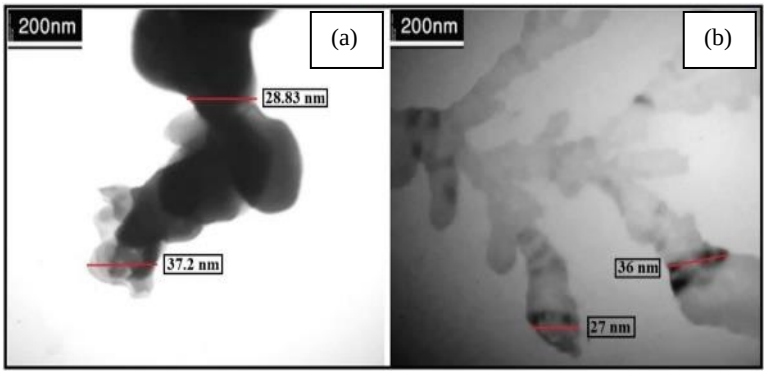


Figure 6: TEM images of CdO nanoparticles, a) CdO NPs from CdSO4, and b) CdO NPs from CdCl2.

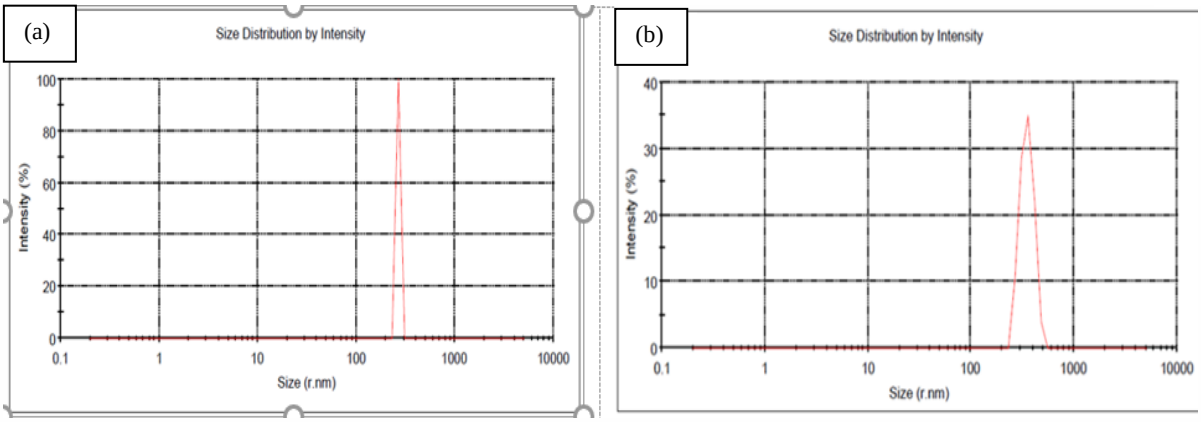


Figure 7: DLS analysis of CdO nanoparticles, a) CdO NPs from CdSO4 salt, b) CdO NPs from CdCl2 salt.

3.6 Antibacterial Activity

The anti-bacterial activities for the synthesized nanoparticles CdO NPs (from CdSO4 and CdCl2) have tested using disk diffusion method and the inhibition zone was measured in mm [20]. Amoxicillin (50 and 100 mg/mL) was used as a reference drug for the test. The synthesized compounds were evaluated for their anti-bacterial activity against the Staphylococcus aureus and Streptococcus pneumonia, Klebsiella Pneumonia, and Escherichia coli for their concentrations (50 and 100 mg/mL) in Muller Hinton agar. The sterilized agar media were poured into Petri dishes and allowed to solidify, and the media microbial suspension were spread on the surface using loop. DMSO was used as a solvent for the cultures. The resulted plates were put in the incubator at 37 oC for 48 hours. The results are provided in Table 2. As seen from Table 2, the antibacterial activity of the test compounds gave excellent activity. The prepared compounds show activity against S. aureus is nearly close to that of the reference in the 50 and 100 mg/mL concentrations. The activities were excellent against S. pneumonia for 50 and 100 mg/Ml. Furthermore, same activity was observed against E. coli and K. pneumonia for both concentrations of 50 and 100 mg/mL since the drug gave resistance to moderate activity against them.

4 CONCLUSIONS

Two CdO nanoparticles have been synthesized using a simple, non-cost-effective, eco-friendly method using two different precursor salts's CdSO4 and CdCl2. The result clearly indicates that the properties of the prepared compounds varied and depended on the type of salt used as a precursor. The formations of two CdO nanoparticles were confirmed using XRD, FE-SEM micrographs, TEM, and DLS. However, the XRD analysis showed that the average size for the CdO nanoparticles was 55.36 nm and 45.69 nm for CdO NPs obtained from CdSO4 and CdCl2, respectively. In addition, the FE-SEM image indicates that the nanoparticles of CdSO4 are found to be cylindrical in shape with an average size of nanoparticles at 77.16 nm, and of CdCl2 found to be spherical in shape with an average size of nanoparticles at 109.57 nm.

Anti-bacterial evaluation of the prepared CdO exhibits a significant inhibition against the four types of bacteria Staphylococcus aureus, Streptococcus pneumonia, Klebsiella pneumonia, and Escherichia coli, compared to the reference.

ACKNOWLEDGEMENTS

The authors appreciate the College of Education for Pure Sciences - University of Diyala - for using laboratories with the necessary equipment to carry out the experiment.

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Synthesis and Characterization of Reduced Graphene Oxide Iron Oxide Tin Oxide Nanocomposites for Biological Applications in Water Treatment

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Keywords: Nanocomposites, Tin Dioxide, Biological Activity, Microorganisms.

Abstract: In this study, the synthesis, characterization, and biological applications of rGO/Fe_3O_4/SnO_2 nanocomposites are discussed for water treatment. The synthesis was conducted via a modified Hummer's method to obtain reduced graphene oxide (rGO), followed by the incorporation of Fe_3O_4 and SnO_2 nanoparticles through a facile co-precipitation process. Various characterization techniques, including Fourier-transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM), and energy-dispersive X-ray spectroscopy (EDX), confirmed successful formation and structural integrity of the nanocomposites. The morphological analysis revealed uniform dispersion of Fe_3O_4 and SnO_2 on the rGO sheets. Biological assays demonstrated significant antimicrobial activity against both Gram-positive and Gram-negative bacterial strains, as well as selected fungal species. Notably, enhanced inhibition was observed against Gram-positive bacteria, which may be due to the synergistic interaction between the components of the nanocomposite. Overall, the study highlights the potential of rGO/Fe_3O_4/SnO_2 nanocomposites as promising candidates for sustainable and effective water purification systems, targeting microbial contamination.

1 INTRODUCTION

Nanocomposites of rGO, Fe_3O_4, and SnO_2, among many others have been prioritized. This is viewed as convincing evidence of their increasing place in modern nanotechnological applications regarding environmental remediation, especially around water treatment [1], [2]. Indeed, in microbial contamination, these materials hold much promise when considered for use in problems regarding public health [3]. The rGO/Fe_3O_4/SnO_2 nanocomposite has a synergetic combination of properties enhancing its biological activity. SnO2 offers a source of enhanced antimicrobial activity because it can efficiently produce reactive oxygen species (ROS) [4]. These ROS can effectively damage the microorganism's cell wall and cellular-internal components upon irradiation with light. Meanwhile, rGO provides a higher surface area and ease of adsorption of microbial contamination [5], [6]. Fe_3O_4 is responsible for its magnetic property, which can make recovery easy from an aqueous system. SnO_2 is also well-known to be highly antimicrobial because it can effectively cause the generation of reactive oxygen species (ROS) [7]. The biological activity of the rGO/Fe_3O_4/SnO_2 nanocomposite sets it apart in wide applicability against different microorganisms like bacteria and fungi responsible for contamination; hence, it provides lower risks of waterborne infections through the control of bacterial and fungal contamination [8], [9]. This study reports the synthesis characterizations of rGO/Fe_3O_4/SnO_2 nanocomposite and their evaluation of biological activity applicable to water treatment [10]. The work will be carried out in pursuit of addressing microbial contamination which is still one of the major challenges toward ensuring accessibility to clean drinking water [11].

2 EXPERIMENTAL PART

The rGO/Fe_3O_4/SnO_2 nanocomposite was synthesized using Hummer's method, the nanocomposite was synthesized using the co-precipitation method [12].

3 RESULTS AND DISCUSSION

3.1 Characterization of rGO/Fe_3O_4/SnO_2

The FT-IR, XRD, FESEM, and EDX were used for the characterization of the synthesized samples. FT-IR spectra have confirmed the formation of nanoparticles of material composites. Surface morphology and nanoparticle size were determined by FESEM. The characteristics of the rGO/Fe_3O_4/SnO_2 nanocomposite powder used in this work were studied by XRD, and qualitative analysis was done by EDX to establish the elemental composition of the samples.

3.2 FT-IR Spectrum of rGO/Fe_3O_4/SnO_2

The IR spectrum of the rGO/Fe_3O_4/SnO_2 composite presents some most salient peaks which can be used to affirm the successful synthesis and incorporation of rGO, Fe_3O_4, and SnO_2. The broad peak centered at 3424 cm^{-1} is associated with O-H stretching vibrations, pointing towards adsorbed water molecules or hydroxyl groups on the substance surface. A peak located at 1610 cm^{-1} gives the stretching vibrations for C=C, thus conformity to the presence of sp^2-hybridized carbon domains in rGO. Peaks near 1444 cm^{-1} may relate to bending vibrations of functional groups on the composite surface. The peak at 1151 cm^{-1} is associated with C-O stretching, indicating residual oxygen-containing groups on the rGO surface. The vibrational bands in the range of 800–400 cm^{-1} confirm the existence of Sn-O and Fe-O bonds. Notably, the band around 606 cm^{-1} can be attributed to Fe-O vibrations within the Fe_3O_4 lattice, while the peak at 412 cm^{-1} is characteristic of Sn-O stretching vibrations in SnO_2 as shown in Figure 1.

3.3 FESEM of rGO/Fe_3O_4/SnO_2

The FESEM micrograph shows the nanoscale morphology of an rGO/Fe_3O_4/SnO_2 composite material. The sheet-like structure of rGO is evident, serving as a conductive matrix, while Fe_3O_4 and SnO_2 nanoparticles are distributed on their surface. The marked measurement (43.46 nm) confirms the nanoscale features, essential for enhanced performance in applications such as energy storage or sensing. The smooth rGO sheets indicate an effective reduction, while irregularities suggest successful nanoparticle deposition. This composite demonstrates a well-engineered structure with potential for advanced technological applications as shown in Figure 2.

3.4 XRD Analysis of rGO/Fe_3O_4/SnO_2

The analysis of XRD data for the rGO/Fe_3O_4/SnO_2 composite confirms the presence of its key phases. The broad peak at 20.5251° indicates the amorphous nature of rGO due to structural defects. The peaks at 26.8778°, 34.2140°, and 52.1276° confirm the well-crystallized rutile tetragonal phase of SnO_2. Additionally, peaks at 31.2697° and 38.2429° validate the cubic spinel structure of Fe_3O_4. This combination of phases demonstrates the successful synthesis of the composite as shown in Figure 3.

3.5 EDX Analysis of rGO/Fe_3O_4/SnO_2

The EDX data confirms the composition of the rGO/Fe_3O_4/SnO_2 composite, showing contributions from carbon (C) primarily from rGO, oxygen (O) from Fe_3O_4 and SnO_2, iron (Fe) from Fe_3O_4, and tin (Sn) from SnO_2. The weight percentages sum to 100%, with carbon being the most abundant element by atomic percentage, indicating the prominence of rGO. The standards are used to ensure accurate calibration. The composition aligns with the expected properties of the composite, where rGO improves conductivity, Fe_3O_4 adds magnetic properties, and SnO_2 contributes to catalytic or sensing functions as shown in Table 1 and Figure 4.

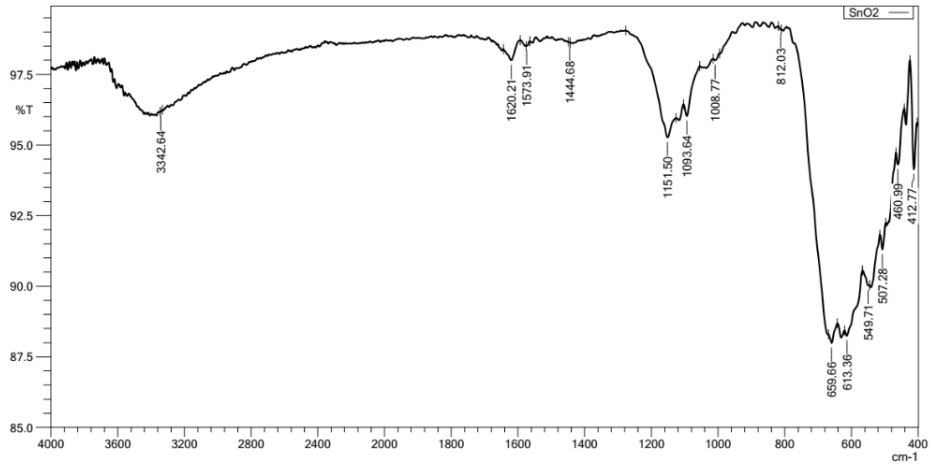


Figure 1: FTIR chart for rGO/Fe_3O_4/SnO_2.

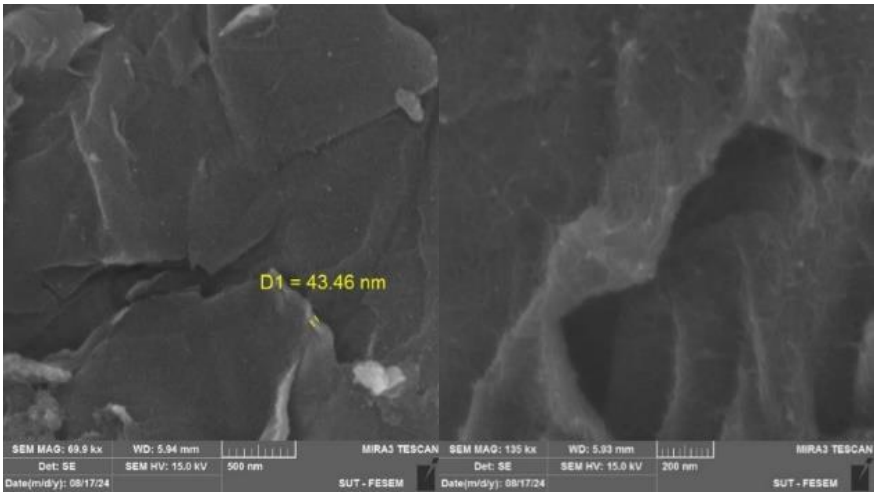


Figure 2: FESEM of rGO/Fe_3O_4/SnO_2.

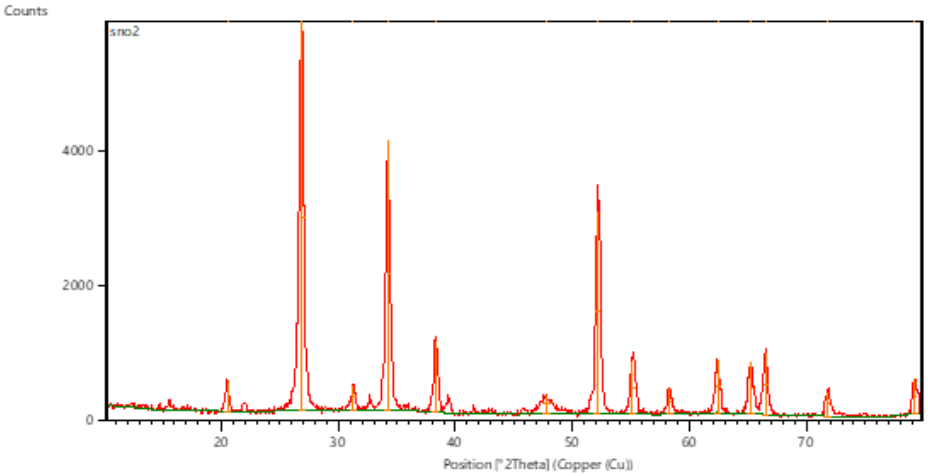


Figure 3. XRD analysis of rGO/Fe_3O_4/SnO_2.

Table 1: EDX analysis of rGO/Fe_3O_4/SnO_2.

Element	Line Type	Apparent Concentration	k Ratio	Wt%	Wt% Sigma	Atomic %
C	K series	1.38	0.0138	29.06	0.30	56.68
O	K series	1.70	0.0057	19.29	0.26	28.25
Fe	K series	2.60	0.026	21.96	0.28	9.21
Sn	L series	3.05	0.031	29.69	0.28	5.86
Total:				100.00		100.00

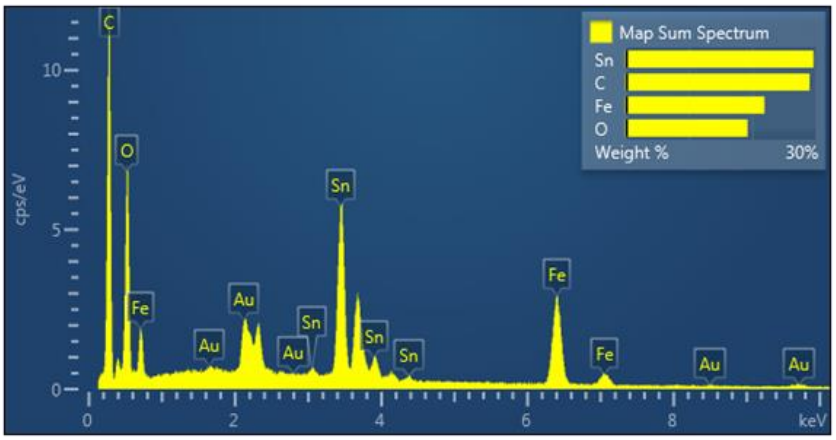


Figure 4: EDX analysis of rGO/Fe_3O_4/SnO_2.

4 BIOLOGICAL ACTIVITY

4.1 Identified Pathogens in Tannery Wastewater

Tannery wastewater is a major public health concern. To control contamination risks, it is necessary that appropriate treatment and monitoring conditions are maintained [13]. For isolation of Aspergillus spp., the principle includes the plating of the samples on PDA, a medium rich in nutrients and supportive to the growth of fungi. After incubation at 25°C for 7 days, the colonies of the fungi are assessed based on their microscopic appearances of colors, smooth or rough textures, and patterns of sporulation. The identity of the species is confirmed based on molecular and microscopic techniques, and thus more accuracy is ensured. Agar well diffusion sensitivity testing of bacteria are used to assess the efficacy of the extract. Bacteria of the order of magnitude of 1.0 x 10^8 cells per milliliter are spread onto Mueller Hinton Agar, and the wells are filled with concentrations of the extract and positive control. The plates are then incubated at 37 degrees Celsius for a period of 18-24 hours, after which time the zones of inhibition are measured so that the antimicrobial activity can be determined. Bacillus spp. from tannery effluents becomes a major health hazard with bacterial counts ranging from 10^6 to 10^9 CFU/mL. Toxins produced by pathogenic strain B. cereus cause gut-related infections, skin infections, and in rare cases, septicemia. There is a need for proper monitoring and treatment of waste to mitigate this potential risk.

Pseudomonas aeruginosa is isolated on Pseudomonas agar, which is a selective medium for adjustment to pH 7.0 ± 0.2. After 24-48 hours of incubation at 35-37°C, the bacteria metabolize and raise the pH, turning the color of agar to purple. This is only a preliminary indicator for P. aeruginosa. But, much more important is that some biochemical testing needs to be done for further confirmation. Biofilm formation by this pathogen proves to be rampant in the resistance of treatment, an aspect that calls for a very healthy monitoring system as well as wastewater management. Health problems associated with bacterial contamination of tannery wastewater are dire at bacterial concentrations within the range of 10^6 and 10^9 CFU/mL. Streptococcus pyogenes is quite dangerous; it attacks the skin and respiratory system leading to necrotizing fasciitis plus Streptococcal Toxic Shock Syndrome (STSS). This risk must be minimized via proper practices in wastewater treatment procedures.

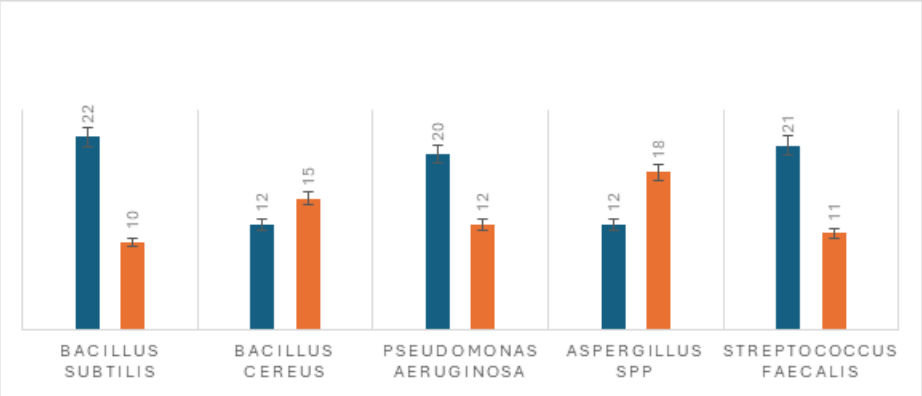


Figure 5: Antimicrobial activity of rGO/Fe_3O_4/SnO_2 nanocomposite against selected microorganisms.

Pseudomonas aeruginosa has an immense effect on the effluents from tanneries at concentrations of 500 CFU/mL. These concentrations are relatively high and permit it to cause skin infection, respiratory condition, systemic infection, especially in immunocompromised people. Biofilm makes it hard to treat; hence, this gives a quick call for good waste control. Aspergillus spp. has a serious pathogenic health effect in tannery wastewater, considered as a potent mycotoxin producer of aflatoxin B1. High concentrations of fungi in contaminated water pose an increased risk of respiratory reaction, asthma, allergy, and aspergillosis to humans; skin disease and systemic toxicity can also result from exposure. Effective monitoring of the fungi and strong waste treatment are found to be very important to keep contamination diseases away. Stronger waste management is very important to reduce contamination risks, better public health, and brown environmental conditions [14].

4.2 Effect of rGO/Fe_3O_4/SnO_2 Nanocomposite on Various Microorganisms

The activity of rGO/Fe_3O_4/SnO_2 nanocomposite against antimicrobial proves that the nanocomposite caused differential growth of microorganisms. A minimum inhibitory concentration (MIC) of 10 mg/mL and an inhibition zone of 22 mm indicate that SnO_2 has moderate susceptibility towards a Gram-positive bacterium, Bacillus subtilis. SnO2 nanoparticles have low MIC at 11 mg/mL and moderate susceptibility (with an inhibition zone of 21 mm) to Streptococcus faecalis. The difference in their sensitivity is also manifested by Bacillus cereus fringe inhibition values of 12 mm and the highest MIC of 15 mg/mL, respectively, thereby indicating much higher resistance compared to B. subtilis and S. faecalis. P. aeruginosa, being a Gram-negative bacterium, has slightly less sensitivity and an MIC of 12 mg/mL with a 20-mm inhibition zone probably as a response to the difficulty in the passage of nanoparticles to the other side, which is regulated by the existence of an outer membrane. Typical of Aspergillus spp. It had no sensitivity, only an inhibition zone of 12 mm and a high MIC at 18 mg/mL. This indicates the complex composition of the fungal cell wall, which does not allow nanoparticles to be effective. Overall, rGO/Fe_3O_4/SnO_2 nanocomposite appears more active, primarily against Gram-positive bacteria, such as Bacillus subtilis and Streptococcus faecalis, but much less active against Gram-negative bacteria and fungi. Such results indicate that SnO_2 nanoparticles hold promise for infections with Gram-positive bacteria and require in-depth further optimization for effective applications against other microbes [10] as shown in Figure 5.

5 CONCLUSIONS

The synthesis and characterization of rGO/Fe3O4/SnO2 nanocomposites were conducted successfully; hence, they show exciting potential for biological applications in water treatment. These composites provide a unique combination of the properties of reduced graphene oxide, magnetic iron oxide, and tin dioxide to ensure that microbial contamination, especially Gram-positive bacteria, is successfully dealt with. Results of this study confirmed the strong antimicrobial activity of the composite, characterized by clear inhibition zones and low MIC values in the challenge pathogens therefore qualifies it for use in reducing health risks

associated with contaminated water. Though showing good antimicrobial behavior against bacteria, the low activity observed against fungi and Gram-negative bacteria opens more avenues for optimization in the future. In sum, this work highlights the sustainability and dependability of $rGO/Fe_3O_4/SnO_2$ nanocomposites as a multifunctional implement for environmental remediation on the part of the broader imperative of access to clean and safe water.

ACKNOWLEDGMENTS

The authors thank the Department of Chemistry, College of Education for Pure Sciences/Ibn Al-Haitham, University of Baghdad, for providing chemicals and support.

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Synthesis and Evaluation of New 1,3,4-Thiadiazole-Benzimidazole Hybrids as Potent Antibacterial Agents

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Keywords: Benzimidazole, 1,3,4-Thiadiazole, Antibacterial Activity, O-Phenylendiamine.

Abstract: Owing to the rapid rise in antibiotic resistance, infectious diseases have become a serious threat to public health. There is an urgent need to develop new antimicrobial agents with diverse chemical structures and novel mechanisms of action to overcome resistance. In recent years 1,3,4-Thiadiazole benzimidazole hybrids have emerged as a new class of antimicrobial agents. In the current study, we designed and synthesized six new 1,3,4-Thiadiazole-benzimidazole hybrids using nucleophilic substitution reaction between 2-mercapto-1H-benzimidazole (A) with Ethyl chloroacetate to produce compound (B), which then reacted with various 2-Amino-5-(substituted phenyl)-1,3,4-thiadiazole(C1-C6) to yield target compounds (D1-D6) and evaluated them for their antimicrobial activity. Synthesized compounds were characterized and elucidated by IR, ^{1}H and ^{13}C-NMR, spectra. Subsequently, the antibacterial activity of the final target compounds (D1-D6) was examined in vitro against four types of bacterial isolates: two gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*). Compounds (D4 and D6) demonstrated promising pharmacological activity in vitro when compared to the standard antibacterial agent Tetracycline.

1 INTRODUCTION

The alarming rise of difficult-to-treat multidrug-resistant bacterial infections has emerged as one of the formidable challenges of the 21st century [1]–[5]. The World Health Organization (WHO) has categorised 12 pathogens as high-risk priority II pathogens that require immediate attention [6]. In particular, S. aureus is known to cause a wide range of diseases, from skin infections to serious illnesses like pneumonia, meningitis, bacteraemia, and sepsis [7]–[9]. Among the various resistant strains, methicillin-resistant (MRSA) and vancomycin-resistant (VRSA) strains are known to be sources of the spread of community infections.

Most benzimidazole derivatives prepared have a wide range of applications as medicinal agents in drug discovery [10]. Thus, benzimidazoles and their condensed systems have attracted much attention as bioconjugated heterocyclic nitrogen systems, which exhibit significant activity against some viruses, such as influenza, [11] herpes (HSV-1), [12] HIV, [13] and human cytomegalovirus (HCMV) [10]. Additionally, they are used as antitumor agents, [14] antimicrobials, [15] strong antiproliferative and DNA binding agents, [16] as well as DNA gyrase inhibitory activities, [17] and antitubercular agents [18]. Recently, most of the heterobicyclic nitrogen systems bearing or containing benzimidazole derivatives have exhibited a wide range of biological and medical properties such as antifungal, [19] antimicrobial, [20] cytotoxicity, [21] antiproliferative, [22] antituberculosis, [23] sulfonamide analogs, [24] and anticancer and antimycobacterial activities [25].

1,3,4-Thiadiazoles represent a prevalent and important class of compounds that exhibit diverse pharmacological activities such as antibacterial, anticancer, antitubercular, and anti-inflammatory properties [26]–[28]. For example, acetazolamide, [29] which contains the 1,3,4-thiadiazole structure motif, has been approved as a diuretic, whereas cefazedone has shown a broad spectrum of antibacterial activity against various pathogens [30]. In addition, another 1,3,4-thiadiazole derivative, BAS0338872, has also been reported as a potent Src/Abl tyrosine kinase inhibitor for the potential treatment of chronic myeloid leukemia [31].

Based on these observations and the search for new highly bioactive compounds, this work reports on the synthesis of new 1,3,4-thiadiazole-benzimidazole hybrids as potent antibacterial agents.

2 EXPERIMENTAL

All starting materials and solvents were purchased from Aldrich and used without purification .Melting points were recorded by Stuart smp3 electronic apparatus and are uncorrected, The FT-IR spectra were recorded on SHIMADZU model FT-IR -8400S, 1H and 13C -NMR spectra were recorded on BRUKER model Ultra shield 500 MHz spectrophotometer using DMSO-d6 as a solvent and TMS as an internal reference. The compounds were evaluated for their purity on silica gel TLC plates and the visualization of spots achieved by UV light:

A: Synthesis of 2-mercaptobenzimidazole.

A mixture of (20 mmol) of *O*-phenylendiamine, (20 mmol) of carbon disulphide, (20 mmol) of potassium hydroxide, (25 mL) of absolute ethanol and (5 mL) of water heated under reflux in 100 ml round bottom flask for 3 hours, then added cautiously (0.5 g) of charcoal and the reflux continued for 10 minutes, then charcoal was removed by filtration and (25 mL) of warm water added after heating the filtrate to 60-70 °C, and then acidified with dilute acidic acid with vigorous stirring. The mixture obtained placed in ice path for 3 hours to complete the crystallization, the product obtained was filtrated, dried and recrystallized from ethanol, the completion of the reaction and the purity of the compounds were checked by TLC (mobile phase: hexane: ethyl acetate (1:1)).

Light beige powder, yield (85%), m.p 305-307 °C, Rf = 0.56; FT-IR (KBr disk, cm-1) 3387 (N-H), 3153 (C-H, aromatic), 2572 (S-H), 1652 (C=N), 1513 and 1467 (C=C, aromatic). 1H NMR (DMSO-d6, 500 MHz, δ)12.58 (s, 1H, benzimidazole-NH),12 .15-12.42 (s, 1H, SH),7.13-7.45 (d,4H, aromatic ring). 13C-NMR (DMSO-d6, 125 MHz, δ). 168.2 (C=N). 115.2-123.0 (aromatic rings):

B: Synthesis of Ethyl-1H-benzo[d]imidazol-2 yl)thio) acetate.

A mixture of (0.03 mole) of 2-mercapto-1Hbenzimidazole A, ((70 ml) of ethanol, and (0.03 mole) of potassium hydroxide was stirred and heated at 78-80 °C for 20 min. Ethyl chloroacetate (0.03 mole) was then added, the reaction mixture was then refluxed for 4 hrs, then the solution was poured into ice-water and stirred for 30 min. The precipitate obtained was collected by filtration, washed with water until free of chloride and dried at 50 °C and recrystallized with water white powder, yield (85%), m.p 95-97 °C, Rf = 0.56; FT-IR (KBr disk, cm-1) 3456 (N-H), 3020 (C-H, aromatic), 1620 (C=N),1741(C=O, ester)1592 and 1405 (C=C, aromatic). 1H NMR (DMSO-d6, 500 MHz, δ) 12.54 (s,1H, benzimidazole-NH), 1.27 (S, 3H, CH3),4.21(q, 2H, CH2), 7.13,7.44(m ,4H, aromatic ring). 13C-NMR (DMSO-d6, 125 MHz, δ). 147.1 (C=N). 123.0-138.9 (aromatic rings). 167.9 (C=O):

C1-C6: *General procedure for the Synthesis of 2-Amino-5-(substituted phenyl)-1,3,4-thiadiazole.*

A mixture of the corresponding carboxylic acid (20 mmol), thiosemicarbazide (1.82 g, 20 mmol) and phosphorous oxychloride (10 mL) was gently refluxed for 3 h. After cooling, water (50 mL) was added slowly and the reaction mixture was refluxed for 4 h and filtered. The solution was neutralized with concentrated potassium hydroxide solution and the precipitate was filtered and recrystallized from ethanol, the completion of the reaction and the purity of the compounds were checked by TLC (mobile phase: hexane: ethyl acetate (1:3)):

C1: 5-phenyl-1,3,4-thiadiazol-2-amine.

Pale yellow powder, yield (95%), m.p 223-225 °C, Rf = 0.46; FT-IR (KBr disk, cm-1) 3421 and 3280 (NH2), 3091 (C-H, aromatic), 1633 (C=N), 1513 and 1468 (C=C, aromatic). 1H NMR (DMSO-d6, 500 MHz, δ) 7.53–8.03 (m, 5H, Ar–H), 7.22-7.52 (s, 2H, NH2). 13C-NMR (DMSO-d6, 125 MHz, δ) 161.6, 174.1 (C=N). 128.7-130.9 (aromatic ring):

C2: *5-(2-chlorophenyl)-1,3,4-thiadiazol-2-amine.*

Pale yellow powder, yield (85%), m.p 213-215 °C, Rf = 0.51; FT-IR (KBr disk, cm-1) 3218 and 3199 (NH2), 3099 (C-H, aromatic), 1666 (C=N), 1514 and 1424 (C=C, aromatic)766 (C-Cl). 1H NMR (DMSO-d6, 500 MHz, δ) 7.38–7.71 (m, 4H, Ar–H), 6.98 (s, 2H, NH2). 13C-NMR (DMSO-d6, 125 MHz, δ) 173.1, 162.6 (C=N). 127.3-130.1 (aromatic ring):

C3: 5-(3-chlorophenyl)-1,3,4-thiadiazol-2-amine.

Pale yellow powder, yield (80%), m.p 204-206 °C, Rf = 0.58; FT-IR (KBr disk, cm-1) 3372 and 3245 (NH2), 3024 (C-H, aromatic), 1639 (C=N), 1514 and 1424 (C=C, aromatic), 714(C-Cl) . 1H NMR (DMSO-d6, 500 MHz, δ) 7.48–7.97 (m, 4H, Ar–H), 7.32-7.63 (s, 2H, NH2). 13C-NMR (DMSO-d6, 125

MHz, δ) 175.1, 162.6 (C=N). 128.8-134.9 (aromatic ring):

C4: *5-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine.*

Pale yellow powder, yield (86%), m.p 226-228 °C, Rf = 0.45; FT-IR (KBr disk, cm-1) 3270 and 3157 (NH2), 3053 (C-H, aromatic), 1630 (C=N), 1593 and 1486 (C=C, aromatic), 827 (C-Cl). 1H NMR (DMSO-d6, 500 MHz, δ) 7.53–8.02 (m, 4H, Ar–H), 7.32-7.62 (s, 2H, NH2). 13C-NMR (DMSO-d6, 125 MHz, δ) 173.2, 165.6 (C=N). 128.9-134.3 (aromatic ring):

C5: *5-(4-nitrophenyl)-1,3,4-thiadiazol-2-amine.*

Yellow powder, yield (82%), m.p 244-246 °C, Rf = 0.62; FT-IR (KBr disk, cm-1) 3426 and 3284 (NH2), 3106 (C-H, aromatic), 1630 (C=N), 1506 and 1456 (C=C, aromatic), 1596 and 1343 (NO2). 1H NMR (DMSO-d6, 500 MHz, δ) 8.11–8.47 (m, 3H, Ar–H), 7.35-7.72 (s, 2H, NH2). 13C-NMR (DMSO-d6, 125 MHz, δ) 176.1, 163.6 (C=N). 124.4-147.9 (aromatic ring):

C6: *5-(p-tolyl)-1,3,4-thiadiazol-2-amine.*

White powder, yield (98%), m.p 265-267 °C, Rf = 0.62; FT-IR (KBr disk, cm-1) 3286 and 3121 (NH2), 3014 (C-H, aromatic), 1631 (C=N), 1510 and 1475 (C=C, aromatic), 1341 and 1425 (CH3). 1H NMR (DMSO-d6, 500 MHz, δ) 7.26–7.72 (m, 3H, Ar–H), 7.27-7.65 (s, 2H, NH2),2.34 (s,3H, CH3). 13C-NMR (DMSO-d6, 125 MHz, δ) 174.1, 161.6 (C=N). 127.4-131.7 (aromatic ring). 21.3 (CH3):

D1-D6: General procedure for the synthesis of the compounds.

The mixture of Ethyl-1H-benzo[d]imidazol-2 yl)thio)acetate [B] 1.05g (5mmol) and 5-(substituted phenyl)-1,3,4-thiadiazol-2-amine (5mmol) are mixed in 60 ml ethanol well in a RBF and heated on water bath for 10 min, the reaction mixture is heated with reflux condenser for 10 hours, cooled to room temperature and the reaction mixture was added to 100gm of ice-water, and kept aside for the crystallization. The colorless crystals are collected by filtration, and recrystallized from water, Melting point is 148-212 oC; the yield is 80 to 90%.

D1: 2-((1H-benzo[d]imidazol-2-yl)thio)-N-(5-phenyl-1,3,4- thiadiazol-2-yl)acetamide.

Pale yellow powder, yield (89%), m.p 148-150 °C, Rf = 0.38; FT-IR (KBr disk, cm-1) 3279 (N-H, amide), 3196 (N-H, benzimidazole),3010 (C-H, aromatic), 2924, 2796 (C-H, aliphatic), 1741 (C=O), , 1635 (C=N), 1557 and 1405 (C=C, aromatic); 1H-NMR (DMSO-d6, 500 MHz, δ) 1.25 (s, 2H, CH2), 7.12-8.31 (m, 8H, Ar-H), 4.16 (s, 1H, NH, amide), 12.78 (s, 1H, benzimidazole-NH); 13C-NMR (DMSO-d6, 125 MHz, δ)) 33.73 (CH2), 126.78-133.57 (aromatic ring),149.62(C=N),156.82(C=O) 169.00-169.10(C=N of 1,3,4-thiadiazole).

D2: *2-((1H-benzo[d]imidazol-2-yl) thio)-N-(5-(2-chlorophenyl)-1,3,4-thiadiazol-2-yl) acetamide.*

Pale yellow powder, yield (88%), m.p 196-198 °C, Rf = 0.43; FT-IR (KBr disk, cm-1) 3222 (N-H, amide), 3101 (N-H, benzimidazole),3086 (C-H, aromatic), 2961, 2925 (C-H, aliphatic), 1741 (C=O), 1633 (C=N), 1512 and 1439 (C=C, aromatic),755 (C-Cl); 1H-NMR (DMSO-d6, 500 MHz, δ) 1.25 (s, 2H, CH2), 7.44-7.61 (m, 8H, Ar-H), 8.04 (s, 1H, NH, amide), 12.88 (s, 1H, benzimidazole-NH); 13C-NMR (DMSO-d6, 125 MHz, δ) 33.72 (CH2), 128.17-131.42 (aromatic ring), 152.07(C=N), 156.92(C=O) 169.08-170.61(C=N of 1,3,4-thiadiazole).

D3: *2-((1H-benzo[d]imidazol-2-yl) thio)-N-(5-(3-chlorophenyl)-1,3,4-thiadiazol-2-yl) acetamide.*

Pale yellow powder, yield (80%), m.p 188-190 °C, Rf = 0.41; FT-IR (KBr disk, cm-1) 3307 (N-H, amide), 3131 (N-H, benzimidazole),3064 (C-H, aromatic), 2972, 2853 (C-H, aliphatic), 1740 (C=O), 1616(C=N), 1565 and 1404 (C=C, aromatic) 763, (C-Cl); 1H-NMR (DMSO-d6, 500 MHz, δ) 1.52 (s, 2H, CH2), 7.03-7.89 (m, 8H, Ar-H), 8.51 (s, 1H, NH, amide), 12.36 (s, 1H, benzimidazole-NH); 13C-NMR (DMSO-d6, 125 MHz, δ) 33.73 (CH2), 121.92-134.29 (aromatic ring), 149.62(C=N), 157.92(C=O) 169.09-169.57(C=N of 1,3,4-thiadiazole):

D4: *2-((1H-benzo[d]imidazol-2-yl) thio)-N-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)acetamide.*

Light beige powder, yield (90%), m.p 210-212 °C, Rf = 0.43; FT-IR (KBr disk, cm-1) 3278 (N-H, amide), 3104 (N-H, benzimidazole),3068 (C-H, aromatic), 2968, 2852 (C-H, aliphatic), 1740 (C=O), 1632 (C=N), 1513 and 1465 (C=C, aromatic), 831 (C-Cl); 1H-NMR (DMSO-d6, 500 MHz, δ) 1.12 (s, 2H, CH2), 7.51-7.80 (m, 8H, Ar-H), 8.18 (s, 1H, NH, amide), 12.62 (s, 1H, benzimidazole-NH); 13C-NMR (DMSO-d6, 125 MHz, δ) 14.46 (CH2), 121.94-134.44 (aromatic ring), 155.58(C=N), 153.92(C=O) 168.32-169.32(C=N of 1,3,4-thiadiazole).

D5: *2-((1H-benzo[d]imidazol-2-yl)thio)-N-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)acetamide.*

Yellow powder, yield (89%), m.p 200-202 °C, Rf = 0.42; FT-IR (KBr disk, cm-1) 3427 (N-H, amide), 3289 (N-H, benzimidazole),3109 (C-H, aromatic), 2924, 2852 (C-H, aliphatic), 1740 (C=O), 1627 (C=N), 1503 and 1440 (C=C, aromatic), 1597 and 1343 (NO2); 1H-NMR (DMSO-d6, 500 MHz, δ) 1.18 (s, 2H, CH2), 7.10-7.89 (m, 8H, Ar-H), 8.50 (s, 1H, NH, amide), 12.12 (s, 1H, benzimidazole-NH); 13C-NMR (DMSO-d6, 125 MHz, δ) 33.74 (CH2), 121.93-137.27 (aromatic ring), 149.60(C=N), 151.82(C=O) 169.08-170.50(C=N of 1,3,4-thiadiazole).

D6: *2-((1H-benzo[d]imidazol-2-yl)thio)-N-(5-(p-tolyl)-1,3,4-thiadiazol-2-yl)acetamide.*

White powder, yield (92%), m.p 192-194 °C, Rf = 0.42; FT-IR (KBr disk, cm-1) 3287 (N-H, amide), 3125 (N-H, benzimidazole),3039 (C-H, aromatic), 2924, 2852 (C-H, aliphatic), 1740 (C=O), 1632 (C=N), 1511 and 1475 (C=C, aromatic); 1H-NMR (DMSO-d6, 500 MHz, δ) 1.49-1.72 (s, 3H, CH3),2.49 (s, 2H, CH2), 7.27-7.50 (m, 11H, Ar-H), 8.50 (s, 1H, NH, amide), 12.12 (s, 1H, benzimidazole-NH); 13C-NMR (DMSO-d6, 125 MHz, δ) 21.72 (CH2), 126.75-136.34 (aromatic ring), 156.143(C=N), 157.82(C=O) 169.08-169.27(C=N of 1,3,4-thiadiazole).

3 RESULT AND DISCUSSION

The synthetic pathway of compounds (D1-D6) is outlined in Figure 1. Compound (A) was obtained by cyclizing *O*-phenylendiamine with CS2 in the presence of potassium hydroxide using ethanol as the medium. This synthesized compound was then characterized using FT-IR and 1H and 13C- NMR. In the IR spectrum of compound (A), stretching bands corresponding to (N-H), (S-H), and (C=N) were observed at 3387 cm-1, 2572 cm-1, and 16152 cm-1, respectively. The 1H NMR spectrum of compound (A) displayed a singlet NH signal of benzimidazole at δ (12.58) ppm, Additionally, a singlet SH signal of (S-H) appeared at δ (12.15-12.42) ppm. The 13C NMR spectrum of compound (A) displayed 168.2(C=N), 115.2-123.0(aromatic ring).

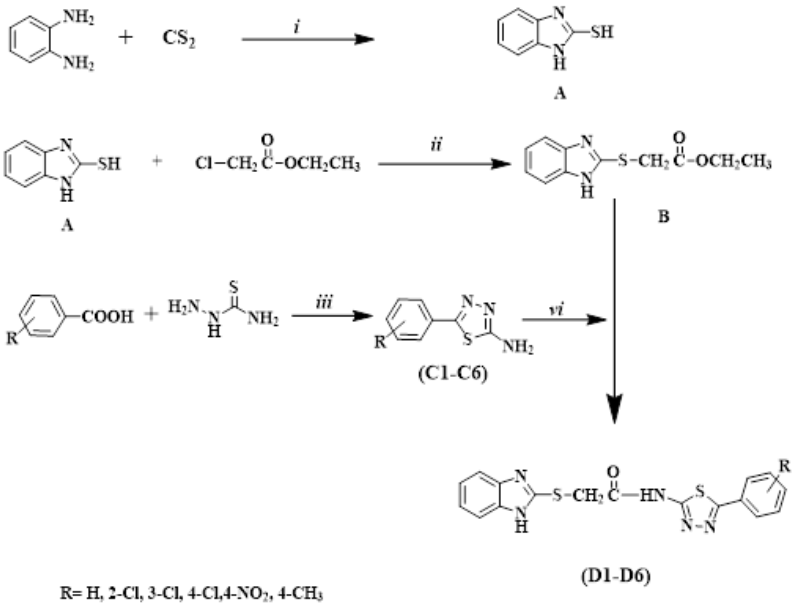


Figure 1: Synthetic route for preparation of compounds (D1–D6).

Compounds (B) was synthesized through neocleophilic substitution reactions of compounds (A) with ethyl chloroacetate in the presence of KOH in EtOH medium, yielding compounds (B). The chemical structures of this compounds were elucidated via FT-IR and 1H and 13C-NMR. In the IR spectrum of compound, the presence of amide group (N-H) bonds was confirmed by bands in the region of (3456cm-1). The presence of a carbonyl group (C=O) in the structure was proven by the presence of a sharp peak at (1741.1cm-1). Additionally, the presence of C=N in the imidazole ring was confirmed by a sharp absorption band at (1620 cm-1). The 1H NMR spectra of these compounds displayed a singlet at δ (6.9) ppm due to the NH (amide) protons and singlet at (12.54) ppm due to the NH(benzimidazole) Additionally, the methylene protons had a downfield value of 4.21 ppm, this can be attributed to the carbonyl group's inductive effect and the chlorine atom's. In the 13C NMR spectrum of the methylene carbon between the sulfur atom and the carbonyl group, a peak was observed at (33.24) ppm. Two carbon atoms in the thiadiazole ring were recorded at (163.85,157.45) ppm. The carbonyl carbon of the ester bond was observed at δ (167.2) ppm.

2-amino-5-(substituted)-1,3,4-thiadiazole (C1-C6) was synthesized by reacting various derivatives of carboxylic acid with thiosemicarbazide in the presence of phosphorousoxy chloride. The FT-IR spectra of compounds (C1-C6) showed the presence of a C=N group at (1630-1666 cm-1) and two bands at (3218-3426 cm-1) and (3121-3284 cm-1), which could be attributed to the asymmetric and symmetric stretching vibrations of the NH2 group. The 1H NMR spectra of these compounds displayed a singlet at δ (6.58-7.63) ppm due to the NH2 protons. In the 13C NMR spectra, two carbons on the thiadiazole ring were observed at δ (173.1, 162.6) ppm.

In the final step, Compound (B) was reacted with compounds (C1-C6) using Ethanol medium and reflux for 10 hrs to yield the target compounds (D1-D6). The chemical structures of all target compounds were established by FT-IR, 1H and 13C-NMR spectra. The IR data obtained for the final compounds (D1-D6) were instrumental in confirming their formation. Upon observing the data for all synthesized compounds, absorption peaks at (3222-3427 cm-1), (3101-3289 cm-1), and (1616-1633 cm-1), confirmed the presence of N-H amide, N-H benzimidazole, and (C=O, amide) groups, respectively. A sharp peak at (29243-2852 cm-1) helped to confirm the presence of C-H bonds in the final products. Additionally, the presence of C=N in the benzimidazole and 1,3,4-thiadazole nucleus was confirmed by a sharp absorption band at (1616-1632 cm-1).

In the 1H NMR spectra of all compounds, the methylene protons resonated at δ (4.14-4.16) ppm, and this downfield shift can be attributed to the inductive effect of the carbonyl group. The singlet NH signal of benzimidazole appeared at δ (12.63-12.64) in all compounds, while the singlet NH signal of (N-H amide) appeared at δ (8.46-8.04) in all compounds. The signals belonging to the aromatic region were observed at δ (7.10-8.30) ppm. In the 13C NMR spectra, the methylene carbon between the sulfur atom and carbonyl group was observed at δ (21.72-33.74) ppm. Two carbons on the thiadiazole ring were observed at δ (149.62-156.13) ppm. The carbonyl carbon of the amide group was observed at δ (168.32-170.61) ppm, all other aromatic carbons were recorded between δ (121.94) and δ (137.27). The physical properties of the synthesized compounds are listed in Table 1.

Table 1: Physical properties of the synthesized compounds.

Comp. No.	R	M.wt	Color	M.P (°C)	Yield %	Rf
A	-	150.20	Light beige	305-307	80	0.46
B	-	236.29	Wight	95-97	93	0.51
C1	H	177.23	Pale yellow	223-225	95	0.58
C2	2-Cl	211.68	Pale yellow	213-215	85	0.45
C3	3-Cl	211.68	Pale yellow	204-206	80	0.62
C4	4-Cl	211.68	Pale yellow	226-228	86	0.48
C5	4-NO_2	222.23	Yellow	244-246	82	0.54
C6	4-CH_3	189.23	White	265-267	98	0.48
D1	H	367.06	Pale yellow	148-150	89	0.47
D2	2-Cl	401.89	Pale yellow	196-198	88	0.53
D3	3-Cl	401.89	Pale yellow	188-190	80	0.38
D4	4-Cl	401.89	Light beige	210-212	90	0.43
D5	4-NO_2	412.45	Yellow	200-202	89	0.41
D6	4-CH_3	381.48	White	192-194	92	0.39

4 BIOLOGICAL EVALUATION

The newly synthesized title compounds (D1, D4, D5 and D6) were evaluated for their antibacterial activity against four types of bacterial isolates: two Gram-positive (Staphylococcus aureus and Bacillus subtilis) and two Gram-negative (Escherichia coli and Pseudomonas aeruginosa). The antibacterial activity of the tested compounds was assessed by zone of inhibition using well diffusion method. The inhibition zones (in millimeters) were measured at three different concentrations (50, 100, and 250 mg/10 mL) using dimethyl sulfoxide (DMSO) as the solvent. The results were compared with the standard antibiotic tetracycline at a concentration of 250 mg/10 mL. The results of antibacterial screening (Table 2) reveal the following information:

A) Compound D1:
   1) 50 mg/10 mL: compound D1 showed no antibacterial activity (n.s = not sensitive) against all tested bacterial strains;
   2) 100 mg/10 mL: the compound exhibited moderate activity against all bacterial strains, with inhibition zones ranging from 9 to 10 mm;
   3) 250 mg/10 mL: a significant increase in antibacterial activity was observed, with inhibition zones ranging from 13 to 16 mm. The highest activity was against B. subtilis (16 mm), followed by S. aureus and P. aeruginosa (14 mm each), and then E. coli (13 mm).

B) Compound D4:
   1) 50 mg/10 mL: compound D4 showed no antibacterial activity (n.s) against all bacterial strains;
   2) 100 mg/10 mL: the compound demonstrated moderate to good activity, with inhibition zones ranging from 8 to 13 mm. The highest activity was against B. subtilis (13 mm), followed by E. coli (11 mm), P. aeruginosa (9 mm), and S. aureus (8 mm);
   3) 250 mg/10 mL: a substantial increase in activity was observed, with inhibition zones ranging from 18 to 22 mm. The highest activity was against B. subtilis (22 mm), followed by P. aeruginosa and E. coli (20 mm each), and then S. aureus (18 mm).

C) Compound D5:
   1) 50 mg/10 mL: compound D5 showed no antibacterial activity (n.s) against all bacterial strains;
   2) 100 mg/10 mL: the compound exhibited good activity, with inhibition zones ranging from 11 to 14 mm. The highest activity was against P. aeruginosa (14 mm), followed by E. coli (12 mm), and then S. aureus and B. subtilis (11 mm each);
   3) 250 mg/10 mL: a notable increase in activity was observed, with inhibition zones ranging from 17 to 21 mm. The highest activity was against P. aeruginosa (21 mm), followed by S. aureus (20 mm), E. coli (20 mm), and B. subtilis (17 mm).

D) Compound D6:
   1) 50 mg/10 mL: compound D6 showed weak activity only against P. aeruginosa (9 mm), with no activity against the other bacterial strains;
   2) 100 mg/10 mL: the compound demonstrated good activity, with inhibition zones ranging from 10 to 14 mm. The highest activity was against P. aeruginosa (14 mm), followed by E. coli (13 mm), and then S. aureus and B. subtilis (10 mm each);
   3) 250 mg/10 mL: a significant increase in activity was observed, with inhibition zones ranging from 18 to 23 mm. The highest activity was against S. aureus (23 mm), followed by E. coli (19 mm), P. aeruginosa (18 mm), and B. subtilis (19 mm).

- Concentration-Activity Relationship: all compounds (D1, D4, D5, D6) exhibited increased antibacterial activity with higher concentrations, indicating a concentration-dependent effect;
- Differences Between Bacterial Strains: in general, Gram-positive bacteria (S. aureus and B. subtilis) were more sensitive to the compounds compared to Gram-negative bacteria (P. aeruginosa and E. coli), particularly at higher concentrations;
- Most Effective Compounds: compound D6 showed the highest antibacterial activity, particularly against S. aureus at 250 mg/10 mL (23 mm), followed by Compound D4, which exhibited strong activity against B. subtilis (22 mm).

Table 2: Diameter inhibition zone (in mm) of the compounds (D1, D4, D5, and D6).

Com-pounds	Conc. (mg/10mL)	Gram-positive		Gram-negative	
		Staphylococcus aureus	Bacillus subtilis	Pseudomonas aeruginosa	E. Coli
D1	50	n.s	n.s	n.s	n.s
	100	9	10	10	9
	250	14	16	14	13
D4	50	n.s	n.s	n.s	n.s
	100	8	13	9	11
	250	18	22	20	20
D5	50	n.s	n.s	n.s	n.s
	100	11	11	14	12
	250	20	17	21	20
D6	50	n.s	n.s	9	n.s
	100	10	10	14	13
	250	23	19	18	19
Tetra-cycline	250	29	27	26	23
n.s = no sensitive					

5 CONCLUSIONS

In this study, a new series of hybrid compounds combining 1,3,4-thiadiazole and benzimidazole scaffolds (D1–D6) were successfully synthesized and characterized using IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopic techniques. The synthetic strategy employed a straightforward approach involving nucleophilic substitution followed by condensation reactions, yielding products in high purity and good yields. The antibacterial activity of the synthesized compounds was evaluated against both Gram-positive and Gram-negative bacterial strains. Among the tested derivatives, compounds D4 and D6 exhibited the most significant antibacterial activity, particularly at higher concentrations, suggesting a clear structure–activity relationship influenced by the nature of the substituents on the phenyl ring. Specifically, the presence of electron-withdrawing groups (such as Cl and NO_2) and electron-donating groups (like CH_3) enhanced the biological efficacy of the hybrids.

ACKNOWLEDGMENTS

The authors express their thanks and appreciation to Department of Chemistry, Collage of Education for pure sciences, University of Diyala for their support and assistance.

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Thiadiazole Derivative Impregnated on Cross-Linked Hydrogel for Water Retention and Adsorption of Nickel Ions for Sustainable Environment

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Keywords: Acrylic Acid, Azobisisobutyronitrile, Hydrogel, MBA, Adsorption.

Abstract: In this study, free radical copolymerization was used to prepare a hydrogel of poly (acrylic acid-co-2-amine -5-(3,5-dinitrophenyl)-1,3,4-thiadiazol) (poly-AA-ANT). The poly-AA-ANT hydrogel was characterized using Fourier transform infrared spectroscopy FTIR, Thermogravimetric analysis (TGA), and scanning electron microscopy (SEM), X-ry diffraction. The swelling of poly-AA-ANT was performed by using two solvents (water and methanol) each alone and found that the swelling ability in methanol was higher than for water. The removal of Ni^{2+} was conducted by immersing the poly-AA-ANT in a solution of Ni^{2+} and after a periol of time the residual we measured by atomic absorption spectrophotometer. The percentage of removal was 93.8%. The poly-AA-ANT hydrogel had a high swelling ratio of 546%. penetration rate of the poly-AA-ANT hydrogel coupled with the high swelling rate exposed to the internal adsorption sites. The high-water holding capacity of Poly-AA-ANT improved the moisture holding limit of soil for irrigation up to 24 days. systems as a result, Poly-AA-ANT has become an important addition to irrigation systems.

1 INTRODUCTION

Nickel is a widely distributed metal in the environment and has many industrial and commercial uses. The public may be exposed to Nickel in air, water, and food. Most nickel in the human body comes from drinking water and food [1]. Nickel is nutritionally essential for many species of animals, microorganisms, and plants, and therefore deficiency or toxicity symptoms can occur when nickel is ingested in too little or too much. Although several cellular effects of nickel have been documented, no deficiency has been described in humans [2]-[7]. Environmental contamination with toxic metals arises from industries and agricultural waste. Many industries, such as metal plating facilities, mining operations, and tanneries, dispose of waste that contains heavy metal ions. Because of their importance, heavy metals have been widely studied. These toxic metals can cause cumulative toxicity, cancer, and brain damage when present at levels above permissible levels. Removal of heavy metals such as cadmium, lead, copper, nickel, and mercury has become very important for environmental remediation [8]. The World Health Organization has recommended strict controls on the levels of various heavy metals in wastewater [9]. In recent years, polymer gels have played an important role in the adsorption of heavy metal ions because they can swell in water and achieve high adsorption [10]

Heavy metal pollution has become a global environmental issue and a major concern due to the toxic, bio accumulative and non-degradable nature of heavy metals. Therefore, this heavy metal must be removed from aquatic wastewater before being discharged into water bodies. Therefore, many technologies have been used to remove heavy metals from wastewater, such as ultrafiltration, reverse osmosis, coagulation, adsorption, electro-precipitation and chemical precipitation [11]-[15]. Among all these strategies, adsorption is frequently applied for removal by taking advantage of its simple operation, high removal rate, low cost and excellent regenerability [16]. Adsorbents are the material basis of adsorption technology; therefore, the development of new adsorbents is a research focus for wastewater treatment. Ion3 hydrogel is a polymeric material with a three-dimensional network structure [17] that can absorb large amounts of water and retain a certain amount of water compared to other similar materials such as cloth, cotton, and sponge [18-19]. Heavy metals,

unlike organic pollutants, are non-degradable and tend to accumulate in living organisms, which may cause serious problems for human health and wildlife [20]. Therefore, it is very necessary to remove these toxic heavy metals from water and soil. Ni^{2+} is widely used in industrial applications such as batteries, electroplating, and coins [21-23]. Ni2+ belongs to the so-called essential metals and is identified as a component of many enzymes, participating in vital metabolic reactions [24].

2 MANUSCRIPT PREPARATION

2.1 Materials and Reagents

3,5-dinitrobenzoic acid(C7H4O6N2,99), thiosemicarbazide(CH5N3S), phosphorous ox chloride (POCL3) acrylic acid (C3H4O2, 98%), Azo bis isobutyronitrile(98%), benzene(C6H6,99%), Nikel chloride nitrogen gas and nickel ion (Ni^{+2}) (99%) were used.

2.2 Instrumentation

The infrared spectra of the compound and poly-AA-ANT were measured in the range of (4000-400) cm-1 using an IR Affinity (SHIMADZU) instrument using a (KBr) disk in the frequency range. The 1H-NMR was measured using a Broker Ultra Shield, 500 MHz, using DMSO-d6 solvent and TMS reference for the compound. A (Rheometric Scientific TGA-1000) instrument was used to record the TGA of the prepared polymer, and a (ZEISS) scanning electron microscope (SEM) was used to measure the prepared poly-AA-ANT, and the absorbance of poly-AA-ANT recorded on a (SHIMADZU-1700) dual UV-Vis spectrometer was measured in the presence of a quartz cell using KOH solvent, and the UV-Vis spectra were measured on a (SHIMADZU 1800 UV) spectrometer.

2.3 Stepwise Synthesis of Poly-AA-ANT Hydrogel

2.3.1 Synthesis of 2-Amine -5-(3,5-Dinitrophenyl)-1,3,4-Thiadiazol

3,5 dinitro benzoic acid (0.01 mol) was refluxed with thiosemicarbazide (0.01 mol) in (10 ml) POCl3 in a 100 ml round bottomed flask. The reaction was then heated for three hours, after that (30 ml) of distilled water was added to it and the mixture was heated for four hours. The mixture was then neutralized using (10%) potassium hydroxide (KOH), the solution was filtered, the resulting precipitate was washed several times with distilled water, and the precipitate was left to dry.

2.3.2 Synthesis of poly-AA-ANT Hydrogel

The polymer were prepared using the solution polymerization technique and the free radical polymerization method by dissolving 2-amine -5-(3,5-dinitrophenyl)-1,3,4-thiadiazol (0.5g) in acrylic acid (5ml), completely dissolving it with occasional heating, then benzene (30ml) was added to the mixture with continuous stirring, methylene bisacrylamide (1g) was added to the mixture. At this point, N2 gas was pumped into the mixture and the gases were removed using a Schlenk device. The mixture was refluxed using a water bath at (75) oC. When this temperature was reached, the initiator azo bisiso butyro nitrile (AIBN) (0.008) was added with stirring rite (300rpm). After a while, the polymer began to form, and it was observed that (N_2) gas was released resulting from the disintegration of the initiator. After the reaction ended, methanol was added to isolate the polymer from the benzene. To complete polymer formation.

2.4 Swelling Studies of Poly(AA-ANT) Hydrogels

(0.5) g of dried hydrogel (Wd) was immersed in 20 mL of distilled water at room temperature. The hydrogel was left to swell until a constant weight was achieved. Then hydrogel was removed from the solution and the final of the hydrogel (Ws) was weighed and recorded. The swelling percentage of hydrogel was calculated by using through (1). Table 1 shows the swelling percentage in water for 24 h, Table 2 shows the swelling percentage in methanol after 24 h, and Figures 1 and 2 show the swelling percentage in water and methanol. as:

$$\%\, Swelling\ degree = \frac{Ws-Wd}{Wd} \times 100. \qquad (1)$$

2.5 Removal of Ni^{2+} Ion

A stock solution of 1000 mg/L Ni2+ was prepared. A standard solution of 5 mg/L was then prepared from this stock solution. Approximately 0.1 g of the hydrogel was immersed in 20 ppm of (5 mg/L) Ni^{2+} solution. After 24 hours, the hydrogel was removed from the solution. The remaining Ni^{2+}

Table 1: Percentage of swelling in water after 24 hours' poly-AA-ANT.

No	Swelling						Percentage of swelling
	0 h	3h	6 h	9 h	12 h	24 h	
poly-AA-ANT	0.5	0.63	0.76	1.99	2.56	3.23	546%

Table 2: Swelling ratio of polymers prepared in methanol poly-AA-ANT.

No	Swelling						Percentage of swelling
	0 h	3h	6 h	9 h	12 h	24 h	
poly-AA-ANT	0.5	0.59	0.68	0.80	1.13	2.34	368%

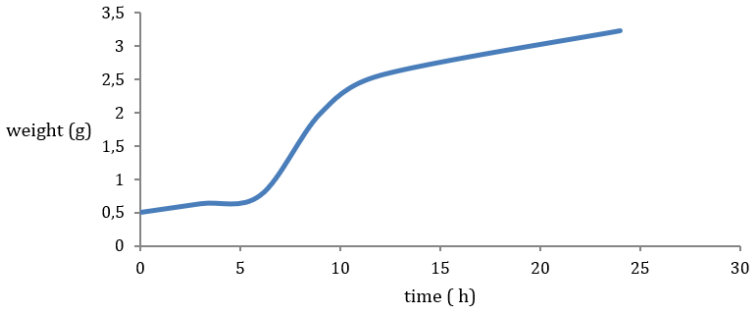


Figure 1: Swelling of poly-AA-ANT in water after 24h.

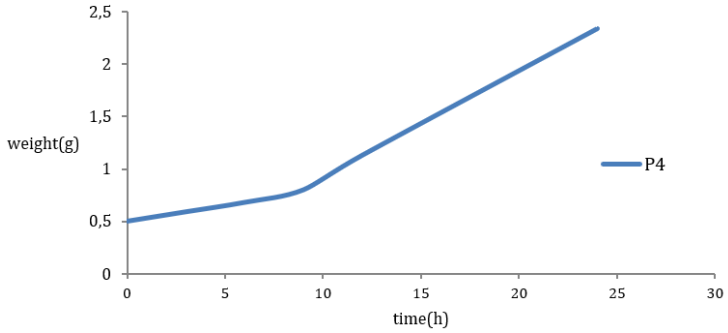


Figure 2: Swelling of poly-AA-ANT in methanol after 24 h.

Table3: The residual and the percentage of adsorption of Ni(II) using poly-AA-ANT hydrogel.

No.	Residual	Absorption rate %
poly-AA-ANT	1.225	%93.8

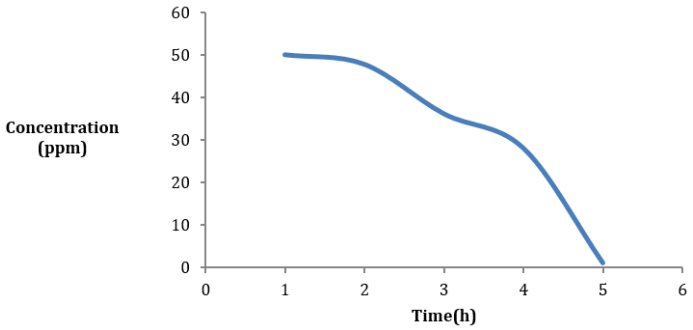


Figure 3: Removal of Ni^{2+}Ion of poly-AA-ANT.

concentration was monitored using atomic absorption spectroscopy. The Ni2+ removal ratio was calculated using (2), where Ci and Cf are the initial and final concentrations (mg/L) of the Ni2+ solution, respectively, as shown in Table 3. Figure 3 shows the nickel ion removal ratio.

$$(\%)Percentage\ removal\ = \frac{Ci-Cf}{Ci} \times 100. \quad (2)$$

2.6 Water-Retention Study of Poly-AA-ANT Hydrogel

The poly- hydrogel water preservation analysis was performed in soil collected from University, Diyala, where 20 g soil and 2 g poly- hydrogel were homogenized in a plastic container, 30 mL water was added gradually, and the weight (W1) wasdetermined using a weighing machine. The container was weighed every day (W2) and kept at room temperature until there was no consistency in weight loss. The water loss ratio (W %) of soil samples was calculated through the standard (3).

$$W\ \% = \frac{W1-W2}{30} \times 100. \quad (3)$$

3 RESULTS AND DISCUSSION

In this study, the reaction between 5,3-dinitrobenzoic acid and AAc in the presence of MBA as a cross-linking agent resulted in the formation of a hydrogel. In the initiation step, radical polymerization occurred by hydrolysis and hydrogen abstraction from the AAc chain [25]. Grafting of AAc onto the backbone of 3,5-dinitrobenzene at the active site occurred. Then the propagation reaction occurred continuously while the cross-linking agent built a cross-linking bond between the growing chains [26] .

3.1 Characterization

FTIR spectra of hydrogel were recorded by using Perkin Elmer Spectrum in the range of 4000–400 cm−1. The sample was completely dried before FTIR analysis. The thermal degradation of hydrogel was conducted by using thermogravimetric analyser TGA. Temperature used was up to800 °C with a heating rate of 10°C/ min under nitrogen atmosphere. SEM at appropriate magnification was used to observe morphology of the sample.

3.1.1 FTIR

The prepared compound was identified by FTIR spectrum, where the absorption bands (3510-3441cm-1) were observed, which belong to the (NH_2) band, the band at (3109) cm-1, which belongs to the aromatic (C-H) group, and the band (1620) cm-1 which belongs to the (C=N) group. As for the aromatic (C=C) band, it appeared at (1543) cm-1. Figure 4 shows the infrared spectrum of the compound.

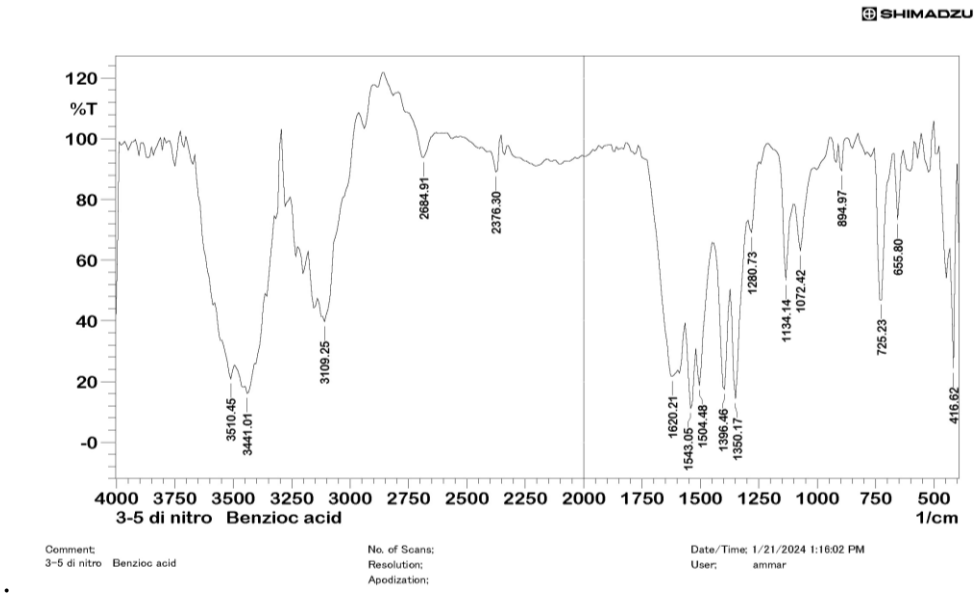


Figure 4: FT-IR Spectrum of 2-amine -5-(3,5-dinitrophenyl)-1,3,4-thiadiazol

3.1.2 1H-NMR Spectroscopy

7.70 ppm associated with the (-NH_2) group (S,2H), the aryl ring showed a vibration at 7.01 ppm associated with the proton (a) as in (d,1H), (b) a chirality at 9.02 ppm as in (t,1H), (c) a vibration at 8.06 associated with (t,1H) and (d) at 8.05 (a,1H) Figure 5 shows the nuclear resonance spectrum of the compound 2-amino-5-(2-chloro phenyl)-1,3,4-thiadiaozle.

3.1.3 FTIR of Polymer

The FTIR spectrum assures the formation of the poly(acrylic) through the presence at a stretching vibration at (3436) cm-1 related to O-H group and anther band at (2939) cm-1 which is attributed to the asymmetric and symmetric stretching vibration for (CH_2) group and consequently the signal at (2252) cm-1 is related to C≡N group. Finally, the peak at (1728) cm-1 is attributed to (C =O) stretching vibration Figure 6 shows the infrared spectrum of the polymer.

3.1.4 TGA Analysis

Study was conducted on the polymer to understand the thermal stability from room temperature to 800oC. Figure 7. The degradation occurred in three steps where the first degradation appeared at 100 ºC related to the removal of moisture resulted from the interaction with COOH group in the polymer. The second step of degradation occurred at 200 ºC. The third step appeared at 400 ºC which involved the scission of C-C bond in the polymer.

3.1.5 SEM Analysis

Scanning Electron Microscopy (SEM) is a sophisticated imaging technique used to observe the surface morphology and topography of samples at high magnifications. Unlike optical microscopy, which uses visible light, SEM employs a focused beam of electrons to interact with the sample, producing detailed images that reveal structural and compositional information. Figure 8 shows the SEM picture for poly-AA-ANT and the holes are presents these holes may be due to the cross linked.

3.1.6 XRD Analysis

XRD patterns of thiadiazole and poly-AA-ACT hydrogel are described in the 2θ range of 5º–90º Figure 9. The thiadiazole spectra showed fundamental diffraction peaks at 19.5º, 26.2º, 30.4º, 34.5º, 48.1º, and 52.3º corresponded to (111), (220), (311), (400), (511), and (440), respectively. Which demonstrated the crystalline characteristic of thiadiazole. The grafting of thiadiazole on polymer showed major transformation in the XRD pattern of poly-AA-ANT hydrogel. The hydroxyl groups of acrylic acid were affected by thiadiazole, which destroyed the crystalline structure and transformed it toamorphous composition.

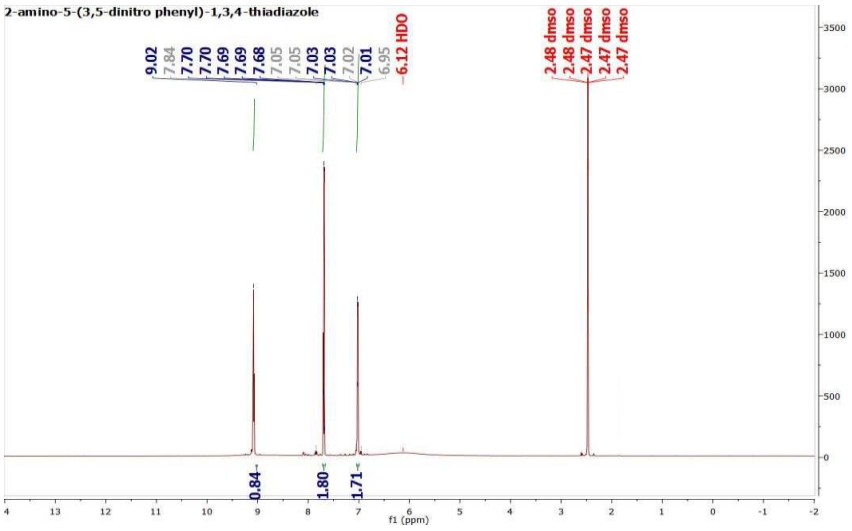


Figure 5: 1H-NMR Spectrum of 2-amine -5-(3,5-dinitrophenyl)-1,3,4-thiadiazol.

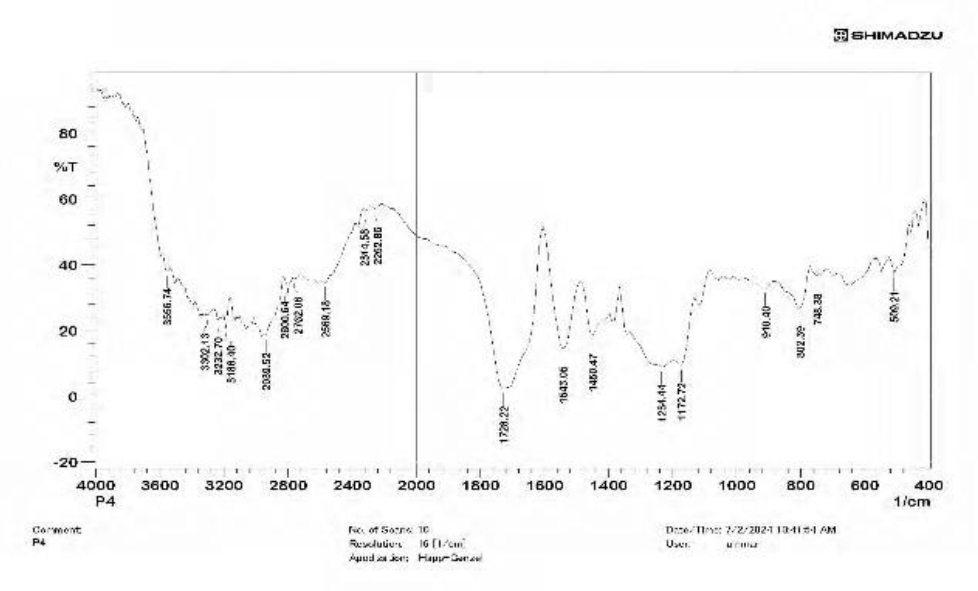


Figure 6: FT-IR Spectrum of poly-AA-ANT.

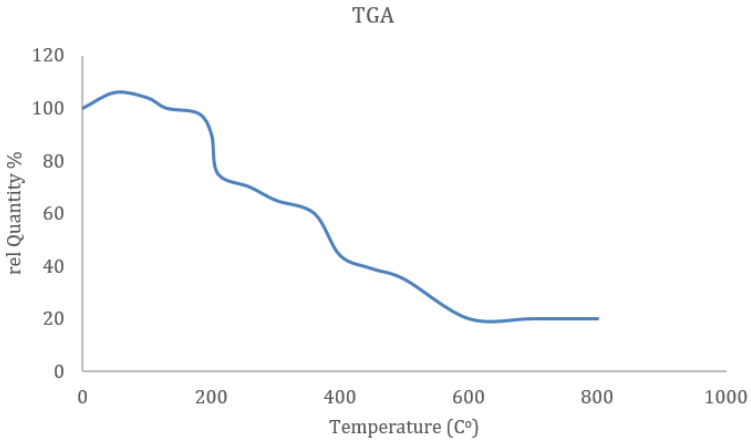


Figure 7: TGA of poly-AA-ANT.

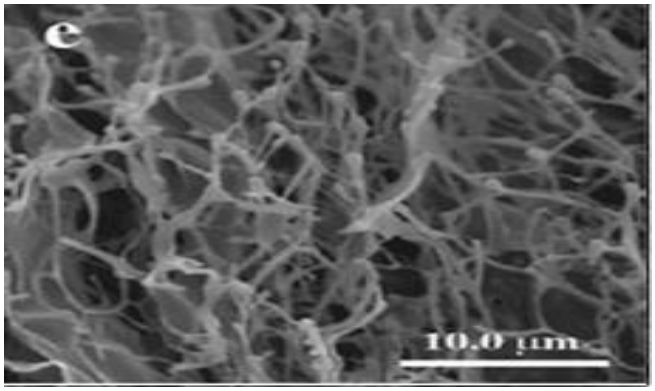


Figure 8: SEM morphology of poly-AA-ANT.

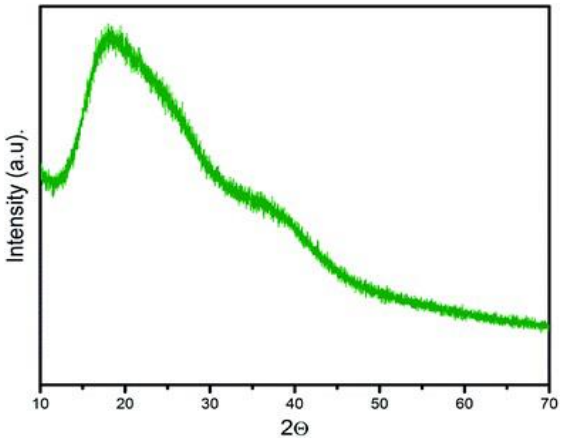


Figure 9: XRD of the poly-AA-ANT.

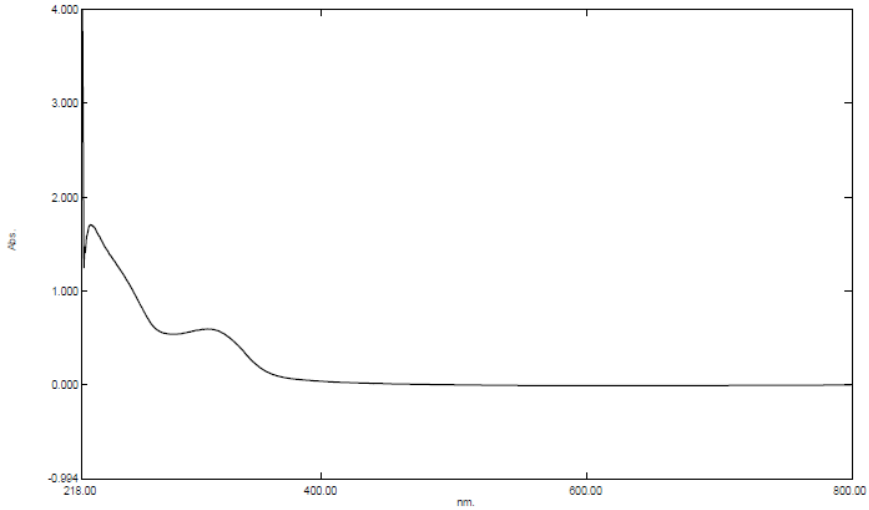


Figure 10: Shows IR short for the retained of 2-amine -5-(3,5-dinitrophenyl)-1,3,4-thiadiazol.

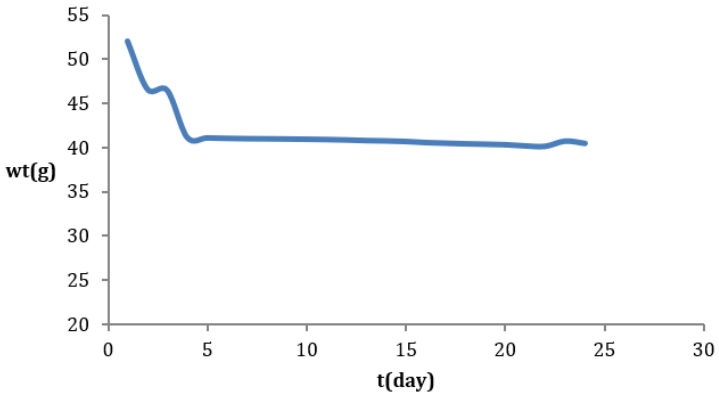


Figure 11: Water retention of soil.

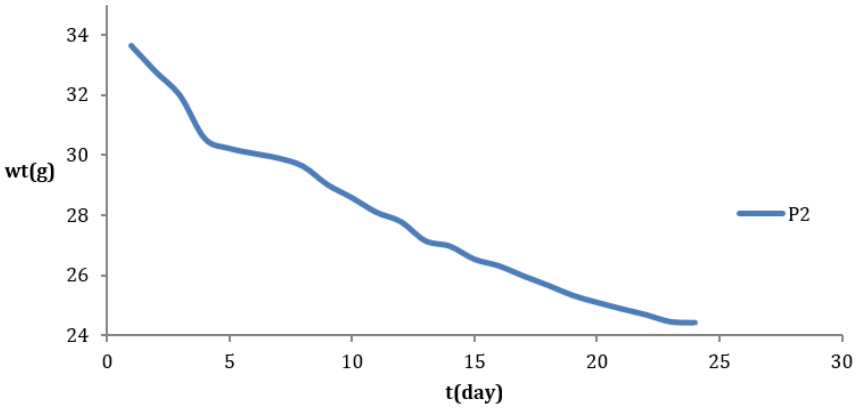


Figure 12: Water retention of poly-AA-ANT-soil.

3.1.7 5-(2-Chloro Phenyl)-2-Amino-1,3,4-thiadiazole Retention

Identification of 1,3,4-thiadiazole complex loaded on polymer using UV-visible technique. A buffer solution of pH = 8 was prepared, then 0.1 g of the polymer was weighed. This amount was added to 20 ml of the buffer solution and left for an hour. After that, the solution was filtered and the filtrate was placed in a UV-visible spectrometer, where the maximum absorption of the decomposed thiadiazole compounds was measured. This indicates that these compounds are loaded on the polymer. Figure 10 shows IR short for the retained shows IR short for the retained of 2-amine -5-(3,5-dinitrophenyl)-1,3,4-thiadiazol

3.2 Water-Retention Study of Polymer

Water retention analysis was performed using polymer in soil collected from University of Diyala, in Diyala province. 20 g of soil and 2 g of polymer were taken in a plastic container and 30 ml of water were added gradually. The weight (W1) was determined using a weighing machine. The container was weighed every day (W_2) and kept at room temperature so that there was no stability in weight loss. The percentage of water loss (W%) for soil samples was calculated using the standard formula. Figure 11 shows water retention using soil and Figure 12 shows water retention using the polymer poly-AA-ANT.

4 CONCLUSIONS

The polymerization with impregnation process was used to synthesize a novel polymer (poly-AA-ANT) Hydrogel that has been used for the adsorption of Ni(II) Ione from water. The swelling tendency was (546%) in water and (368%) in methanol. The adsorption which is the highest of Ni(II) ions was (%93.8) compared to the reported Ni(II) adsorption data. The prepared poly (AA-ANT) showed 1.2 times better soil -water retention. penetration rate of the poly-AA-ANT hydrogel coupled with the high swelling rate exposed to the internal adsorption sites. The high-water holding capacity of Poly-AA-ANT improved the moisture holding limit of soil for irrigation up to 24 days. systems as a result, Poly-AA-ANT has become an important addition to irrigation systems.

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Spectrophotometric Determination by Azo Coupling Reaction of Bisphenol A Using Benzidine Reagent

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Keywords: Spectrophotometric, Diazotization, Bisphenol A.

Abstract: New spectrophotometric method for Bisphenol A determination has been developed according to the current study. A colored complex called Bisphenol A can be obtained through the combination of benzidine in an alkaline solution. The chemical reaction produces a dark yellow compound which shows absorbance at 452 nm. The analysis showed Beer's law compliance from 5–14 g/mL with R2 value at 0.999 and LOD at 0.3470 g/mL while LOQ at 0.1190 g/mL. This reaction solution demonstrated a molar absorptivity value of 2872.8 $L \cdot mol^{-1} \cdot cm^{-1}$ accompanied by a relative standard deviation of 0.00125%. The recovery value for this method reached 102.2311% and the Sandal sensitivity measurement produced a result of 0.07937 g/mL. Bisphenol A detection through this method succeeded in a series of wastewater samples which included measurements from Tap water along with Diyala, Khresan, and Mahroot Bridge. The technique exhibited a strong correlation and excellent conformity to Beer's law while ensuring high precision, accurate detection, and low measurement limits. The ability to detect BPA at trace levels was achieved by optimizing reaction parameters and reagent dosages. This method proved to be a reliable tool for assessing BPA contamination in drinking water sources and industrial effluents, as demonstrated by its application to environmental samples from various sources. Its cost-effectiveness, ease of implementation, and high sensitivity make it an effective monitoring approach for environmental assessments.

1 INTRODUCTION

The industrial organic molecule known as Bisphenol A (BPA) serves as a component for producing epoxy resins and polycarbonate plastics. BPA functions as a vital organic substance in numerous consumer items including plastic bottles together with food can linings as well as medical devices and electrical equipment and dentistry supplies [1]. BPA provides plastics with better strength and transparency together with thermal stability yet its widespread use leads to critical environmental problems since it shifts into food containers and natural environments where humans can encounter it multiple ways [2]. Human consumption of BPA is common because research proves that this chemical gets into food and drinks through plastic storage containers that handle heat or acid exposure [3]. BPA ranks as an endocrine-disrupting chemical (EDC) since it duplicates hormones by becoming active in estrogen receptors and disturbs the body's natural hormone activity thereby producing possible dangerous biological impacts [4]. Studies link chronic exposure to low BPA concentrations to an elevated danger of heart disease plus infertility and brain development problems and metabolic conditions and fetal congenital defects [5].

The toxicological and epidemiological study of BPA continues because scientists have established a connection between the chemical and hormone-related cancer development including prostate and breast cancer [6], [7]. BPA functions both as a significant environmental danger point while impacting human wellness negatively. BPA migrates to surface then groundwater sources after its entrance through industrial waste, plastic degradation and wastewater effluents thus contaminating ecosystems while harming aquatic life [8]. Research shows that BPA causes its accumulation in marine organisms leading to disruption of hormonal balance and inhibited development and reproduction until it disrupts the natural ecosystem balance [9]. The European Chemicals Agency (ECHA) and U.S. Food and Drug

Administration (FDA) established strict regulations regarding BPA usage in food contact materials especially baby bottles and infant food containers because of public concern [10]. Other countries such as Canada along with Japan and France have moved beyond European legislation by banning BPA applicability or supporting BPA-free polymers for substitution purposes [11]. The continuous detection of BPA remains challenging because its substitute chemicals BPS and BPF might produce equivalent biological consequences [12].

The accurate analysis provided by gas chromatography-mass spectrometry alongside high-performance liquid chromatography demands expensive specialized tools along with prolonged analytical periods that reduce their suitability for daily commercial work [13]. The analytical methods used for BPA detection have experienced substantial progress throughout recent years. UV-Vis spectrophotometry has gained popularity as a BPA detection method because it offers easy operation and low cost and rapid analysis time which fulfil requirements of environmental and regulatory applications [14], [15]. The identification of phenolic chemicals including BPA finds success through Azo coupling reactions which have become one of the most successful spectrophotometric detection techniques [16]. Azo coupling detects BPA through its reaction with specific reagents such as benzidine that yields a measurable colored compound at a precise spectroscopic wavelength [17].

The detection method proves suitable for measuring BPA in water and plastic items and industrial waste because of its high sensitivity combined with easy operation[18]. The research develops and optimizes a spectrophotometric assessment method through azo coupling processes to accurately measure BPA concentrations because BPA detection requires immediate attention [18]. The research analyzes vital reaction-sensitive and accuracy-affecting parameters by studying various reagent concentrations alongside temperature control and acid types and sequence order of reagent addition [19]. The method's detection capacity together with its sensitivity to low doses of BPA is checked through LOD and LOQ measurements [20]. A dependable analytical tool aimed at better environmental and industrial BPA monitoring will assist regulatory bodies in their efforts to minimize human exposure while addressing health and ecological problems according to this study [21].

2. EXPERIMENTAL

2.1 Material

All substances and reagents used during the study received first-rate quality selection.

2.2 Equipment Used

The Shimadzu 800 UV-Visible Spectrophotometer produced in Japan served as the main device, European-made Velp Scientific water bath, Geemy Plc-03 centrifuge, Taiwan, Electrical Balance: China's Kern & SOHN GmbH.

2.3 Preparation of Standard Solution

All standard solutions were prepared in volumetric flasks using analytical-grade reagents and distilled water, with concentrations verified gravimetrically/ volumetrically:

1) The preparation involved dissolving 0.1 g of benzidine into 25 mL of ethanol to make a 100 µg/mL standard solution. The dissolved solution was poured into a 100 mL volumetric flask while adding the needed quantity of distilled water to reach the specified measurement level.
2) A 100 mL volumetric flask received its maximum capacity using distilled water following the dissolution of 0.1 g Bisphenol A into a 100 µg/mL standard solution.
3) 1 g of sodium nitrite dissolved in a 100 mL volumetric flask received additional distilled water to create a 1% w/v solution which filled the capacity.
4) A 1% w/v solution of urea was prepared by dissolving 1 g sulfamic acid in water which filled a 100 ml volumetric flask. An additional amount of distilled water was added to reach the appropriate level of solution.
5) A 100 mL volumetric flask received distilled water until it reached the brim followed by the addition of 5.4 mL of 18.41 M concentrated phosphoric acid to establish a 1 M phosphoric acid solution.

6) 5.46 ml of 18.29 M concentrated sulfuric acid was added in a 100 mL volumetric flask and complete with distilled water for preparing a 1 M solution of sulfuric acid.
7) The solution of 1 M acetic acid prepared by adding 5.74 mL of 17.431 M acetic acid and complete of 100ml volumetric flask with distilled water.
8) In 100 mL volumetric flask added 8.40 ml of 11.96 M hydrochloric acid followed by distilled water addition for creating a 1 M hydrochloric acid solution.
9) A 4 g of sodium hydroxide added in a 100 mL volumetric flask and complete with distilled water until the flask reached the mark to yield a 1 M sodium hydroxide solution.
10) A 1 M solution of potassium hydroxide was prepared by dissolving 5.6 g of potassium hydroxide into a 100 mL volumetric flask and adjusting it to volume with distilled water.
11) A solution with one molar barium hydroxide was prepared by dissolving 1.71 g of barium hydroxide with a small amount of distilled water and then filling a 10 mL volumetric flask to the mark.
12) A 1 M solution of sodium bicarbonate was prepared by dissolving (8.4 g) of the compound in water before adjusting its volume to 100 mL with distilled water.

2.4 General Azo Coupling Procedure

The prepared azo compound accomplished by adding 0.9 mL of 100 µg/mL benzidine in a volumetric flask 10mL in ic bath, followed by adding 0.6 mL of 1 hydrochloric acid, 0.4 mL of 1% sodium nitrite, 0.6 mL of urea 1% solution to eliminated excess sodium nitrite, 1mL of 100 µg/mL of Bisphenol A, and 0.8mL of 1mL of potassium hydroxide solution, the absorption was determined using uv-vis. The maximum wavelength absorption was determined.

2.5 Suggested Protocol for Environmental Water Samples

The waste water samples were collected from some locations including Diyala, Khresan, Mahroot Bridge and tap water, the samples kept on plastic bottles and filtered with filter papers before analysis and then in series of 10 mL volumetric flasks added the optimal volumes, 0.9 mL benzidine, 0.6 mL of HCl, 0.4mL $NaNO_2$, 0.6mL urea, 1 mL of 10, 20 µg/mL of Bisphenol A, 0.8mL KOH, Apply each solution to make up the total volume of the solution. The spectrophotometer served to measure the quantitative absorbance of the solution.

3 RESULTS AND DISCUSSION

3.1 Bisphenol A Spectrophotometric Determination in an Aqueous Solution Utilizing the Azo Coupling Reaction

The diazotization of benzidine and its coupling with BPA to form a dark yellow product was reported at 452 nm, Figure 1 show the spectrum of absorption of dark yellow product versus blank.

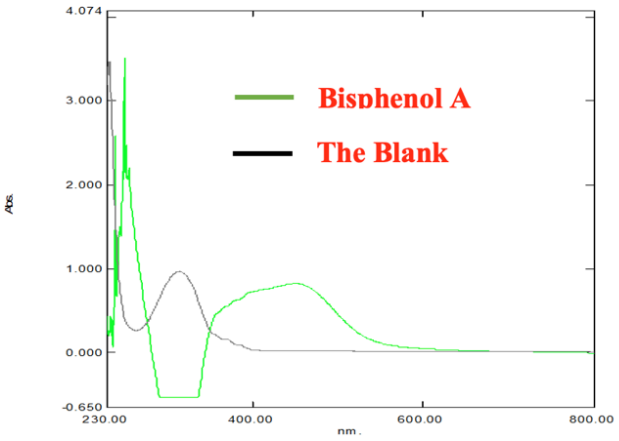


Figure 1: The green line show how Bisphenol A absorbs dye spectra while the black line compares the blank solution.

3.2 Study the Optimal Conditions for the Reaction

This study featured multiple tests that evaluated different parameters affecting the absorption of colored azo dye products.

3.2.1 Effect of the Type of Acid

The experimental procedures involved creating acids solutions with 1 M concentration. The data in Table 1 and Figure 2 demonstrate that hydrochloric acid 1 M achieves maximum Bisphenol A absorption with an absorbance value of 0.481.

Table 1: Data on absorption for the effect of acid type.

Acid Type	Absorbance at 452 nm
HCl	**0.481**
H_2SO_4	0.446
H_3PO_4	0.465
CH_3COOH	0.404

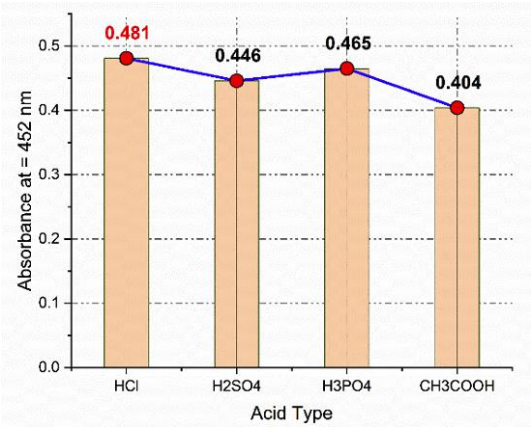


Figure 2: Acid type's effect on absorbance value.

3.2.2 The Effect of Acid Volume

A series of experiments contained 1 M hydrochloric acid solutions prepared with different volume quantities. Table 2 shows that the combination of 0.6 mL hydrochloric acid at 1 M concentration results in the best color product absorption 0.501. Figure 3 shows how the absorbance increases as acid volume rises until it drops sharply because the basic phenol molecule becomes unreactive. The combination of 0.7 mL hydrochloric acid with color product yielded the maximum absorption during subsequent testing sessions to verify the peak absorption volume.

Table 2: The effect of acid volume on the colored Bisphenol A product's absorbance.

Acid volume (1M)	Absorbance at 452nm
0.1	0.423
0.2	0.431
0.3	0.445
0.4	0.463
0.5	0.484
0.6	**0.501**
0.7	0.498
0.8	0.493
0.9	0.486
1.0	0.481

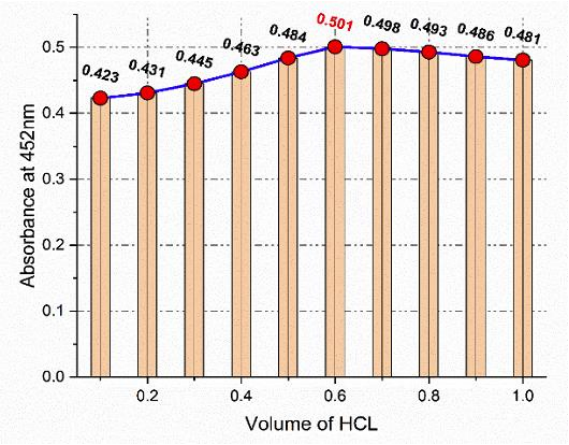


Figure 3: The effect of the hydrochloride acid volume at a 1 M concentration on absorbance value.

3.2.3 The Influence of the Base Type

Colored azo product solution preparation used potassium hydroxide at a concentration of 1 M. Results in Table 3 and Figure 4 show that potassium hydroxide 1 M proved to be the most efficient base choice for Bisphenol A compound since it exhibited an absorbance reading of 0.527.

Table 3: Effect of the type of base on the absorbance of the colored products of Bisphenol A.

Base Type (1M, 1mL)	Absorbance at 452nm
NaOH	0.502
KOH	**0.527**
$NaHCO_3$	0.439
$Ba(OH)_2$	0.487

3.2.4 The Effect of the Ideal Volume of 1 M of KOH

The base volume in potassium hydroxide solution range from 0.1 mL to 1 mL at a 1 M concentration. The data in Tables 4 and Figure 5 indicate that using

0.8 mL of 1 M potassium hydroxide solution produces the best results for colored product by achieving an absorbance level of 0.544.

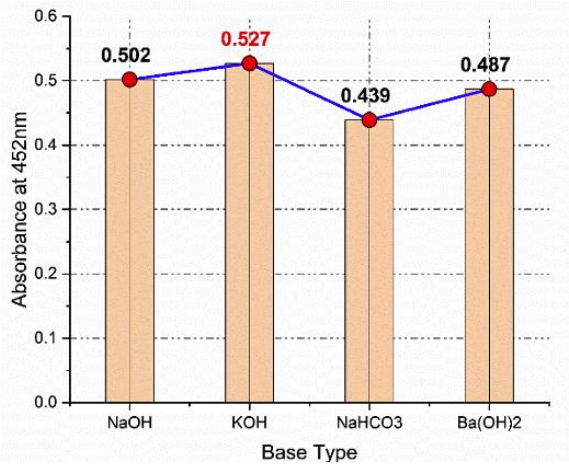


Figure 4: Base type's effect on the colored product's absorbance.

Table 4: Effect of volume on absorbance of the colored product of Bisphenol A.

Base volume (1M)	Absorbance at 452nm
0.1	0.487
0.2	0.491
0.3	0.508
0.4	0.517
0.5	0.520
0.6	0.538
0.7	0.540
0.8	**0.544**
0.9	0.535
1.0	0.527

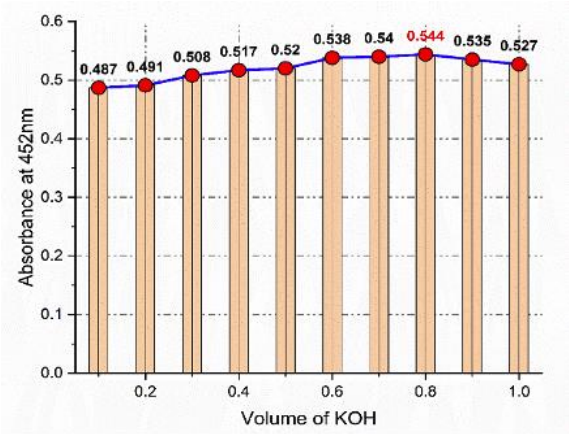


Figure 5: The effect of base volume KOH on the colored Bisphenol A's absorbance.

3.2.5 The Effect of the Optimal Volume of 1% of Sodium Nitrite

A 1% sodium nitrite solution displayed various concentration levels within the experimental setup that resulted in data display in Table 5. The amount of 1% sodium nitrite solution determines how much colored complex Bisphenol A the solution accepts according to the data presented in Figure 6. The experimental results demonstrate that using 0.4 ml of sodium nitrite produces the maximum absorption of 0.631 making it the optimal condition for Bisphenol A detection. The data shows absorption grows when sodium nitrite volume increases up until absorption starts to decrease.

Table 5: Effect of 1% volume of sodium nitrite on the absorbance of the colored product of Bisphenol A.

V of 1% Sodium Nitrite	Absorbance at λ max = 452
0.1	0.603
0.2	0.610
0.3	0.627
0.4	**0.631**
0.5	0.624
0.6	0.609
0.7	0.589
0.8	0.567
0.9	0.559
1.0	0.543

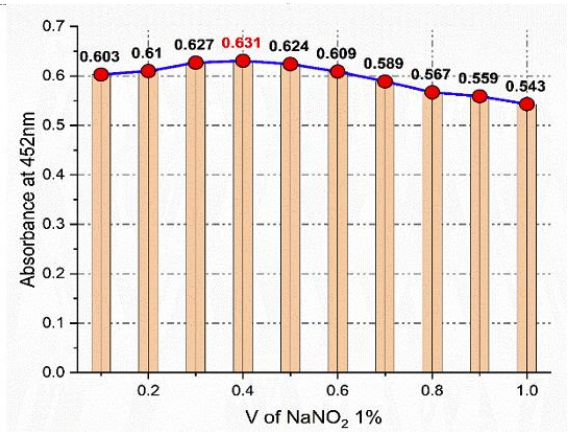


Figure 6: Effect of 1% sodium nitrite volume on the colored complex Bisphenol A.

3.2.6 Effect of Optimal Volume of 1% Urea

The data from Table 6 and Figure 7 demonstrate that using 0.6 mL of 1% urea solution yields the greatest absorbance of 0.755 for the Bisphenol A

product. The study reveals that increases in urea concentration result in higher absorbance readings until a turning point where nitrite gas formation produces rapid absorbance decreases.

Table 6: Effect of 1% volume of urea on absorbance value.

V of 1% urea	Ab at λ max = 452
0.1	0.681
0.2	0.695
0.3	0.708
0.4	0.731
0.5	0.741
0.6	**0.755**
0.7	0.734
0.8	0.692
0.9	0.650
1.0	0.634

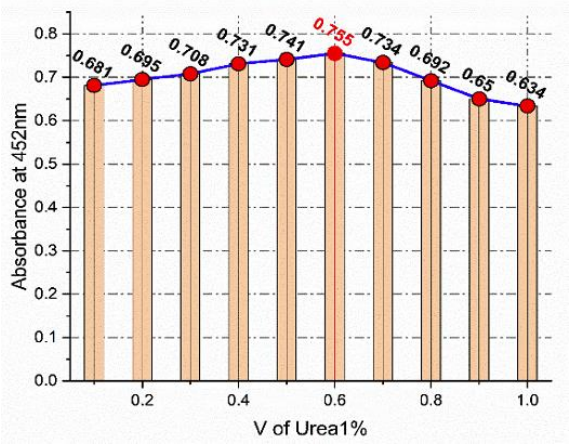


Figure 7: Effect of 1% urea volume on the colored product Bisphenol A.

3.2.7 Effect of Reagent Volume 100 µg/mL

Bisphenol A solution was added to different amounts of benzidine reagent with concentration 100 µg/mL. Results in Table 7 with Figure 8 show that the most suitable amount of benzidine reagent 0.9 mL with Bisphenol A at its peak absorbance 0.791. The absorption reaction increases with increasing reagent volume although excessive volume leads to reduced absorbance since an excessive volume limits the conjugation capacity for phenol.

3.2.8 The Effect of Reaction Time on the Product's Color Stability

The reaction period from 5-60 minutes generated multiple testing outcomes that researchers documented using Table 8 and Figure 9. The experimental results indicated a reaction completion time of 40 minutes because Bisphenol A showed the highest absorbance value at 0.822.

Table 7: The effect of reagent volume at a concentration of 100 µg/mL on absorbance value.

V of (100 µg/mL) Reagent	Absorbance at λ max = 452
0.1	0.691
0.2	0.710
0.3	0.726
0.4	0.734
0.5	0.758
0.6	0.764
0.7	0.771
0.8	0.784
0.9	**0.791**
1.0	0.751

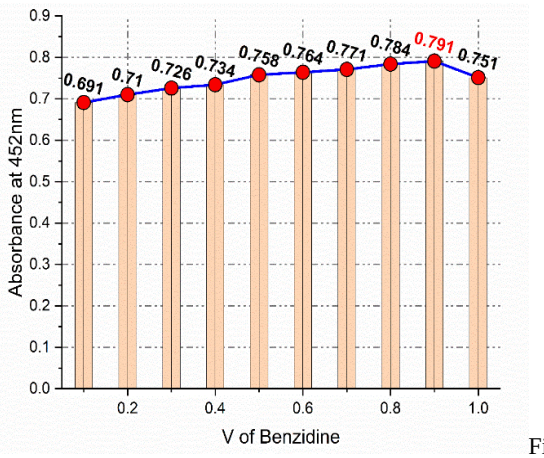


Figure 8: Effect of Benzidine volume 100 µg/ml on colored product absorption.

Table 8. Reaction time's effect on the stability of the Bisphenol A colored product.

Time (min)	Ab at λ max = 452
5	0.491
15	0.556
20	0.621
25	0.708
30	0.732
35	0.810
40	**0.822**
45	0.818
50	0.783
55	0.745
60	0.705

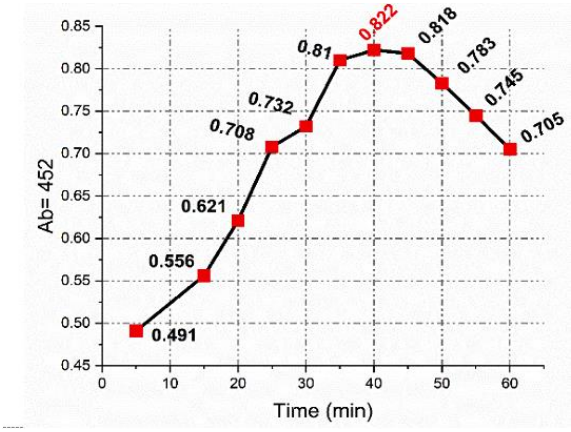


Figure 9: Reaction time's effect on the stability of a phenol complex's colored product.

3.2.9 Effect of Adding Sequence

The experimental results appear in Table 9 along with Figure 10. The optimal addition sequence for Bisphenol A production involved 1, which resulted in a maximum absorbance reading of 0.821.

Table 9. Shows the Effect of the addition sequence.

No.	Addition	λmax=452
1	ABCDEF	**0.821**
2	ABCDFE	0.440
3	EABCDF	0.750

A: Benzidine, B: Hydrochloride acid, C: Sodium nitrite, D: Urea, E: Bisphenol A, F: Sodium carbonate.

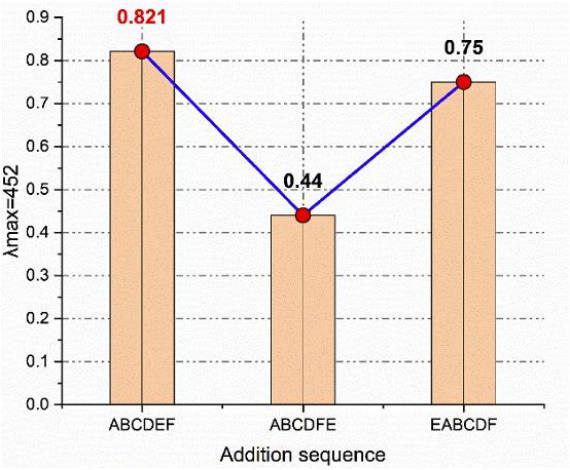


Figure 10: Effect of the addition sequence on the absorbance of the colored product of Bisphenol A.

3.2.10 Solvent Effect

The study employed a various of solvents including water and both ethanol and methanol alongside acetonitrile, 1-propanol and acetone. Data in Table 10 together with Figures 11 proves water provides optimal conditions for dissolving Bisphenol A at 0.820 as the main solvent. Water solvent stands as a top choice because it is cheap to obtain and easily accessible. The solvent operates as one of the environment-friendly solutions in existence.

Table 10: Effect of the solvent on the absorption of the colored product of Bisphenol A.

No.	Solvent	Abs
1	Water	**0.820** (in red)
2	Ethanol	0.754
3	Methanol	0.691
4	Acetonitrile	0.813
5	1-Propanol	0.793
6	Acetone	0.784

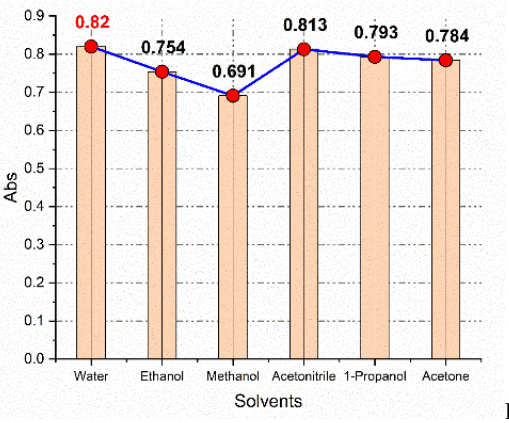


Figure 11: Effect of the solvent on the absorption of the colored product of the phenol complex.

3.2.11 Temperature's Effect on the Formation and Stability of the Colored Product

Multiple temperature conditions combined with different scientific tests formed the foundation of this study. The analysis in Table 11 and Figure 12 confirms that 30 °C represented the best temperature for Bisphenol A compound evaluation based on the highest observed absorption 0.805. Higher temperatures lead to decreased absorption strength during dissociation of the product which is evidenced through hue intensity.

Table 11: Effect of temperature on the absorption of colored product.

Temp.°C	Abs of BPA
5	0.612
10	0.744
15	0.698
20	0.782
25	0.667
30	0.805
35	0.636
40	0.783
50	0.749
60	0.694

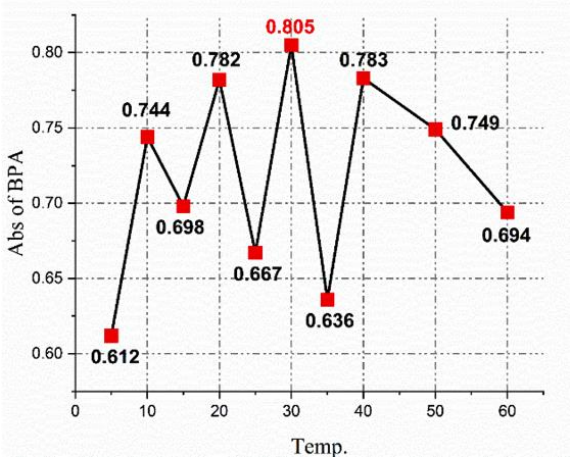


Figure 12: Temperature's effect on the colored product Bisphenol A absorbance.

3.3 The Nature of the Resulting Product

3.3.1 Method of Continuous Variation (Job's Method)

The obtained data in Table 12 and Figure 13 show that the ratio of BPA to benzidine reagent is 1:1.

Table 12: Continuous variation data for benzidine and Bisphenol A.

V of phenol/mL	V of Reagent/ mL	Abs of BPA
0.1	0.9	0.134
0.2	0.8	0.210
0.3	0.7	0.278
0.4	0.6	0.354
0.5	0.5	0.434
0.6	0.4	0.354
0.7	0.3	0.302
0.8	0.2	0.210
0.9	0.1	0.123

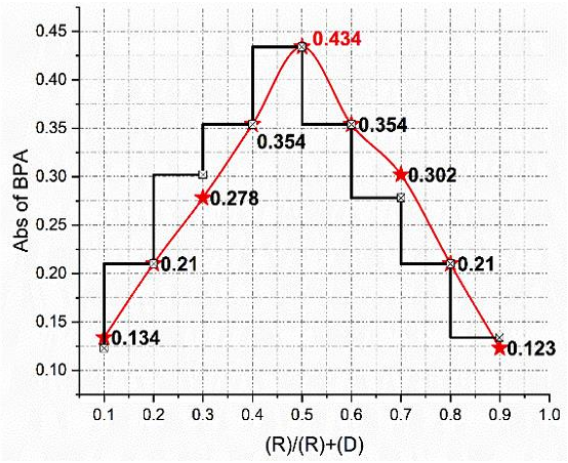


Figure 13: The method of continuous variation (JOB) for Bisphenol A.

3.3.2 Mole Ratio Method

The data presented in Table 13 and Figure 14 show that the stiochemistry ratio between reagent and phenol results 1:1. Figure 15 show the proposed.

Table 13: Absorbance values for the results of the mole ratio method for Bisphenol A, Benzidine.

Volume of phenol/mL	Volume of Reagent/ mL	Abs of BPA
0.1	0.9	0.134
0.2	0.8	0.210
0.3	0.7	0.278
0.4	0.6	0.354
0.5	0.5	0.434
0.6	0.4	0.354
0.7	0.3	0.302
0.8	0.2	0.210
0.9	0.1	0.123

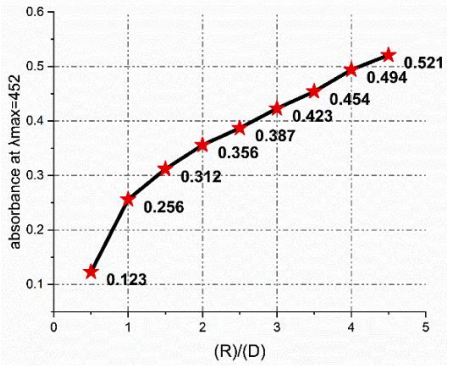


Figure 14: Curve of the Bisphenol A molar ratios method.

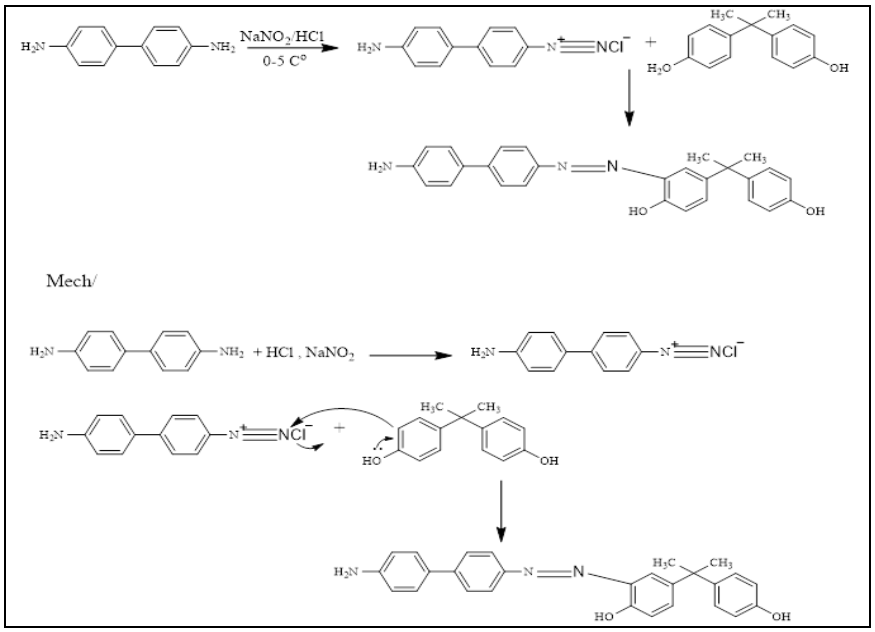


Figure 15: Proposed mechanism for the formation of the colored product of Bisphenol A.

3.4 Calibration Curve for Bisphenol A Complexed with Benzidine

The measurement of absorbance occurs at the wavelength that shows the best variation when compared to the blank solution. In a volumetric flask with 10 mL capacity added different amounts of Bisphenol A solution ranging from 1 mL at 5–14 µg/mL concentration and contained 0.6 mL of hydrochloric acid, 0.4 mL of sodium nitrite, 0.6 mL of urea, and 0.8 mL of potassium hydroxide. Table 14 and Figure 16 show a calibration curve for Bisphenol A consisting of concentrations that obeys with Beer's law within the 5 to 14 µg/mL range. The sensitivity of Sandal's reaches 0...07937 mg/cm^2 and the molar absorption coefficient for the product measures 2872.8 L/mol.cm.

Table 14: Calibration data for Bisphenol A complexed with Benzidine.

M (µg/mL)	Ab at λmax =452
5	0.135
6	0.146
7	0.161
8	0.173
9	0.184
10	0.20
11	0.210
12	0.223
13	0.234
14	0.250

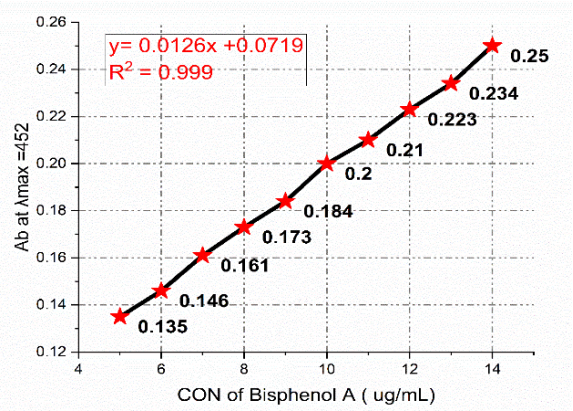


Figure 16: Calibration curve for Bisphenol A.

3.5 Interference Effect

A 1000 µg/mL solution of phenol contained 1 M of each interfering compound (Naphthol, Aniline, Benzoic acid, Nitrophenol, without interference) for testing how these substances affect the phenol. The volumetric vial containing 10ml receives further diluted distilled water after all additives reach their precise amounts. The spectrophotometer measures absorbance of Bisphenol A at wavelength 452 nm. Table 15 displays the findings for Bisphenol A. The measurement results from this table show that samples components do not influence Bisphenol A detection levels.

Table 15: Effect of interactions on the absorption of Bisphenol A.

No.	100ppm interference	Abs.
1	Naphthol	0.201
2	Naphtol	0.182
3	Aniline	0.049
4	Benzoicacid	0.137
5	Nitrophenol	0.486
6	Nitrophenol	0.399
7	Without interference	0.580

3.6 Detection Limit and Quantitative Limit for Drugs

The determination of detection and quantitative detection limits required performing ten blank solution measurements. Depending on Table 16.

3.7 Colored Output Stability Constant

The ratio between phenol and reagent stands at 1:1 according to the established method and previous calculations. Data from Table 17 demonstrates a high stability constant of the complex thus indicating its final dye remains enduring.

3.8 Accuracy and Precision Testing

The calibration curves allowed four different standard solutions (12, 9, 6, 3) to test the precision and accuracy levels of Bisphenol A. The data in Table 18 displays the influence of these characteristics. The best possible testing methods were employed to analyze five experimental replicates. Bisphenol A exhibits a recovery rate of 102.2311 which confirms high accuracy and precision in the obtained results.

Table 16: Calculating the detection limit and the quantitative limit for Bisphenol A.

Parameters	Bisphenol A
$\bar{x}^B$	0.320
$S^B = [(X_i - \bar{x})^2 / (n - 1)]^{1/2}$	0.145052
$LOD = \bar{x}^B + 3S^B$	0.3470
$LOQ = \bar{x}^B + 10S^B$	0.1190

Table 17: Stability constant data for the colored product of Bisphenol A.

V (3×10^{-4} M of Bisphenol A) /mL	As*	Am*	α	K ($L\cdot mol^{-1}$)	Mean of K ($L\cdot mol^{-1}$)
0.5	0.135	0.130	0.0384	2114.4578	9208.5843
0.7	0.161	0.159	0.0125	19276.3554	
0.9	0.184	0.180	0.0222	6234.9397	

Table 18. Accuracy and precision data for the proposed method for the determination of Bisphenol A.

Amt.BPA	12	9	6	3
F	12.1160	9.1780	6.1100	3.1244
R %	100.9667	101.9778	101.8333	104.1467
AvgR %	102.2311			
E_{rel}%	0.9667	1.9778	1.833	4.1467
Avg E_{rel}%	2.2311			
RSD%	0.0016	0.0019	0.0012	0.0003

Table 19: Data for determining Bisphenol A in samples.

Sample								
Stat. params	Water Tab		Diyala		Khresan		Mahroot	
M	10	20	10	20	10	20	10	20
F	9.2292	19.3310	9.854	19.770	9.4314	19.5242	9.3840	19.4178
R%	92.2920	96.6550	98.54	98.8530	94.3140	97.6210	93.8400	97.0890
Avg R %	94.4735		98.6965		95.9675		95.4645	
Erel %	-7.7080	-3.3450	-1.46	-1.1470	-5.6860	-2.3720	-6.1600	-2.9110
Avg Erel %	-5.5265		-1.3035		-4.029		-4.5355	
RSD %	0.0011	0.0004	0.0003	0.0016	0.0008	0.0006	0.0102	0.0077

3.9 Applications of Real Samples

A method to detect Bisphenol A measures its concentration in water solutions. The analytical technique is applied to various Bisphenol A samples that have a concentration between 10 and 20 mg/Meto1. The experimental findings in Table 19 demonstrate how the proposed testing method performs successfully to detect Bisphenol A in various samples. The results obtained are shown in Table 20.

Table 20: Statistical results of the proposed spectral method for estimation Bisphenol A.

Parameter	Bisphenol A
Colour of Product	Dark yellow
λ max	452 nm
Regression equation	y = 0.0126x + 0.0719
Standard deviation of regression	0.145052
Correlation coefficient (r)	0.99
C.L for slope (b±tSb) at 99%	0.012600 ± 0.3225475
C.L for Intercept (b±tSb) at 99%	0.07190 ± 0.61550
Concentration range (µg mL^{-1})	(5–14) mg/mL
Limit of Detection (µg mL^{-1})	0.3470
Limit of Quantitative (µg mL^{-1})	0.1190
Sandell's Sensitivity (µg mL^{-1})	0.07937
Molar absorbance ($L \cdot mol^{-1} \cdot cm^{-1}$)	2872.8
Composition of product	1:1
Recovery %	102.3211
RSD% n=4	0.00125
C.L for con.12 (µg mL^{-1})	12.1160 ± 0.04014
C.L for con.9 (µg mL^{-1})	9.1780 ± 0.03683
C.L for con.6 (µg mL^{-1})	6.1100 ± 0.01456
C.L for con.3 (µg mL^{-1})	3.1244 ± 0.0184

4 CONCLUSIONS

A reliable and sensitive spectrophotometric method was developed for the quantitative determination of Bisphenol A (BPA) based on an azo coupling reaction with benzidine. The method is characterized by a stable and intensely colored azo dye product, formed under optimized conditions of pH, reagent concentration, and reaction time. It exhibits excellent linearity over a broad concentration range, obeying Beer's law with a high correlation coefficient ($R^2 > 0.999$), and demonstrates high molar absorptivity, precision (low RSD%), and accuracy (recovery within acceptable limits). Low detection and quantification limits confirm the method's capability for trace-level analysis of BPA. Application to real environmental samples, including drinking water and industrial effluents, confirmed its practical applicability and reliability. Furthermore, the technique is simple, cost-effective, and does not require advanced instrumentation, making it a suitable tool for routine environmental monitoring of BPA contamination in water sources.

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Determination of Tetracycline Hydrochloride by Using Dapsone as a Reagent in Developed Spectrophotometric Analysis

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Keywords: Spectrophotometric, Dapson, Diazotization, Tetracycline Hydrochloride.

Abstract: Inexpensive spectrophotometric method has been developed to estimate its pure concentration. This method is based on the azo coupling reaction using Diaz onium dapsone, which is formed in a solution containing hydrochloric acid and sodium nitrate. The resulting salt reacts with tetracycline hydrochloride to form a Tetracycline hydrochloride is considered an antibiotic, and a sensitive, easy, fast, and orange azo dye at a wavelength of 380 nanometres. After optimizing the reaction conditions, a recovery rate of 100% was achieved, with a Beer-Lambert limit between (2 - 26) µg /mL, and a Sandal sensitivity of 0.0238 µg.cm². The method also demonstrated high sensitivity without any interference from the excipients present in the formulation, with a sensitivity value of 20160 $L \cdot mol^{-1} \cdot cm^{-1}$ and a correlation coefficient of 0.9992. The limit of detection (LOD) was found to be 0.094 and the limit of quantification (LOQ) was found to be 0.28. The reaction was stable for a sufficient period, making the method suitable for estimating tetracycline in its pure form and in pharmaceutical preparations, such as capsules, without any interference.

1 INTRODUCTION

Tetracycline in IUPAC nomenclature (4S,4aS,5aS,6S,12aR)-4- (dimethyl amino)-1,6,10,11,12a- pentahydroxy-6-methy l-3, dioxo-4,4a,5,5,5a -tetrahydrotetracene-2- carboxamide; hydrochloride 12- (Fig. 1).

They are yellow crystals [1], soluble in water, resulting in a clear yellow solution that can become cloudy if left for a long time. It acts as an antibiotic to eliminate bacterial growth such as acne and urinary tract infections, as it is active against a variety of gram-positive and gram-negative bacteria. It can be used as an anti-ulcer medication in the treatment of acute bronchitis [3], [4], it is economical [5], and in the treatment of certain types of diseases, including cholera, trachoma, rickettsia [6], and others. Due to the importance of this drug, there are many analytical methods to estimate the purity of tetracycline hydrochloride, the most important of which are spectroscopic methods [4]-[9], HPLC [10], flow injection techniques [11]-[14], and TLC [15]. Multispectral determination [16], effective absorption and photo degradation using visible light [17], fluorescence spectrum [18], as well as nanotechnology methods [19], [20].

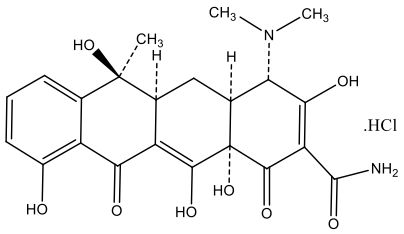


Figure 1: Structure tetracycline hydrochloride [1], [2].

2 EXPERIMENTAL

2.1 Preparation of Solutions

A solution of tetracycline hydrochloride with a concentration of 1000 µg/ml.It was prepared with a weight of 0.1020 grams of the pure substance, which was later dissolved in distilled water. The solution was then transferred to a 100 ml flask, where the volume was completed with the solvent, water, until reaching the mark on the flask. This is the stock solution, and a solution with a

concentration of 250µg /ml is prepared from the stock solution.

2.2 Preparation of a 4× 10-3 M Dapsone Solution

0.1 g of the substance were weighed and dissolved in 25 ml of ethanol in a 100 ml glass flask. Then, the volume of the flask was completed with distilled water to the marked line of the flask.

2.3 Sodium Nitrate 0.01 M

0.06901 grams of the solid were weighed and then dissolved in distilled water. After that, the volume of the flask was filled to the specified mark of 100 ml.

2.4 Preparation of a 1N Hydrochloric Acid Solution

The dilution law was used for the concentrated solution with a concentration of 11.9 M, and from this solution, using the same law, a 0.05 N solution with a volume of 100 ml was prepared. The solvent was distilled water.

2.5 Preparation 1N Sodium Hydroxide Solution

A weight of 4.001 grams of the solid was taken and dissolved in distilled water. The volume was completed until the mark on the 100 ml bottle was reached.

3 SETTING THE OPTIMAL CONDITIONS

A study was conducted to determine the optimal amount of hydrochloric acid solution, which gave the highest absorption of the colored product. 1 ml of the reagent and 1 ml of sodium nitrite were added, along with increasing volumes of acid ranging from 0.5 to 2.5 ml of hydrochloric acid, 1 ml of the drug, and 1 ml NaOH. The results indicated that 1.5 ml of acid is the most suitable volume, and this volume was used for subsequent experiments. Table 1.

3.1 Study of the Effect of Sodium Nitrite

A study was conducted to determine the optimal amount of sodium nitrite solution, which gave the highest absorption of the colored product. 1 ml of the reagent and increasing volumes of sodium nitrate from (0.5-2.5) ml, and 1.5 ml of hydrochloric acid, 1 ml of the drug, and 1 ml NaOH. The results indicated that 1 ml of sodium nitrite is the most suitable volume, and this volume was used in subsequent experiments. Table 2.

Table 1: Results in the table No. (1) Quantitative study of acid.

Volume ml (HCl) 0.05 N	0.5	1	1.5	2	2.5
ABS	0.453	0.468	0.489	0.485	0.487

Table 2: Study of the effect of sodium nitrite.

NaNO2(ml)	0.5	1	1.5	2	2.5
0.01 M ABS	0.461	0.479	0.471	0.472	0.470

3.2 Study of the Effect of Sodium Hydrochcide

A study was conducted to determine the optimal amount of sodium hydroxide solution that gave the highest absorption of the colored product. 1 ml of the reagent, 1 ml of sodium nitrite, 1.5 ml of hydrochloric acid, and 1 ml of the drug were added, along with increasing volumes of sodium hydroxide (2.5-0.5 ml). The results indicated that 1 ml of sodium hydroxide is the most suitable volume, and this volume was used in subsequent experiments. Table 3.

Table 3: Study of the effect of Sodium hydrochcide.

NaOH (1N): ml	0.5	1	1.5	2	2.5
ABS nm	0.468	0.488	0.485	0.487	0.484

3.3 Study the Effect of Dapsone (Reagent) con. 4× 10-3 M

A study was conducted to determine the optimal amount of reagent solution, which gave the highest absorption of the colored product. Increasing volumes of the reagent (0.3 - 2) ml, 1 ml of sodium nitrite, 1.5 ml of hydrochloric acid, 1 ml of the drug, and 1 ml sodium hydroxide were added. The results indicated that 1 ml of the reagent is the most suitable volume, and this volume was used in subsequent experiments. Table 4.

Table 4: To study the effect of dapsone (Reagent) Reaction sequence.

Reagent 4×10-3M	0.3	0.6	1	1.5	2
1000µg/ml ABS	0.366	0.393	0.483	0.480	0.482

3.4 Study of the Effect of the Formed Interaction Time

The effect of time was studied to find the necessary time to reach the stable product in the equilibrium state and completion of the reaction under optimal conditions (1 ml of the reagent, 1 ml of sodium nitrite, 1.5 ml of hydrochloric acid, 1 ml of the drug, and 1 ml of sodium hydroxide). It was observed that under these conditions, the colored product is formed as the absorption increases over time, and the color stabilizes after at least 10 minutes and remains stable for 40 minutes due to the stability and permanence of the product formed over time. Table 5.

4 FINAL ABSORPTION SPECTRUM

When the drug is coupled with the reagent under experimental conditions, the resulting product has an orange color. The result is measured after 10 minutes, and the highest absorption was measured at a wavelength of 380 nanometers compared to the reference solution. Figure 2 illustrates this, showing the resulting product against the reference solution and the absorption spectrum of the reference solution against distilled water and the reference solution against distilled water.

Table 5: Study of the effect of the formed interaction time.

Time minute	0	10	20	30	40	50	60
Abs/ nm	0.454	0.485	0.483	0.438	0.444	0.434	0.399

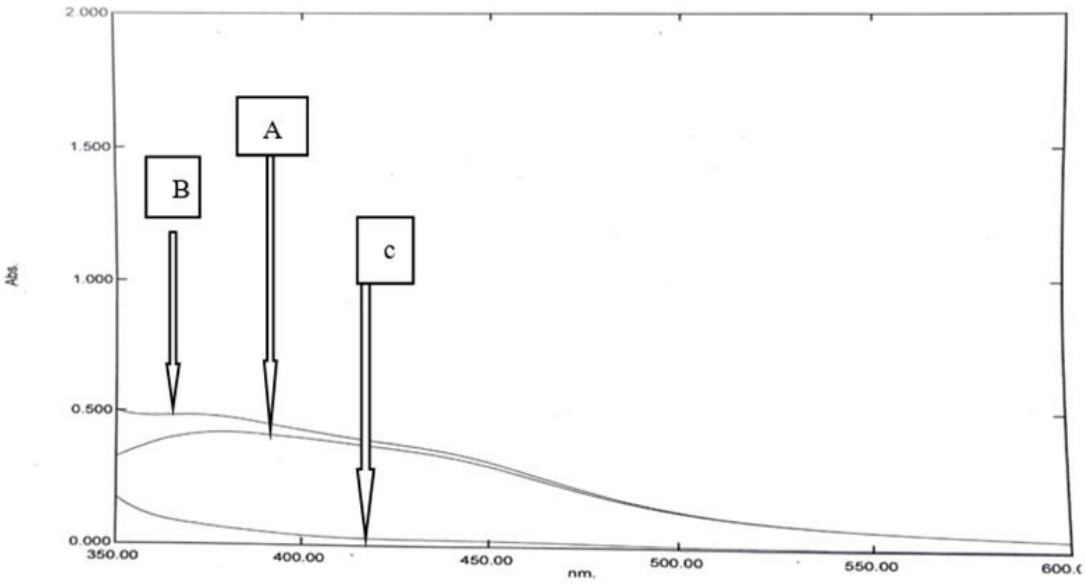


Figure 2: The final (A) absorption spectrum of tetracycline hydrochloride under optimal conditions against the blank solution, (B) absorption spectrum of tetracycline hydrochloride against distilled water, and (C) the blank solution against distilled water.

5 CALIBRATION CURVE

After establishing the optimal conditions, a series of 25 ml glass vials were taken, and 1 ml of the reagent, 1 ml of sodium nitrite, and 1.5 ml of hydrochloric acid were added, along with increasing volumes of the drug 0.2 – 26 ml and 1 ml of 1 M sodium hydroxide. The volume was then completed to the marked line with distilled water. The samples were left for a period of time, and then the absorbance was measured at a wavelength of 380 nm against the blank solution, as shown in Figure 3, which demonstrates Beer's Law for the drug tetracycline hydrochloride at concentrations of 2 - 26 µg/ml in a final volume of 25 ml. The molar absorptivity was calculated from the (1) - (7):

$$Ɛ = a \times 1000 \times M = 20160, \quad (1)$$

$$S = M/Ɛ = 0.0238, \quad (2)$$

$$L.O.D = 0.094, \quad (3)$$

$$Ɛ = L.mol\text{-}1.cm\text{-}1, \quad (4)$$

$$a = ml.µg\text{-}1.cm\text{-}1, \quad (5)$$

$$M = g.mol\text{-}1, \quad (6)$$

$$S = µg.cm\text{-}2. \quad (7)$$

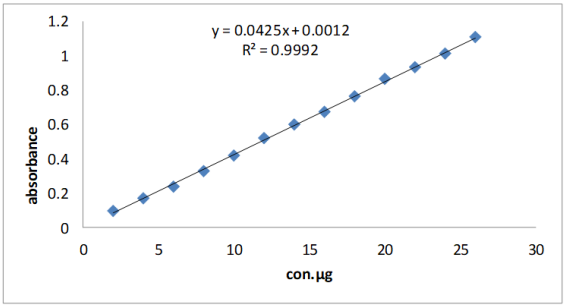


Figure 3: Calibration curve for tetracycline hydrochloride.

6 PRECISION AND COMPATIBILITY OF PURE MATERIAL

Accuracy is the extent to which the measured result is close to the true or accepted value.
Repeatability refers to the degree of repeatability and consistency in the results when repeated measurements are made on the same sample The analysis is considered good if the results are close to each other Table 6.

Table 6: Precision and compatibility of pure material.

RSD%	Average recovery%	Recovery%	Average RE%	Average Abs nm	Con. µg/ml
1.52	-	100	0.011	329	8
2.62	-	100	0.016	609	15

7 THE NATURE OF THE RESULTING OUTPUT

7.1 Job Method

The continuous variable method was used to determine the interaction percentage of the drug tetracycline hydrochloride with the reagent. This method relies on the changes that occur in the solutions with the drug and the reagent. This method was carried out by following the procedure outlined below. Figure 4. Different solutions were prepared containing increasing volumes of the drug from 1-9 ml, and the reagent from 1-9 ml, both at a concentration of 250µg/ml (5.4×10-4 M) in a final volume of 25 ml. The remaining solutions were added under optimal working conditions, and the reaction output was measured against the blank solution at a wavelength of 380 nanometers, indicating a reaction ratio of 1:1.

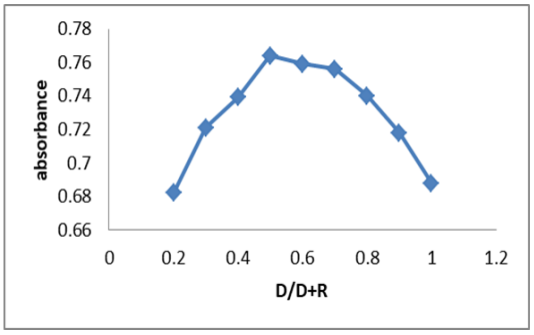


Figure 4: The curve of continuous variables (Job's method of the result formed from the interaction of the solvent and the reagent) between the drug and the reagent-(Dapson)/(tetracycline hydrochloride) + (Dapson).

7.2 Mole Ratio Method

A series of 25 ml volumetric flasks were prepared, and varying volumes of the reagent were added. The remaining variables obtained under optimal conditions were added, followed by the addition of 1 ml of the drug to each flask at the same drug concentration. The absorbance was then measured against the blank solution at a wavelength of 380 nanometers. Figure 5.

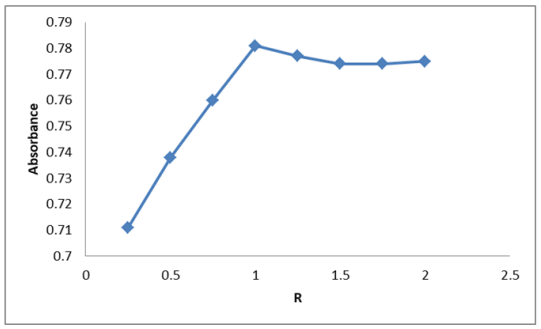


Figure 5: The molar ratio curve of the product formed by the nitration reaction and the coupling, between the drug and the reagent. () tetracycline hydrochloride /(Dapson).

8 REACTION EQUATION

The azo coupling reaction between tetracycline hydrochloride and Dapsone is depicted in Figure 6.

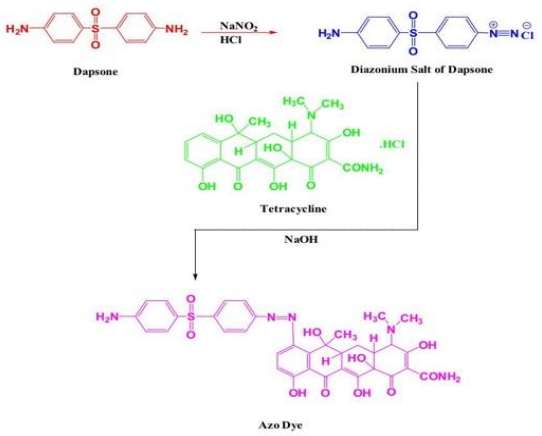


Figure 6: Reaction equation.

9 APPLICATIONS

After the developed method was successful, it was applied to the preparation and different concentrations (30, 15, 8) micrograms/ml were taken. The same working steps followed when preparing the standard curve were applied, and the efficiency of the method was demonstrated as shown in the following Table 7.

$$A1 / A2 = C1 / C2 \quad (8)$$

$$C2 = A2\ C1 / A1 \quad (9)$$

Table 7: Determination for six.

	Amount taken µg/ml	Amount found µg/ml	Recovery,* %	Average Recovery	RSD,* %
Tetracycline Hydrochloride, Company: SDI	8	8.2	102	101.3 %	2.2
	15	15.1	100		2.1
	30	30.8	102		4.0

10 THE STANDARD ADDITIONS METHOD

The experiment was conducted using standard additives to prove that the developed method is reliable, achieves high efficiency, and is not affected by interferences, as shown in the following Figure 7.

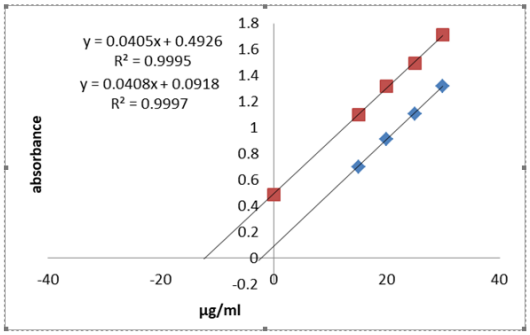


Figure 7: The standard additions method.

From the graph of the standard addition method, the following results emerged Tables 8 and 9.

Table 8: Standard additions method.

Tetracycline hydrochloride	Amount taken	Amount found	recovery	company
	10 ppm	9.95	99.5%	SDI

Table 9: Optical characteristics of the method.

Optical characteristics of the method	
λmax	380 nm
The limits of linearity µg/ml	2-26
Molar absorptivity L.mol-1.cm-1	20160
Color of the dye	orange
Recovery	100%
Sandal allergy µg.cm-2	0.0238
Slop	0.0425
Intercept	0.0012
LOD µg/ml	0.09
LOQ µg/ml	0.2

11 CONCLUSIONS

A simple and accurate spectrophotometric method was developed for the determination of tetracycline hydrochloride, based on the azo coupling reaction between tetracycline hydrochloride and a suitable chemical reagent, resulting in the formation of a colored complex with easily measurable absorbance. Experimental results demonstrated that this method exhibits high reproducibility and excellent accuracy, along with resistance to potential interferences from other components in pharmaceutical samples. The method's linear range was evaluated, showing a proportional response across a wide concentration range, thereby enhancing the reliability of the results. Moreover, this technique is characterized by its simplicity and cost-effectiveness, making it suitable for routine application in quality control laboratories and pharmaceutical product monitoring. Based on these attributes, this method can be considered a reliable and efficient analytical tool for the determination of tetracycline hydrochloride in various dosage forms, contributing to the assurance of pharmaceutical product quality and safety.

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Comparative Analysis of Vegetable Chemical Compounds from Orange Peel Extract and Thuja Plants Using Gas Chromatography Mass Spectrometry Technique

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Keywords: Thuja Leaves, Orange Peel, Gas Chromatography Mass Spectrometry , Phytochemical Analysis, Alcoholic Extract.

Abstract: The present study was carried out to investigate the chemical composition of the alcoholic thuja and oranges peels extract using Gas Chromatography – Mass Spectrometry (GC-Ms). Fifty-five compounds were determined for Thuja leaves extract, most notably the D-Limonne, Ferrgoinol, and Terpine-4-IL, which are known as its antioxidant and microbial properties. In the orange peel extract, it was determined 78 compounds, including nootkatone, Squalene and D-Limonne, known as its role in the pharmaceutical industries and cosmetics. The current study analyzes indicated that the use of methanol alcohol in the Soxhlet system increased the efficiency of extracting semi -polar compounds such as phenols and glycosides and flavonoids. The current study also emphasized the sensitivity of the GC-MS technology in detecting volatile and non-polar compounds, such as D-Limonne, which appeared in both extractors. The study emphasized the importance of choosing the technique of extraction and the type of solvent in enhancing the efficiency and quality of the extracted vehicles. the present study also offers a valuable chemical database for the optimal exploitation of these plants in the medical, agricultural, and industrial fields, in line with the principles of environmental sustainability.

1 INTRODUCTION

Plant extracts are natural treasures rich in various chemical compounds that play a vital role in various fields, medical, industry and agriculture [1]. These compounds are derived from the secondary metabolism of the plant, such as phenols, rectaloids, alkaloids, and flavonoids, which show distinctive biological activities such as antioxidants, antimicrobials, and anti -inflammatory properties. It is also alternatives that can be applied as natural insecticides, which seemed industrial, due to the diversity of the active chemical compounds that contain and subject to biological decomposition, which reduces the harmful environmental effects [2]. Thuja is an important plant in proportion to scientists because it contains phenolic compounds with anti - bacterial and antioxidants [3]. Because of its association with proteins, it is used in industries and also on compounds that prevent the growth of germs and fungi with high efficiency [4]. Orange peels, which are taken from orange fruit, are widely used because they contain volatile compounds such as lemon, flavonoids and glycosides, which work to promote heart health and reduce oxidative stress [5]. Essential oils extracted from orange scales - rich in chemical compounds - can be used as effective natural pesticides, which helps protect soil and water from chemical pollution caused by artificial pesticides [6]. The active oils extracted from the leaves of the thuja plant and orange peels are one of sustainable products to reduce the harmful environmental effects as their ability to manage agricultural pests effectively due to the chemical components in both extractors. The GC-MS is the separation and identification of volatile and semi-volatile chemical compounds that make it a very accurate analytical tool for the detection of chemical compounds in plant extracts [7]. This technique has been successfully used to analyze secondary

compounds in different plants, providing visions about its chemical diversity and potential applications [8]. This study is compared chemical compounds for the extract of the leaves of Thuja plant and orange peel using gas- chromatography technique. This analysis is important for understanding the differences in phenolic, triny and aromatic compounds, which may explain the contrast in its biological activity. These results contribute to enhancing our understanding of how these plants are optimally used in industrial or medicinal environments due to their effective chemical properties.

2 MATERIALS AND METHODS

The fruits of orange plants (family Rutaceae.) were harvested in the Bohriz, Baquba district, Diyala Governorate, Iraq, from November to February for its orange peels and the leaves of the thuja plant (cypress family).

2.1 Preparing the Sample

Thuja and orange peels were washed to remove dust and dirt using sterile distilled water, then it was dried with air at room temperature ((25°C ± 2°C)) in a dark place to avoid the decomposition of active compounds, according to standard protocols to prepare plant samples [6].

Thuja leaves and orange peels were left to dry in room temperature. After drying, the two plants were ground using an electric grinder to obtain the powder of thuja leaves and orange peel to increase the contact area with the solvent as shown in Figures 1 and 2.



Figure 1: Steps for preparing ground thuja leaves.



Figure 2: Steps for preparing ground orange peels.



Figure 3: Steps for extracting thuja leaves using a Soxhlet.



Figure 4: Steps for preparing orange peel extract using the Soxhlet device.

2.2 Soxhlet Preparation

The samples were weighed using a sensitive balance. Fifty grams of the dried samples were put inside a Whatman no1 filter paper funnel and sealed tightly. The Soxhlet was used with 400 mL of methanol for 6 hours. The sample extract was cooled and transferred to a rotary evaporator for 7 minutes to concentrate the sample to 50 mL, as shown in Figures 3 and 4.

2.3 Chemical Analysis

The chemical analysis of the thuja leaf and orange peel extracts was performed. Chemical compounds were identified using gas chromatography-mass spectrometry (GCMS), using Agilent Technologies A GC System GC-MS 7890, manufactured in the USA, from the Basra Oil Company - Laboratory Division. After starting at 40°C, the temperature in the column was raised by 10°C every minute until it reached 300°C. The helium carrier gas flow rate was 1 ml per minute. One microliter of the extract was injected, and peaks were identified using the National Institute of Standards and Technology (NIST) database and the device's memory list [9].

3 RESULTS AND DISCUSSION

3.1 Results

The chemical composition of the plant extract was analyzed using GC-MS, an advanced technology characterized by its high efficiency, specificity, and

sensitivity in analytical chemistry applications. As indicated in Table 1 and 2, this technique was used to investigate the compounds found in the alcoholic extract of orange peels and thuja leaves. For the chemical compounds determined in thuja extract, the fifty five peaks obtained are displayed in the gas chromatography-mass spectrometry diagram in Figure 5. D-limonene, terpinen-4-ol, p-cymene, ferruginol, gamma-terpinene, trans-4-(hexyloxy) chalcone, citronellol, alpha-bisabolol, 2-tridecanone, and myo-inositol 4-C-methyl-were the most effective chemical compounds (Fig. 5). The most ten bioactive chemical compounds were identified for extract of thuja (Table 1).

The most ten effective chemical compounds identified were ibuprofen, nootkatone, D-Limonene, squalene, 1-Methyl-5-fluorouracil, lactose, megastigmatrienone, fercomin, 2-Methoxy-4-vinylphenol, and thymine (Table 2). A total of 78 peaks were obtained for the chemical compounds used, which are displayed in the gas chromatography-mass spectrometry diagram shown in Figure 6.

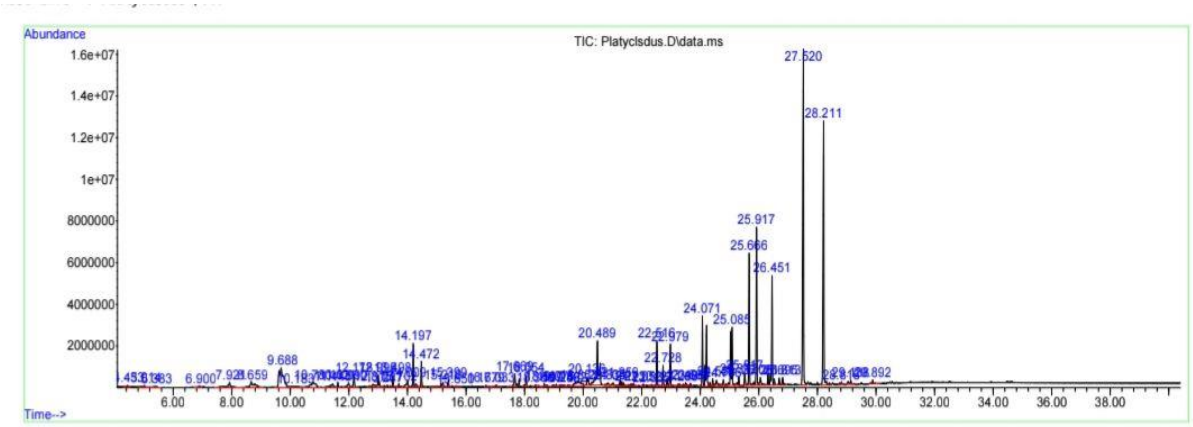


Figure 5: Gas chromatography-mass spectrometry of the alcoholic extract of Thuja leaves.

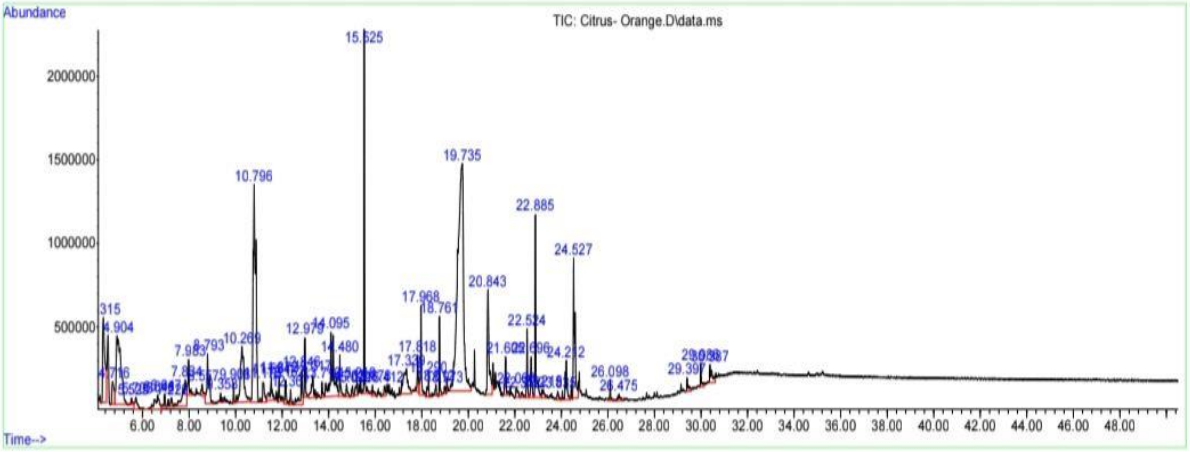


Figure 6: Gas chromatography-mass spectrometry of the alcoholic extract of orange peels.

Table 1: Phytochemical compounds identified in the thuja leaf extract.

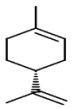
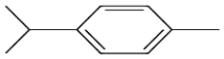
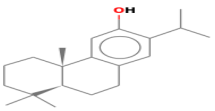
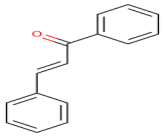
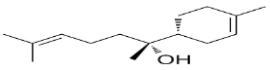
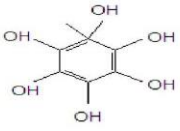
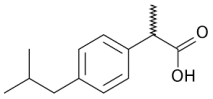
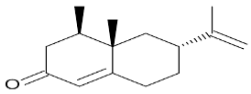
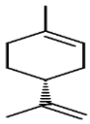
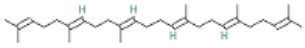
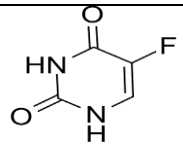
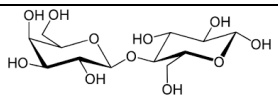
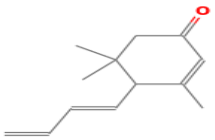
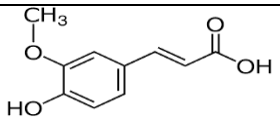
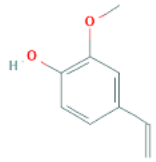
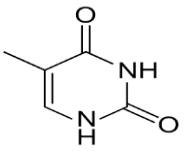
Number	Name	Formula	M.W	CAS ID	Area	RT	Formula for structure
1	D-Limonene	$C_{10}H_{16}$	136	5989-27-5	932689	10.768	
2	Terpinen-4-ol	C10H18O	154	562-74-3	987614	13.5	
3	p-Cymene	C10H14	134	99-87-6	611922	10.802	
4	Ferruginol	C20H30O	286	514-62-5	8430109	26.448	
5	. gamma. -Terpinene	C10H16	136	99-85-4	872086	11.447	
6	trans-4-(Hexyloxy)chalcone	C21H24O2	308	99259-59-3	12150399	25.919	
7	Citronellol	C10H20O	156	106-22-9	3538529	14.198	
8	. alpha. -Bisabolol	C15H26O	222	515-69-5	855408	20.134	
9	2-Tridecanone	C13H26O	198	593-08-8	540903	17.807	
10	Myo-Inositol, 4-C-methyl-	C7H14O6	194	472-95-7	3127481	20.518	

Table 2: Phytochemical compounds identified in orange peel extract.

#	Name	Formula	M.W	CAS ID	Area	RT	Formula for structure
1	Ibuprofen	C13H18O2	206	15687-27-1	169070	19.367	
2	Nootkatone	C15H22O	218	4674-50-4	223622	21.607	
3	D-Limonene	$C_{10}H_{16}$	136	5989-27-5	6801365	10.794	
4	Squalene	C30H50	410	111-02-4	189969	29.986	
5	1-Methyl-5-fluorouracil	C5H5FN2O2	140	1000427-92-0	1113101	12.977	
6	Lactose	C12H22O11	342	63-42-3	607049	22.696	
7	Megastigmatrienone	C13H18O	190	38818-55-2	249880	19.539	
8	Fercomin	C23H30O5	254	104758-20-5	163536	26.095	
9	2-Methoxy-4-vinylphenol	C9H10O2	150	7786-61-0	3541874	15.527	
10	Thymine	C5H6N2O2	126	65-71-4	310941	11.869	

4 DISCUSSION

Advanced analytical methods, such as GC-MS, help identify chemical compounds in plant extracts and understand their different biological properties better [10]. Thuja leaf extract analysis was performed in GC-MS, which had determined numerous chemical compounds, including D-Limonne, Terpine-4-WOL and Ferruginol. These results correspond to Sharma study and his colleagues [11] . They demonstrated that the Thuja extract contained a lot of turbine components, especially the D-Limonne, which constitutes 15-25 % of the total essential oil. Moreover the results of [12] supported what was obtained from the current results that terpinene-4-IL is one of the main components of Thuja extracts, as they confirmed its presence in concentrations ranging from 8 to 12%. The presence of these compounds has biological importance, as Wang and others [13] reported that the presence of virogenel enhances a high capacity against free radicals and infection-causing pathogens. Furthermore, in accordance with the extraction technique, the study pointed out that the concentration of virogenol is greater in the alcoholic in comparision with the aqueous extracts. In a study conducted by Naguin and others [14], they were reported that gamma-terpinene had antimicrobial activity against both gram-positive and gram-negative bacteria, which enhanced the bioactive properties of the extract as a natural antibiotic. The analysis of orange peel extract showed 78 chemical compounds, the most important chemical compounds were D-Limonne, Squalene and Nootkatone. The presence of D-Limonne is compatible with Liu et al. [15] who they reported this substance makes 68-98 % of the essence of orange peel therapeutic oil. The presence of D-limonene is directly correlated with the quality of the extract and its antioxidant efficacy. The finding that orange peel extract contains squalene is significant because of its various health benefits. Recently, Martinez-Rodriguez et al. [16] suggested that squalene had numerous biological effects such as anti-inflammatory and antioxidant action and proposed that it should be used to improve heart health. The study also stated that using plant products like orange peels to extract squalene is more environmentally friendly than using animal sources. The findings from the study have confirmed the presence of a phenolic compound called 2-methoxy-4-vinylphenol in orange peel extract, which is recognized for its antioxidant properties. This aligns with the essay conducted by Kim and his team [17], where they found this powerful phenolic antioxidant in various plant extracts, essay serving in protect against oxidative stress. In examining Warumuh Zimat's extractors, it was observed that both contained D-Limonene, highlighting the significance of this compound as a key element in aromatic plants. This observation Supported by Mahato and colleagues [18], who noted that D-Limonene is a common component in many plant extracts, particularly those from the Citrus and Pine families. Despite similarities in presence numerous compounds , there is considerable variation in the composition of extracts from different sources. For instance, fruit extracts tend to have higher levels of certain compounds like Terpine-4-OL and GAMMA-TERPINENE, while orange peel extracts are rich in squawen, nootkatone, and other unique compounds. These variations are further explored in a recent study by Chen et al. [19], which highlighted that the differences in chemical composition of plant extracts arise from genetic factors, environmental influences, and the extraction methods used. The striking similarity notwithstanding, there exists great diversity in the composition in the extracts of the different extractors. For one, the fruit extracts contain higher amounts of turbine compounds such as Terpine-4-IL and GAMMA-TERPINENE, whereas orange peel extracts contain squawen, nootkatone, and other compounds. These differences provide scope to a recent study conducted by Chen et al [19] [20] that pointed out that the differences in the chemical composition of plant extracts are the resultant of inheritance, influences of the surroundings, and also the techniques and methods of extraction that employed.

5 CONCLUSIONS

This research has effectively showcased the chemical abundance and phytochemical variety of extracts from Thuja leaves and orange peels via GC-MS analysis. This study confirms that Thuja occidentalis (Thuja) leaf and Citrus sinensis (orange) peel extracts contain bioactive phytochemicals with promising applications in medicine, agriculture, and industry. Methanol-based Soxhlet extraction demonstrated high efficiency in isolating these bioactive compounds. GC-MS analysis identified several key constituents—including D-limonene, ferruginol, terpine-4-ol, nootkatone, and squalene—which exhibit properties relevant to pharmaceuticals (e.g., antimicrobial and anti-inflammatory agents),

cosmetics (fragrances and skin-care formulations), and eco-friendly agrochemicals (biopesticides). Which confirms the efficiency of this technology. In the Thuja extract, 55 phytochemicals were identified, while 78 compounds were found in the orange peel extract. Essential components like D limonene, terpinen-4-ol, ferruginol, nootkatone, and squalene demonstrate well-established biological effects, featuring antimicrobial, antioxidant, and anti-inflammatory characteristics. The existence of overlying compounds like D-limonene in both extracts indicates a common therapeutic potential, whereas distinctive compounds such as squalene in orange peel and ferruginol in Thuja emphasize plant-specific benefits.

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Synthesis, Spectral Characterisation and Biological Evaluation of a New Azo-Ligand Derived from Aminoacetophenone with its Metal Complexes

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Keywords: (E)-3-((4-Acetylphenyl)Diazenyl)-2-Hydroxy-1-Naphthaldehyde, Metal Complexes, Azo Ligand, Proteus Mirabilis, 4-Aminoacetophenone.

Abstract: Synthesis and characterisation of a new azo ligand based on a 4-aminoacetophenone moiety, along with its metal complexes. The synthesis of the ligand, ((E)-3-((4-acetylphenyl)diazenyl)-2-hydroxy-1-naphthaldehyde) (HL), was obtained from the interaction of 4-aminoacetophenone with 2-hydroxynaphthalene-1-carbaldehyde in a one-to-one molar ratio. Subsequently, the interaction of HL with various metal ions (Cr(III), Mn(II), Co(II), Ni(II) and Cu(II) in the ratio of one ligand to one metal, produced monomeric coordination connections. These compounds have been fully characterised using analytical methods and spectroscopic techniques, involve elemental microanalysis,^{1}H and ^{13}C-nuclear magnetic resonance, Fourier transform infrared spectroscopy, electronic and mass spectroscopy, as well as magnetic susceptibility and Conductivity measurements. The analytical data verified the formation of coordination compounds exhibiting six-coordinate geometries. The antibacterial evaluation of the ligand and its coordination complexes against bacterial strains and fungus species showed a significant enhancement in the effectiveness of the antimicrobial ligand when forming complexes with metal ions. In addition, the study evaluated the antibacterial activity of synthesised compounds with a number of bacteria and fungi. The complex formation significantly enhanced the antibacterial efficacy of the ligand.

1 INTRODUCTION

The chemical compounds characterised by the functional group RN=NR called Azo dyes′, where R and R′ are typically aryl groups [1]. It is frequently used to create mono-azo dyes, which are marketed as coloring agents [2]-[6]. They could include [1]-[3] azo linkages connecting phenol group and naphthalene. Strong colours distinguish azo dyes, they are thought to constitute the majority of the synthetic dyes generated today. This class of commercially available organic dyes has the highest structural variety and a wide range of uses [7], [8]. Azo dyes are primarily synthesised through the diazotisation of a primary amine, followed by conjugation with one or more electron-rich nucleophiles, including amines or hydroxyl groups [9]. Azo dyes have special characteristics, including molecular aggregation and optical data storage capacity. In addition, they have been used in various fields of activity, such as textile fibre colouring, plastic, leather, metal chips, food products, cosmetics, toys, plastics, biological and clinical research, and even organic composition [10], [11]. Azo-dyes exhibit biological activity, including antibacterial, antifungal, anti-HIV, and anticancer properties. Therefore, they are of enormous importance in medical chemistry [12], [13]. A considerable amount of work was devoted to the advancement and characterisation of excellent azohydroxyl ligands, which possess the capability to chelate metal ions and create complexes with different configurations of structures [14]. In recent years, we have documented the production of azo chemicals and the complexes they create [15]-[17]. This chemical interaction of 2-hydroxynaphthalene-1-carbaldehyde with 4-aminoacetophenone generated the ligand. The produced compounds' antimicrobial and antifungal properties were also examined.

2 MATERIALS AND EXPERIMENTAL PROCEDURES

The ligand's nuclear magnetic resonance spectra, which include (^{1}H and ^{13}C) spectrum, Utilising dimethyl sulfoxide-d6 as the solvent, the results have been obtained with a Bruker 400 MHz spectrometer. The measurement frequencies had been 400 MHz for ^{1}H and 100 MHz to obtain ^{13}C, using tetramethylsilane as the internal standard. Fourier transform infrared spectroscopy The spectrum has been obtained utilising KBr granules and collected by using the FTIR-600 infrared Fourier spectrometer throughout a spectrum range of 4000 to 200 cm^{-1}. Analyses utilising positive ion electrospray mass spectrometry were performed with a Sciex ESImass spectrometer. We used a Stuart SMP4 electrothermal device to determine the melting points of compounds. The spectrum of UV-visible has been obtained within the wavelength range of 1000 to 200 nm utilising a Shimadzu UV-160A spectrophotometer. At room temperature, using dimethyl sulfoxide, the solutions were prepared at a concentration of 10^{-3} mol L^{-1} and analysed in a one-centimetre quartz cuvette. The Eutech Instruments Cyber Can CON 510 digital conductivity meter was employed to determine the conductivity of the solution in DMSO with concentrations between 10^{-1} and 10^{-5}. At the Heraeus Vario EL and Shimadzu AA-7000 atomic absorption, elemental (C, H, N) and metal content were utilised for the analysis. Utilising a Metrohm 686 Titro processor and a 665 Dosim unit, the quantification for chloride ions in compounds was conducted by potentiometric titration. Finally, using a magnetic balance from Johnson Matthey, the magnetic characteristics have been assessed at 30°C.

3 SYNTHESIS

3.1 Synthesis of ((E)-3-((4-Acetylphenyl) Diazenyl)-2-Hydroxy-1-Naphthaldehyde) (HL)

Preparation of HL was based on the described method [18]-[20] as follows; In a 250 ml round flask containing 1.35g (10 mmol) of 4-aminoacetophenone and 0.69g (10 mmol) of sodium nitrite, 20 ml of one-to-one (EtOH:H_2O) mixture was added. The mixture was cooled to a temperature range of 0 to 5°C in an ice bath. Subsequently, 3 ml of hydrochloric acid (36%) was placed in a beaker with 10 ml of cold distilled water, then added dropwise with stirring over one hour. Diazonium salt solution is the product of this process, then reacts with a coolant mix that has 0.8g (20mmol) of sodium hydroxide and 1.72g (10 mmol) of 2-hydroxynaphthalene-1-carbaldehyde in 20ml of ethanol. The mixture of reaction was allowed to stir for two hours. Following to the reaction, the precipitate had been filtrated at pH 4, washed with cold water until its pH became 6-7, and then allowed to dry. The precipitate was filtered and was orange-red in colour. The precipitate was washed with 5 ml of cold ethanol and then allowed to dry. The yield: 2.293g (72.10%), having a melting point among 136-138°C (Fig. 1). ^{1}H nuclear magnetic resonance (400 MHz,DMSO-$_6$,ppm). $H_{(a)}$ at δ 9.67 ppm. 8.54; 8.45 ppm (s, 1H), which equivalent to $H_{(b)}$ and $H_{(c)}$ protons, respectively. $H_{(g.g-)}$ at 8.33 (d, J = 9.1 Hz, 2H). $H_{(h,h-)}$ displays at 7.79 (d, J = 7.9 Hz, 2H). $H_{(d,f)}$ at 7.48, 7.39 (t, J = 7.5 Hz, 2H). at 7.01 (d, J = 9.2 Hz, 2H); 6.76 (d, J = 9.6 Hz, 1H) belongs to $H_{(j,k)}$. $H_{(i)}$ at 2.60 ppm. Finally, at 15.27 ppm belongs to the impact of tautomerism. The ^{13}C-nuclear magnetic resonance (100 MHz, DMSO-d_6, ppm). Resonances at δ_c = 197.11 and 193.27 ppm were assigned to carbonyl carbon: (ketonic C_a); (aldehydic C_b), respectively. The Signal of phenolic carbon (C_c) was detected at 177.23 ppm. Resonances assigned for N- (C_n), was observed at 164.44 ppm, when the other N- (C_q) chemical shifts appeared at 147.06 ppm. The two signals displayed at 143.02; 138.89 were related to (C_w), (C_t), respectively. The peaks which related to (C_e), (C_x) and (C_k) showed with 134,54; 132.14; 130.62 ppm frequency respectively. The two groups of Carbon nucleuses of ($C_{g,g-}$); ($C_{h,h-}$), assigned at 129.75 and 124.71 ppm. Resonance of (C_u); (C_d) signals appears at 128.61; 122.71 ppm, respectively. The assignments of (C_f) and (C_j) resonance appeared at 117.70 and 119.20 ppm, respectively. The methyl group (C_m), appeared as a one peak at 27.15 ppm.

3.2 Synthesis of Complexes

The synthesised complexes process was a similar process used to synthesise the chromium(III) complex. The process will be as follows: It has been placed 0.3g (0.94 mmol) of HL has been dissolved in 10 ml in a mixed solution (5:5) (ethanol-benzene), and then 10ml of ethanol solution KOH 0.05g (0.94 mmol) was added in a 100 ml round-bottomed flask.

We stirred the mix. Subsequently, the slow addition of a solution of Chromium(III) chloride hexahydrate 0.25g (0.94 mmol) in 5 ml of EtOH was performed. The reaction mixture had been subjected to reflux heating for two hours. Following the heat process, the substance underwent filtration, washed to remove any remaining unreacted material used a cold ethanol and allowed to air dry. 0.262g (60.83%) was the yield of the Cr(III) complex, with a melting point above 300°C. The synthesis process has been described in Figure 2. See Table 1 for further information on yields, colors, the amounts of metal salts utilised, and m.p. of the resulting complexes.

4 MICROBIOLOGICAL EVALUATION

The susceptibility of bacteria and fungi to the generated chemicals has been assessed utilising the Kirby-Bauer disk diffusion method. Organism colonies were suspended in an 85% sodium chloride solution to achieve a turbidity comparable to 0.5 McFarland standard. over the surface of Mueller Hinton agar in a Petri dish, this suspension was evenly spread. Wells on the agar were created with uniform spacing and concentration. In each well, 100 ml of the test sample diluted to 1 mg/ml DMSO was added. After incubating, zones of inhibition were evaluated for 24 hours at 37°C and compared to reference values [21]. Control experiments using DMSO solutions confirmed the absence of intrinsic anti-microbial activity. Any of the tested bacteria or fungus.

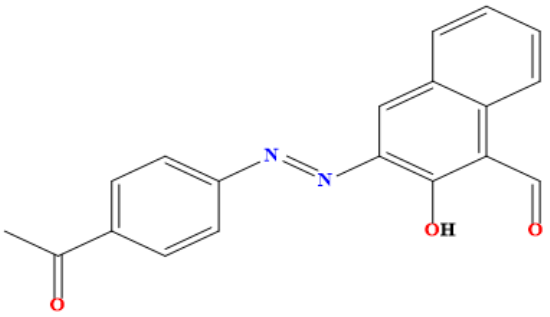


Figure 1: Chemical structure of azo ligand.

Table 1: Yield, colors, metal salts quantites and m.p. of ligand complexes.

Complexes	Quantity of metal salts (g)	Quantity of complex(g)	Colors	m.pºC	Yield (%)
$[Cr(L)Cl_2H_2O]$	0.25	0.43	Yellowish-green	>300*	60.83
$[Mn(L)Cl(H_2O)_2]$	0.19	0.42	Dark orange	>300*	70.00
$[Co(L)Cl(H_2O)_2]$	0.22	0.42	Dark brown	>300*	75.82
$[Ni(L)Cl(H2O)_2]$	0.22	0.42	Dark green	>300*	79.14
$[Cu(L)Cl(H_2O)_2]$ *=Decomposed	0.23	0.43	Reddish-brown	>300*	66.38

Table 2: Microanalysis and physical characteristics of complexes.

Complexe	Molecular formula	M.Wt	Microanalysis found, (calculated) %				
			C	H	N	M	Cl
$[Cr(L)Cl_2H_2O]$	$C_{19}H_{15}Cl_2CrN_2O_4$	458.27	(49.80) 49.51	(3.30) 3.13	(6.11) 6.00	(11.35) 11.11	(15.47) 15. 05
$[Mn(L)Cl(H_2O)_2]$	$C_{19}H_{17}ClMnN_2O_5$	443.74	(51.43) 51.24	(3.86) 3.40	(6.31) 6.01	(12.38) 12.05	(7.99) 7.61
$[Co(L)Cl(H_2O)_2]$	$C_{19}H_{17}ClCoN_2O_5$	447.74	(50.97) 50.67	(3.83) 3.53	(6.26) 6.12	(13.16) 13.01	(7.92) 7.70
$[Ni(L)Cl(H_2O)_2]$	$C_{19}H_{17}ClNi\ N_2O_5$	447.50	(51.00) 50.87	(3.83) 3.62	(6.26) 6.15	(13.12) 13.00	(7.92) 7.41
$[Cu(L)Cl(H_2O)_2]$	$C_{19}H_{17}ClCuN_2O_5$	452.35	(50.45) 50.23	(3.79) 3.56	(6.19) 6.01	(14.05) 13.88	(7.84) 7.67

5 RESULTS AND DISCUSSION

The azo ligand ((E)-3-((4-acetylphenyl)diazenyl)-2-hydroxy-1-naphthaldehyde) (HL). In this approach, $NaNO_2$, HCl and EtOH were used as a catalyst and reaction medium, respectively.4-aminoacetophenone and 2-hydroxynaphthalene-1-carbaldehyde reacting in EtOH at a one-to-one ratio. The ligand functions as a tridentate species, supplying the azo's nitrogen atom, hydroxyl, and carbonyl oxygen as donor atoms. The interaction of the ligand with the chlorides of metal of Cr(III), Mn(II), Co(II), Ni(II), and Cu(II) occurs in a mole ratio of one to one (Ligand to Metal) yielded the segregation of six-coordinate monomeric compound of the universal formula $[M(L)Cl_2H_2O]$ with Cr(Ш), $[M(L)Cl(H_2O)_2]$ with Mn(II) and Cu(II), Co(II) and Ni(II) ions (Figure 2). The isolated compounds exhibit stability in the air, exist as solids, and are soluble in DMSO and DMF. However, it is not soluble in other common organic solvents. Based on their physico-chemical data, complexes' coordination geometries and complexation behavior were assumed. Results shown in Table 2 are well suited to the proposed formula. Conductance measurements of the compounds in dimethylsulfoxide solutions showed that the complexes are non-electrolytic. The entity of Azo was confirmed by C, H, N, M (Table 2), Fourier transform infrared spectroscopy (Table 3), and Electron spectroscopy (Table 4).

5.1 FT-IR and NMR Data

In Table 3, the main IR absorption bands of the complexes are listed with their duties. The peak is observable in the ligand spectrum at 3194 cm^{-1} due to ν(OH) stretching vibration of the phenol hydroxyl group [22]. at 1621 and 1492 cm^{-1}. The noted bands correspond to the stretching of the carbonyl group ν(C=O) and the stretching of the azo group ν(N=N), respectively [23]. The complex spectrum had a noticeable extent between 1617 and 1612 cm^{-1}.which is related to the ν (C=O) stretching vibration. The ligand's spectra appeared the band at 1621 cm^{-1}. Interaction involving the metal ion and the carbonyl oxygen atom is explained by the bands that form upon complexation [22]. Band observed at 1492 cm^{-1} in HL, which belongs to the azo group ν(N=N) [20], [22]. It was displaced and appeared at 1463, 1466, 1489, 1465, and 1465 cm^{-1} in the five complexes in order. The occurrence might have been associated with the involvement of the nitrogen atom in complexation. In addition, the metal complex spectra revealed new bands around (646-629), (464-419), and (289-223) cm^{-1} that were not visible in the ligand spectrum associated to ν(M-O), ν(M-N), and ν(M-Cl), respectively [22], [23]. Finally, the complexes of Cr(Ш), Mn(II), Co(II), Ni(II), and Cu(II) showed peaks at (3439), (3432), (3337), (3450), and (3547) cm^{-1}, respectively. were bound to aqua molecules. At 742, 749, 756, 740, and 742 cm^{-1}, complex bands 1, 2, 3, 4, and 5 can be found. These are linked to water-coordinated ν(M-O) for 1,2,3,4 and 5 [20], [25]. The identification of peaks in the nuclear magnetic resonance spectra corresponds to the numbering scheme shown in Figure 1. The spectra ^1H nuclear magnetic resonance of ligand with dimethyl sulfoxide (d_6) as a solvent, is shown in Figure 3. The chemical shift at δ 9.67 ppm (s, 1H), that equivalent to one proton attributed to phenolic proton $H_{(a)}$. The chemical shift that appeared as a singlet signal at 8.54; 8.45 ppm (s, 1H), which equivalent to $H_{(b)}$ and $H_{(c)}$ protons, respectively. The spectrum shows doublet signal refers to $H_{(g.g-)}$ at 8.33 (d, J = 9.1 Hz, 2H). The doublet signal of attributed to $H_{(h,h-)}$ displays at 7.79 (d, J = 7.9 Hz, 2H). The value of $H_{(d,f)}$ appear as a triplet signal at 7.48, 7.39 (t, J = 7.5 Hz, 2H). when another doublets signals displayed at 7.01 (d, J = 9.2 Hz, 2H); 6.76 (d, J = 9.6 Hz, 1H) belongs to $H_{(j,k)}$ protons. The aliphatic region revealed a singlet peak belongs to $H_{(i)}$ protons appeared at 2.60 ppm. At 2.51 and 3.37 ppm, peaks were seen corresponding to the dimethyl sulfoxide-d_6 solvent and trace water molecules present in the solvent, respectively. Finally, the proton signal which appeared at a very low chemical shifting area, near 15.27 ppm belongs to the impact of tautomersim which phenols suffer from [26]-[29]. Obesely the spectrum don't displays a signals between 4-5 ppm which proven the transformation of amine group to diazo moiety. Figure 4 shows the ^{13}C nuclear magnetic resonance spectrum in DMSO-d_6, which shows the correct number of carbon atoms in a molecule. Resonances at δ_c = 197.11 and 193.27 ppm were assigned to carbonyl carbon: (ketonic C_a); (aldehydic C_b), respectively. The Signal of phenolic carbon (C_c) was detected at 177.23 ppm. Resonances assigned for N- (C_n), was observed at 164.44 ppm, when the other N- (C_q) chemical shifts appeared at 147.06 ppm. The two signals displayed at 143.02; 138.89 were related to (C_w), (C_t), respectively. The peaks which related to (C_e), (C_x) and (C_k) showed with 134,54; 132.14; 130.62 ppm frequency respectively. The two groups of Carbon nucleuses of ($C_{g,g-}$); ($C_{h,h-}$), assigned at 129.75 and 124.71 ppm. Resonance of (C_u); (C_d) signals appears at 128.61;

122.71 ppm, respectively. The assignments of (C_f) and (C_j) resonance appeared at 117.70 and 119.20 ppm, respectively. The methyl group (C_m), appeared as a one peak at 27.15 ppm, when the solvent signals of the DMSO-d_6 resonances appeared at 40.50-39.33.

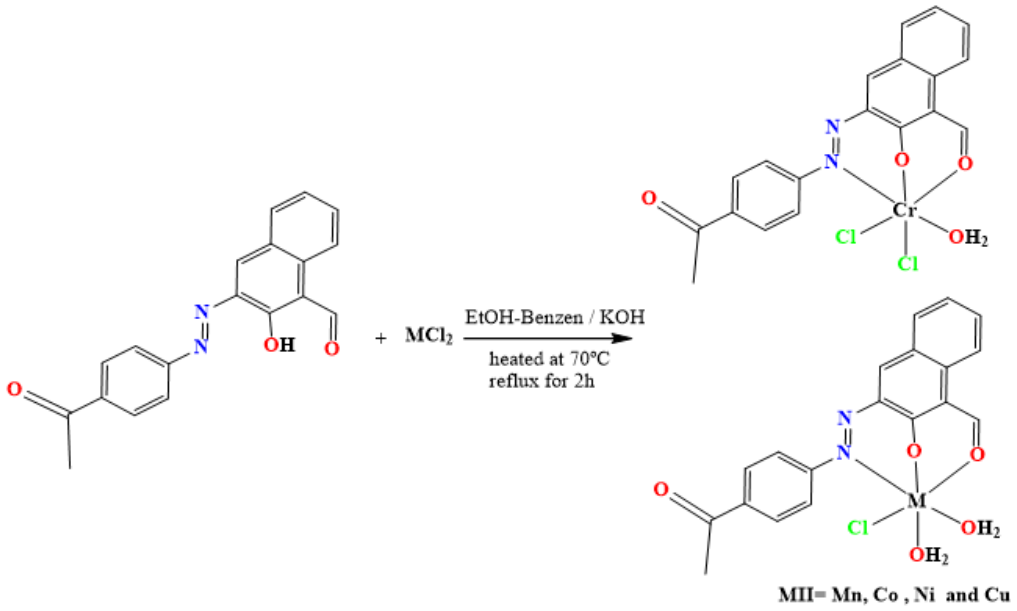


Figure 2: General route for the synthesis of HL complexes.

Table 3: FT-IR data of the most prominent peaks of complexes (cm^{-1}).

Compounds	ν(C=O) ke ν(C=O)al	ν (C=C)	ν N=N	ν C-O ν C-N	ν(H_2O) ν(M-OH_2)	ν (M-O) phenol ν (M-O) aldeh.	ν M-N	ν M-Cl
$C_{19}H_{15}Cl_2CrN_2O_4$	1674 1612	1593 1544 1500	1463	1357 1265	3439 742	646 590	464	279 260
$C_{19}H_{17}ClMnN_2O_5$	1671 1617	1597 1581 1538	1466	1357 1261	3432 749	629 536	449	281
$C_{19}H_{17}ClCoN_2O_5$	1674 1615	1598 1577 1558 1551	1489	1334 1262	3337 756	629 558	419	262
$C_{19}H_{17}ClNiN_2O_5$	1674 1614	1593 1543 1500	1465	1355 1267	3450 740	623 572	451	289
$C_{19}H_{17}ClCuN_2O_5$	1678 1614	1593 1544 1502	1465	1357 1265	3547 742	646 569	420	223

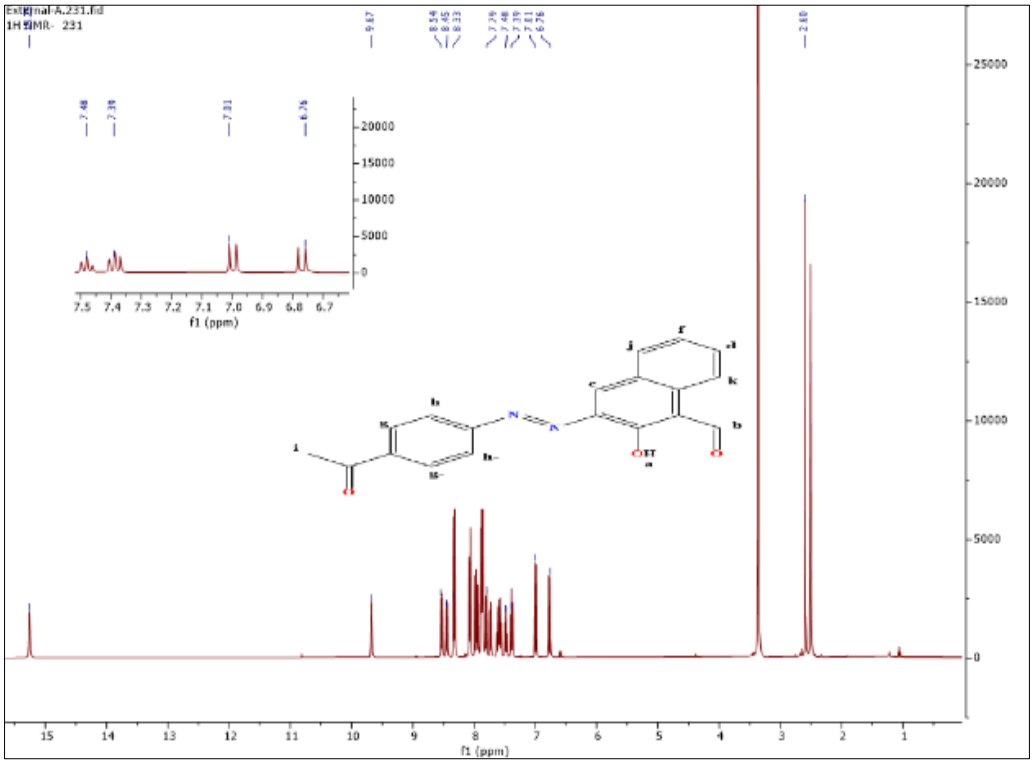


Figure 3: ^{1}H-NMR spectrum in DMSO-d_6 solutions of azo ligand.

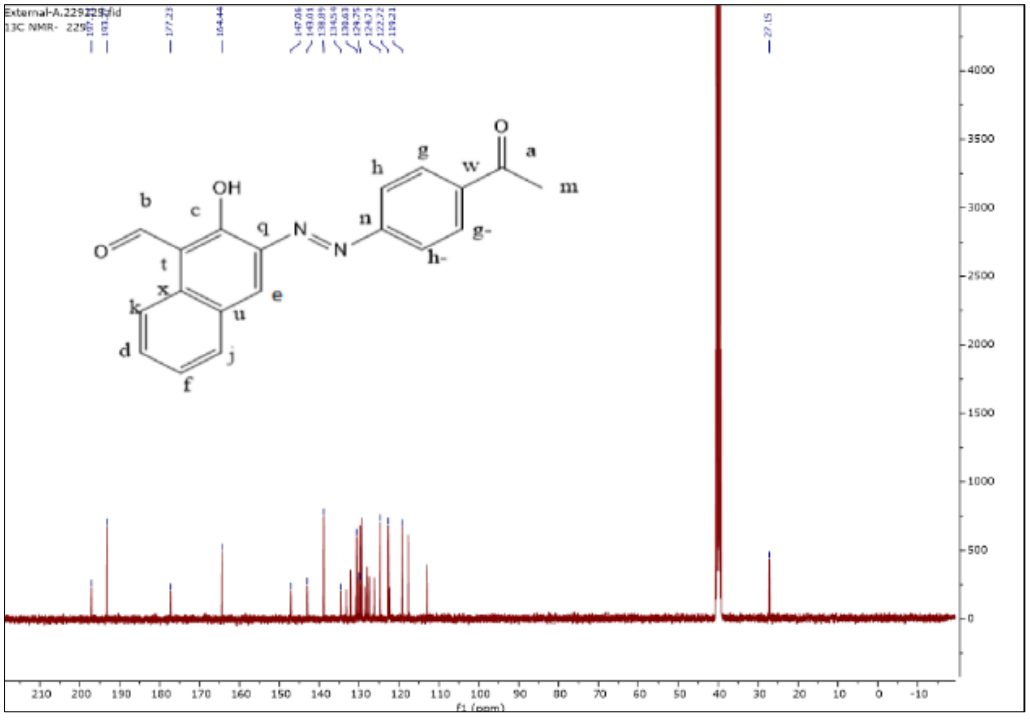


Figure 4: ^{13}C-NMR spectrum in DMSO-d_6 solutions of azo ligand.

Table 4: Uv-vis data for complexes in DMSO solutions.

Complex	ʎnm	Molar extinction coefficient εmax (dm^3 mol^{-1} cm^{-1})	Assignment	Suggested geometry
$C_{19}H_{15}Cl_2CrN_2O_4$	301 436 512	1760 597 22	Intra-ligand π → π*, n→π* C.T $^4A_2g \rightarrow {}^4T_1g^{(p)}$	Distorted octahedral
$C_{19}H_{17}ClMnN_2O_5$	287 316 374 401 488	1090 771 559 459 85	Intra-ligand π → π*, n→π* C.T C.T C.T $^6A_1g \rightarrow {}^4T_1g^{(G)}$	Distorted octahedral
$C_{19}H_{17}ClCoN_2O_5$	299 374 520	1220 220 60	Intra-ligand π → π*, n→π* C.T $^4T_1g^{(F)} \rightarrow {}^4T_1g^{(P)}$	Distorted octahedral
$C_{19}H_{17}ClNiN_2O_5$	241 317 498	2006 230 29	Intra-ligand π → π*, n→π* C.T $^3A_2g^{(F)} \rightarrow 3T_1g^{(F)}$	Distorted octahedral
$C_{19}H_{17}ClCuN_2O_5$	255 386 564	1550 260 90	Intra-ligand π → π*, n→π* C.T $^2B_1g \rightarrow {}^2A_2g$	Distorted octahedral

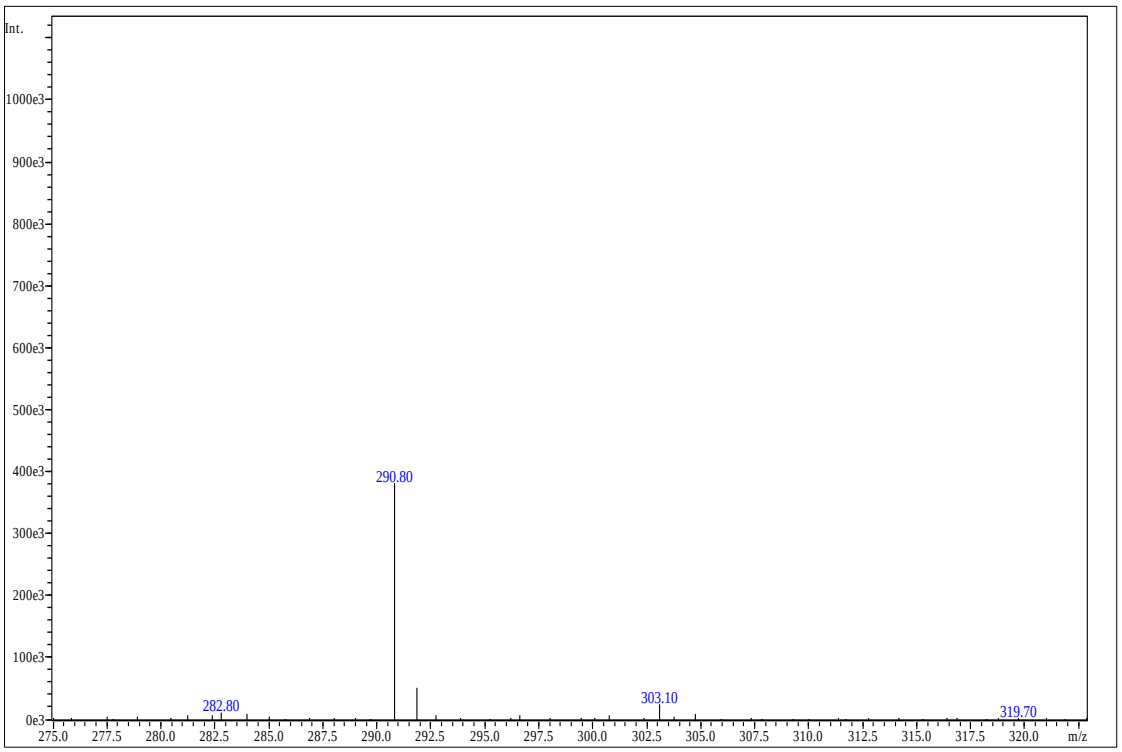


Figure 5: The ESI (+) mass spectrum of azo ligand.

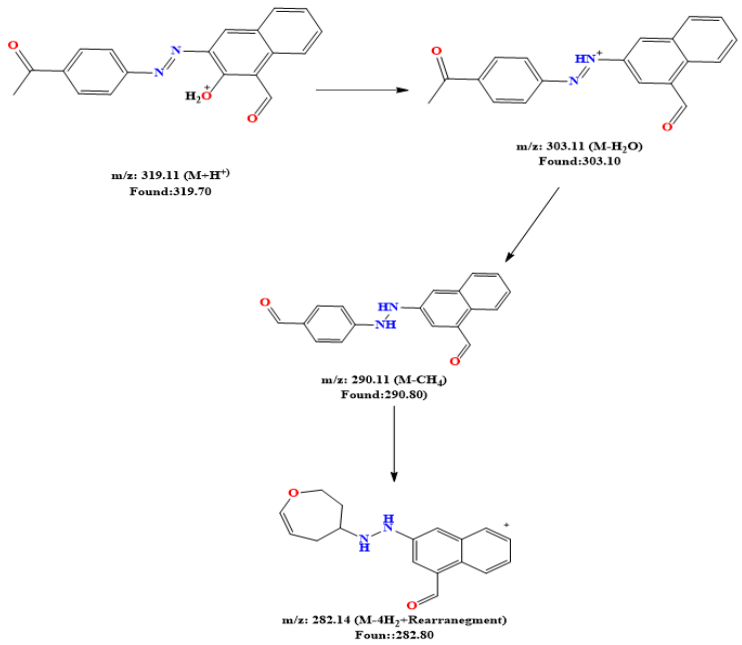


Figure 6: The fragmentation pattern of azo ligand.

Table 5: It shows the areas of antibacterial activity (mm) for HL and compounds.

Compounds	Bacillus cereus (G+)	Staphylococcus aureus (G+)	Proteus mirabilis (G−)	Escherichia coli (G−)
DMSO	-	-	-	-
Tetracycline	30	27	14	1
HL	14	9	10	9
$C_{19}H_{15}Cl_2CrN_2O_4$	14	17	18	22
$C_{19}H_{17}ClMnN_2O_5$	10	9	11	9
$C_{19}H_{17}ClCoN_2O_5$	17	18	18	20
$C_{19}H_{17}ClNi\ N_2O_5$	14	9	11	9
$C_{19}H_{17}ClCuN_2O_5$	16	17	18	17

5.2 Mass Spectrum

Mass spectrum HL was obtained by electron scattering positive mass spectroscopy, spectrum in Figure 5 showed the presence of a parent ion molecule $(M+H)^+$ at m/z = 319amu (2%), measured for $C_{19}H_{14}N_2O_3$, which requires 318.33 amu. Figure 6 showed the fragmentation pattern of azo ligand.

5.3 Electronic Spectra and Magnetic Moment

Magnetic moment and UV-visible is summarised in Table 4. Electron spectrum of the complexes displayed characteristic peaks in the range 241-301 nm, indicating transitions π→π* and n→π*. Further, Charge transfer phenomena are allow for the observed peaks in an array between 316-436 nm. [30,31]. The electron spectra of the Cr(III) complex exhibit a special band at 512 nm, indicative of the transition $^4A_2g \rightarrow {}^2T_1g$, indicating that a distorted octahedral structure around the Cr(III) centre. This explanation agrees to the value of magnetic moment: (3.81) BM for the Cr(III) complex. The Mn(II) complex exhibits a band at 488 nm in the region of d-d, ascribed to the transition $^6A_1g \rightarrow {}^4T_1g(G)$, indicating the geometry around the Mn ion was distorted octahedral. This result corresponds to the magnetic moment measurement for the Mn(II) complex: (5.90) BM. For the Co(II) complex, the spectrum exhibits bands at 520 nm of the d-d region

that correspond with the transition $^4T_1g^{(F)} \rightarrow {}^4T_1g^{(P)}$. A six-coordinate complex suggested by these bands indicates a distorted geometry around the ion of Co(II). The value of magnetic moment: (4.62) BM, agree an octahedral arrangement around the Co ion [30], [32]. Ni(II) complex shows a peak at 498 nm, assigned to $^3A_2g^{(F)} \rightarrow {}^3T_1g^{(P)}$, indicating octahedral geometry around the Ni atom. The value of the magnetic moment: (3.29) BM confirms the octahedral geometry around the Ni ion. Finally, The Cu(II) complex has a peak at 564 nm, ascribed to the $^2B_1g \rightarrow {}^2A_2g$ transition, which suggests a distorted octahedral structure surrounding the ion Cu. The magnetic moment value for ion Cu(II): (1.93) BM is consistent with this structural interpretation [30], [32].

5.4 Biological Activity

By using *Tetracycline* as a reference medication. were utilised four bacterial strains to evaluate the antibacterial activity of the synthesised ligand along with their metal complexes: *(Escherichia coli and Proteus mirabilis (G−))* and also (*Staphylococcus aureus and Bacillus cereus (G+))*. Control experiments separately using dimethyl sulfoxide showed the absence of any intrinsic antibacterial effects [33]-[35]. Table 5 shows the inhibiting area's diameters have been in comparison with those of the antibiotic Syntriaxone. The main results were:

1) HL shows antibacterial activity against (Escherichia coli and Proteus mirabilis (G−)), (Staphylococcus aureus and Bacillus cereus (G+)).
2) The (C19H17ClCoN2O5), (C19H17ClCuN2O5) and (C19H15Cl2CrN2O4) showed stronger effectiveness against selected strains.
3) The metal complexes of HL exhibited significant antibacterial activity comparable to Tetracycline. This might result in potential biological uses of the synthesised compounds.

Antifungal testing has been performed on the yeast (*Candida albicans*), utilising *fluconazole* as the reference medication. DMSO controls did not exhibit antifungal activity [36]-[39]. The observed antifungal activity. The values for the tested substances have been shown in Table 6. The subsequent findings have been discerned;

1) against Candida albicans. All substances exhibited antifungal activity.
2) Complexity substantially enhanced antifungal activity of the free ligand, possibly as a result of the chelate process.
3) Fluconazole has shown greater effectiveness than complexes against Candida albicans.

Table 6: The demonstrates of antifungal inhibition (mm) zones of azo ligand and the compounds.

Compounds	*Candida albicans*
DMSO	-
Fluconazole	9
HL	14
$C_{19}H_{15}Cl_2CrN_2O_4$	17
$C_{19}H_{17}ClMnN_2O_5$	15
$C_{19}H_{17}ClCoN_2O_5$	12
$C_{19}H_{17}ClNi\ N_2O_5$	14
$C_{19}H_{17}ClCuN_2O_5$	16

6 CONCLUSIONS

The azo ligand (HL) and its paramagnetic complexes involving the metals Cr(III), Mn(II), Co(II), Ni(II) and Cu(II) have been reported. The synthesised ligand HL from reaction of the 4-aminoacetophenone with 2-hydroxynaphthalene-1-carbaldehyde in mole ratio one-to-one. The interaction of ligand with metal ions at a (one-to-one) L-to-M ratio generated isolated compounds. They have been structurally characterised via several Physical and chemical techniques. The six-coordinate geometries for the metal complexes' coordination environments given by analytical techniques involve elemental microanalysis,1H and 13C-nuclear magnetic resonance, Fourier transform infrared spectroscopy, electronic and mass spectroscopy, as well as magnetic susceptibility, Conductivity measurements and Biological Evaluation. The ligand Prepared had yield: (72.10%), having a melting point among 136-138□C, As for the complexes resulting from the reaction of the ligand with metal chlorides, the melting point was higher than 300□C. The FTIR of HL displayed characteristic peaks for phenolic, carbonyl, and azo groups. Complexation resulted in carbonyl peak shifts, indicating back-bonding, with additional bands related to the ν(M-N), ν(M-O), ν(M-Cl) and ν(M-OH2) observed in the complex spectra. Mass spectrum of ligand showed the presence of a parent ion molecule (M+H)+ at m/z = 319amu, measured for C19H14N2O3, which requires 318.33 amu. The UV/Vis of complexes supported a distorted octahedral structure. The value of magnetic moment is 3.81, 5.90, 4.62, 3.29 and 1.93BM for Cr, Mn, Co, Ni and Cu, respectively, this explanation agrees to the electron spectra of the complexes. At last, anti-bacterial and anti-fungal

assays demonstrated enhanced bioactivity for the resulting complexes comparing to free ligand, emphasising the plus-side outcomes of complex formation.

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Synthesis Structural Study and Spectroscopic Characterization of Azo Compound Derived from Mercaptothiadiazole and Methoxybenzaldehyde

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Keywords: Azoaldehyde , O-Vanillin , IR, Gussian, Vibration, Bending.

Abstract: The vibrational and electronic properties of 2-hydroxy-5-((5-mercapto-1,3,4-thiadiazol-2-yl)diazenyl)-3-methoxy benzaldehyde were theoretically studied in the present paper by carrying out quantum chemical calculations. Vibrational wavenumbers and their respective assignments were computed at the Hartree-Fock and DFT/B3LYP levels with the 6-311++G(d,p) basis set using Gaussian03 software. The theoretical IR and Raman spectra were calculated and compared with the experimental IR spectrum, which showed good agreement. The vibrational assignments were made by PEG analysis to ensure proper characterization of the modes. Theoretical IR and Raman intensities have also been calculated to provide further spectroscopic insight. Frontier molecular orbitals have been estimated to obtain an in-depth analysis of the electronic structure. (FMOs) were analyzed. The HOMO and LUMO energies have been computed thereby giving HOMO–LUMO energy which, in turn, gives insight into the reactivity and stability of the molecule. Also, Mulliken charges and Molecular Electrostatic Potential (MEP) analysis were computed for charge transfer character and electrostatic interaction within the molecule, respectively. This study is also supplemented by Natural Bond Orbital (NBO) analysis of charges to investigate intramolecular and intermolecular charge delocalization effects on their mean stabilization interactions in the system. Thus, it can parse from the electronic and vibrational features that it can be useful in spectroscopic applications further in molecular design. The paper would, therefore, reveal the power that computational chemistry brings in the prediction and interpretation of vibrational and electronic properties that would go a long way to unravel compounds structurally similar to the heterocyclic compound.

1 INTRODUCTION

Azo compounds comprise a vital group of organic compounds characterized by their containing the azo functional grouping (-N=N-), - that is, the double bonding of two nitrogen atoms bonded to some organic groupings (R-N=N-R'). Powerful coloration and abundantly varied chemical properties account for their wide use in the manufacture of dyes, chemical indicators, and pharmaceuticals [1], [2]. The most popular synthetic dyes for coloring textiles, plastics, and inks are the azo dyes on account of their bright colors and stability. Among them are methyl orange, acid red, and direct blue [3], [4]. Among other azo compounds are sulfa drugs, an important class of drugs because of its antibacterial activity [5]-[11]. the five-membered heterocyclic compound called thiadia zole contains two nitrogen and one sulfur atoms. It emanates from the union of a benzene ring and a heterocyclic group of sulfur atoms and nitrogen atoms. In medicinal chemistry, the thiadiazole and its derivatives are interesting because they usually present many other biological activities, including antimicrobial, antifungal, anti-inflammatory, and antitumor properties [12], [13]. Different thiadiazole derivatives, like 1,3,4-thiadiazole, have been studied for possible use in the field of drug development and are also used to synthesize agrochemicals, which include herbicides and pesticides [14]-[16]. Nonlinear chemical phenomena occur as a result of the interaction of applied electromagnetic radiation with different molecular species and new responses to electromagnetics are generated. In these systems, the induced molecular polarization does not scale linearly with the intensity of the incident field, leading to modifications at wavenumber, phase, and other related properties. Efficient organic molecules

can control photonic signals, which therefore play a role dynamically in optical communications, optical computing, and dynamic image processing. Phenyl substitution can enhance molecular hyperpolarizability-an effect said to be not obvious in most cases. Most of the organic molecules are comprised of conjugated π electrons have large molecular first hyperpolarizabilities and are large values of molecular first studied by using vibrational spectroscopy [17]-[19]. The IR spectrum is currently simulated by some ab initio quantum mechanical method, and these are the simulations that will help perform normal coordinate analysis. There is no place for contemporary vibrational spectroscopy without such simulations; these are necessary tools. The wavenumbers and hyperpolarizability of the title compound are the subjects of theoretical calculations in the present work, which are also compared with experimental results.

2 EXPERIMENTAL

2.1 Chemicals

All chemicals were supplied by Fluka, Merck, and Aldrich Chemicals Co. and used as received.

2.2 The Techniques

FTIR spectra were recorded using KBr pellets on a Nexus 400 Shimadzu FT-IR Spectrometer with a spectral resolution of 16 cm^{-1}.

2.3 Synthesis of (E)-2-Hydroxy-5-((5-Mercapto-1,3,4-Thiadiazol-2-yl) Diazenyl)-3- Methoxy Benzaldehyde

Compound 2-amino-5-marcapto-1,3,4-thiadiazole (0.44 g, 0.0017 mol) was dissolved by heating and stirring in 8 mL of 85% phosphoric acid. The solution was cooled to 0 ºC in an ice bath, and then concentrated nitric acid 4 mL and a solution of sodium nitrite (0.10 g, 0.0017mol) in water 2 mL The mixture was stirred and allowed to stand at below 5oC for 10 minutes. Thereafter, a solution of o-vanillin (0.15g, 0.0017mol) in water (0.5mL) was added dropwise with stirring. The brown solid was filtered, washed with several portions of water, and then taken up in 30 mL of 10% hydroxyl soduim solution. The solution was filtered, and the crude product precipitated during neutralization with 10% hydrochloricacid [20], then filtered and washed with water several times, and recrystallized from ethanol to give 78% yield, m.p = 60-62oC.

Computational details were carried out with the Gaussian program G09 using the HF/6-31G* and B3LYP/6-31G* basis sets for predicting the molecular structure and wave numbers. The molecular geometry was fully optimized using Berny's optimization algorithm including redundant internal coordinates. Harmonic vibrational wavenumbers were calculated using the analytic second derivatives to confirm the convergence to the minimum on the potential surface. Also, the wave number is associated with known systematic errors due to the negligence of electron correlation. The W values computed at the Hartree-Fock and DFT level contain a scaling factor value of Hartree-Fock level 0.8929; therefore, the scaling factor value is taken as the counterpart for HF/6-31G*. The Parameters corresponding to optimized geometry imaginary wave number on the calculated (Fig. 1).

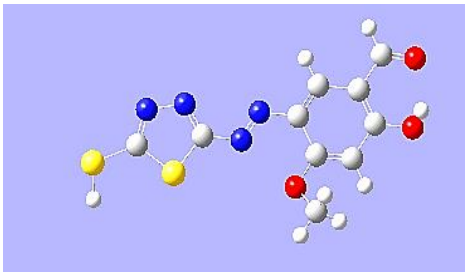


Figure 1: Optimization of 2-hydroxy-5-((5-mercapto-1,3,4-thiadiazol-2- yl)diazenyl)-3- Methoxy benzaldehyde.

3 RESULTS AND DISCUSSION

The broad band seen in the spectrum at 3450 cm-1 is of very high frequency, indicating a strong stretching vibration of the hydroxyl (-OH) group. The absorption intensity (324.66) is high, meaning this mode is clearly visible in the infrared spectrum. The aromatic compound, this vibration is associated with aromatic ring stretching. The absorption intensity (20.76) is much lower than the previous one, meaning the IR absorption is weaker. Raman activity (85.38) is medium (see Table 1, mode 1). It was experimentally measured at 3355 cm^{-1}. This frequency corresponds to the stretching vibration of the thiol (-SH) group. The absorption intensity (10.57) is relatively low. Raman activity (183.50) is high, meaning this group may appear more prominently in the Raman spectrum. It was

experimentally measured at 2882 cm^{-1} (see Table 1, mode 7).

The range from 420-575 cm^{-1} includes eight fundamental vibration modes, all of which are structural. It is noted that the methyl group is observed at 497 cm^{-1} is weak. The 1600-1800 cm^{-1} region exhibits a strong absorption band centered at 1666 cm^{-1}, which corresponds to multiple C-H bending vibrations appearing at 1501 cm^{-1} (see Table 2, mode 16). Additionally, a C=O stretching vibration is observed, attributed to an acetate 1666 cm^{-1} (see Table 2, mode 13), which corresponds to the C-H vibration, is weak due to a polarization of 9.3°. and has merged under the previously mentioned strong band. The frequency 1672 cm^{-1} corresponds to the out-of-plane C-H vibrational motion. The frequency 1677 cm^{-1} is attributed to the hydrazine group functional group at the same spectral region (see Table 2, mode 15). The absorption band at 1708 cm^{-1} is attributed to the skeletal vibrational modes of the aromatic ring and appears as an envelope-shaped band in the infrared (IR) spectrum. This feature may result from conjugation effects or electronic interactions within the aromatic system, leading to slight shifts or broadening in the absorption profile (Fig, 2).

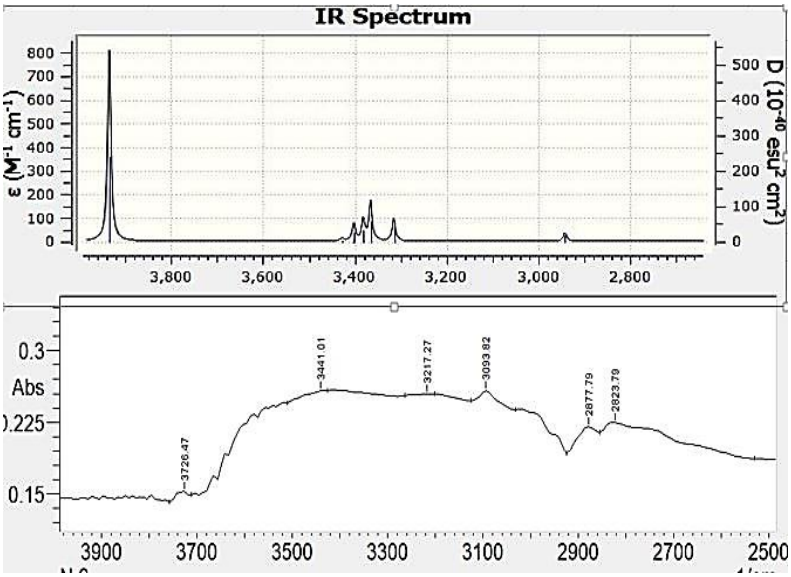


Figure 2: IR and predicted spectra a) and b) for the region 2700 cm^{-1} to 4000 cm^{-1} respectively.

Table 1: Calculated vibrational wave numbers, measured band positions and assignments of hydroxy and aromatic ring using (HF/6-31G*-B3LYP/6-31G*).

Mode	Predicted frequency υ cm^{-1}	Intensity	Raman activity	Frequency υ cm^{-1}	IR intensity	Raman activity	Found υ cm^{-1}	Assignments
1	3934.32	324.66	234.66	3831	57.83	147.93		υO-H
2	3428.01	3.58	75.76	3828	56.97	145.39	3401b	υO-H
3	3405.18	0.425	61.499	3358	11.25	152.57		
4	3402.55	20.76	85.38	3353	17.19	198.99	3093w	υph*[22]
5	3381.37	26.31	37.71	3346	20.88	112.94	3024w	υph
6	3365.65	49.19	112.87	3340	21.01	60.75		
7	2942.14	10.57	183.50	2880	45.01	69.52	2823	υS-H*[21]

υ refer to stretching vibration, υph* refer to aromatic C-H stretching, υS-H* refer to S-H bond stretching

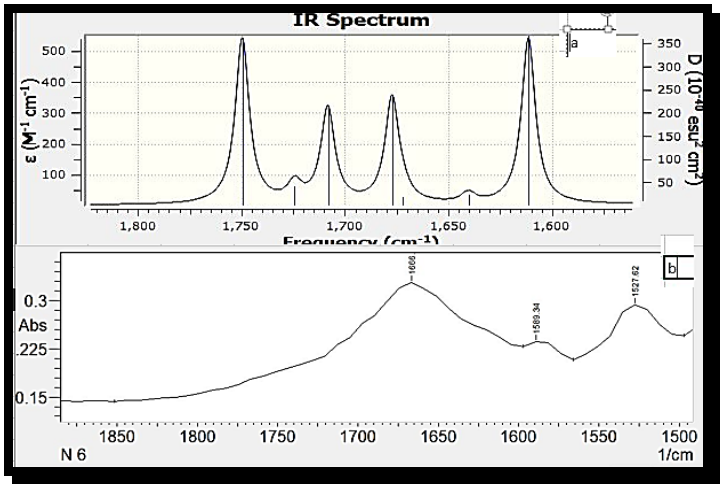


Figure 3: IR and predicted spectra a) and b) for the region 1800 cm^{-1} to 1500 cm^{-1} respectively.

Table 2: Calculated vibrational wave numbers, band positions and assignments of carbonyl and aromatic ring (HF/6-31G*-B3LYP/6-31G*).

	Predicted frequency, υ cm^{-1}	Intensity	Raman activity	υ cm^{-1}	Intensity	Raman	υ cm^{-1}	Assignments
9	1749.77	155.59	5.73	1705.71	72.99	7.88		
10	1724.65	17.68	51.38	1765.98	66.90	2.15		
11	1708.74	89.63	135.22	1700.88	23.55	8.21	1527m	υc=c[25]
12	1677.33	98.63	98.29	1695.44	77.99	10.70		
13	1672.88	7.15	44.77	1665.88	45.99	19.79	1666s	υC=O[26]
14	1640.45	9.37	18,81	1635.06	67.88	2.17		
15	1611.14	156.72	30.06	1607.67	34.87	67.90	1598s	υN=N[27]
16	1501.99	80.53	0.27	1494.77	45.88	18.30	1450s	υc=c

Methylen Group Vibrations: Symmetrical and asymmetrical bending vibrations of C-H bonds in a methylen group are discussed The asymmetrical deformations are expected in the range of 1400–1463 cm^{-1} with calculated values at 1463 and 1422 cm^{-1} (see Table 3, mode 17 and 18). Symmetric deformations are expected around 1391 ±25 cm^{-1} [21]. HF (Hartree-Fock) calculations predict the δasCH2 mode at 1463 cm^{-1}. Observed IR bands at 1572, 1473, and 1440 cm^{-1} are assigned to deformation bands of the methylen group (see Table 3) [22]. Primary Aromatic Amines: These are chemical compounds that contain an amino group (-NH_2) directly attached to an aromatic ring (such as benzene). Primary aromatic amines are significant in organic chemistry and are used in the synthesis of various organic compounds, including dyes and pharmaceuticals [23], [24]. Infrared (IR) spectroscopy is an analytical technique used to study chemical interactions and molecular structures by measuring the absorption of infrared radiation Each chemical bond in a molecule has a characteristic vibrational frequency that can be measured using IR spectroscopy. Carbon-Nitrogen Stretching Vibration (C-N Stretch): Primary aromatic amines absorb IR at 1260 ± 60 cm^{-1} in the carbon-nitrogen bond within the aromatic ring (Fig. 3). It is assigned to the stretching vibration of the C-N bond. (C-N stretch). Experimental and Theoretical Results: In experimental observations, absorption peaks were noted at wavenumbers 1277, and 1205 cm^{-1} (see Table 3, mode 23 and 25). Theoretical calculations, such as quantum chemical computations, predicted absorption peaks at about 1297, 1296, and 1299 cm^{-1} [26]. Significance of These Findings: Such results can be very imperative in identifying the presence of aromatic amines as chemical samples

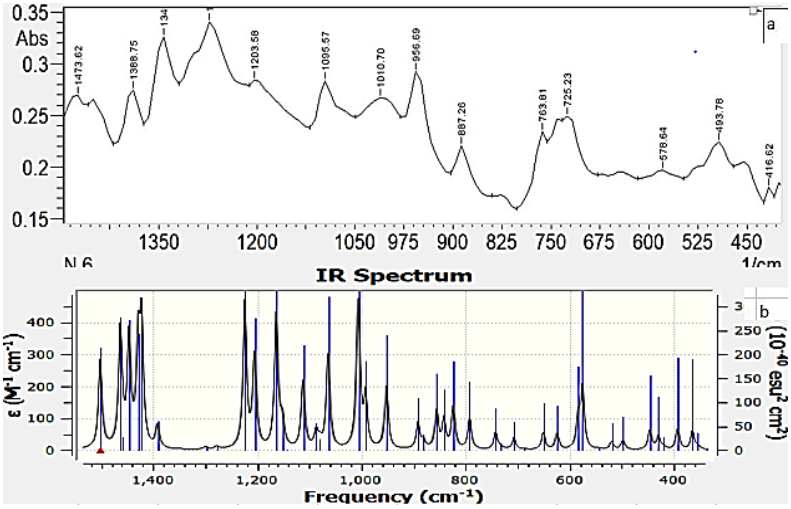


Figure 4: IR and predicted spectra a) and b) for the region 400 cm^{-1} to 1400 cm^{-1} respectively.

Table 3: Calculated vibrational waves numbers and measured bands positions and assignments of carbonyl and aromatic ring using (HF/6-31G*-B3LYP/6-31G*).

Mode	υ cm^{-1}	Intensity	Raman activity	υ cm^{-1}	Intensity	Raman activity	υ cm^{-1}	Assignments
17	1463.42	87.007	3.3	1455	84.03	14.7	1473m	υ-C-C-H [31]
18	1459	2.28	19.48	1450	72.60	3.92	1440m	υC-C -H
19	1428	20.98	11.97	1424	6.03	38.88		υC-O [32]
20	1422	2.83	53.40	1405	30.55	66.19		
21	1391	83.14	99.39	1371	14.26	81.99	1388s	υC-O-H [33]
22	1298	132.88	50.48	1270	16.63	66.99	1342s	υC-O [341]
23	1277	87.77	396.388	1260	21.90	99.8		
24	1223	101.17	16.55	1218	16.63	82.88		Pph
25	1205	13.89	12.87	1205	14.63	89.90		Pph
26	1163	80.007	101.14	1150	72.88	15.99	1095w	
27	1151	156.78	98.28	1164	3.88	55.90		
28	1142	155.98	89.77	1130	85.77	88.90		
29	1111	69.89	51.99	1105	12.90	90.88		
30	1078	80.53	89.28	1060	55.27	0.78		
31	1079	22..005	69.52	1056	16.88	19.30		
32	1063	133.88	183.77	1078	15.98	14.88		
33	1006	57.08	120.61	1010	30.81	19.45	1010s	pph
34	992	26.55	53.16	982	14.16	16.70		
35	951	34.31	54.93	978	14.76	11.81	956m	pph
36	891	121.82	16.55	887	15.78	1.98		
37	880	22.05	396.38	874	67.99	30.90	887s	pph
38	855	61.36	18.99	840	16.97	89.89		
39	841	85.45	53.16	824	21.33	76.90		
40	823	46.99	46.77	876	76.90	65.09		
41	792	34.31	55.90	706	09.99	98.90	763m	pph
42	742	28.9	2.8	734	0.45	0.87		

In turn, the above information may find general applicability in pharmaceutical analysis and characterization of chemical materials. Bending vibrational band of the hydroxyl group (C-OH), normally in the 1220-1440 cm^{-1} region, is characterized by composite bands of different appearances, arising from diversity. This is assigned based on the band appearing at 1370 cm^{-1}, and thus the C–O–H bending modes in this region are highly sensitive to changes in the chemical environment mainly through the variations in the hydrogen bonding and neighboring groups [27]. The presence of the band at 1370 cm^{-1} can be associated with bending vibrations of the hydroxyl group and sometimes could change its intensity or position due to interaction between molecules, which could include tautomeric equilibria common in azo-phenolic systems [28], [29]. If one were looking at an IR spectrum of a compound, though also useful to check the region 3200-3600 cm^{-1}, where generally the stretching vibrations of an O–H group appear, such information further confirms the hydroxyl group in this compound. The stretching vibration of C–S generally may vary between 600-750 cm^{-1} depending on molecular structure and chemical environment. The intensity of the band at 727 cm^{-1} suggests that the bond has moderate dipole, making the absorption band neither too strong nor too weak. In-plane bending vibrations of C-H bonds are usually seen above 1000 cm^{-1}. They appear in the IR spectrum at 1111, 1162, and 1219 cm-1 in this case (Fig. 4 and Table 3, mode 24, 26, 29). The such-calculated frequencies are 1113, 1171, and 1209 cm^{-1} respectively. Out-of-Plane Deformations CH Modes: Generally, out-of-plane deformations of C-H bonds, CH, are observed within the region 1000-700 cm Typically of the out-of-plane deformations, those with a higher wavenumber such as those at lower wavenumbers are always of weak intensity than the low wavenumber ones. The IR spectrum shows these CH modes at 956, 900, 887, and 858 cm^{-1} (see Table 3, mode 35 - 38) [30]. Theoretically, these modes are calculated to be around 895 ± 60 cm^{-1}) [31]-[34].

4 CONCLUSIONS

This study presents a comprehensive analysis of the vibrational properties and geometry of 2-hydroxy-5-((5-mercapto-1,3,4-thiadiazol-2-yl)diazenyl)-3-Methoxy benzaldehyde -. The investigation employed both theoretical and experimental methodologies to provide a thorough understanding of the compound's IR spectrum. It was a quantum mechanical description of the geometry and vibrational wave numbers. Within the laser Raman spectral wavenumbers, calculated values kept relatively close to the experimental values, thereby confirming the accuracy of the theoretical model. Although minor discrepancies came up between the theoretical and experimental results, those discrepancies are usually attributed to the intrinsic anharmonicity of molecular vibrations and the well-known property of overestimation of the force constants at the equilibrium geometry by quantum mechanical methods.

It can be a very important hyperpolarizability value of the compound for applications in nonlinear optics and as a potential ligand for the synthesis of biologically active metal complexes [35], [36], [37]. This fact really gives us only that 2-hydroxy-5-((5-mercapto-1,3,4-thiadiazol-2-yl) diazenyl)-3-Methoxy benzaldehyde is an easy shortcut to future works tabling the nonlinear optical properties. To sum up, the present study merges theoretical and experimental analyses in the explanation of vibrational and geometric properties of the titled compound. The observed agreement between these two methodologies further compounds the promises of the compound in the prospects for more explorations in the field of nonlinear optics.

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New Metal Complexes with Azo-Schiff Base Ligand Synthesis, Characterisation and Biological Activity

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Keywords: ((E)-3-((4-Acetylphenyl)Diazenyl)-2-Hydroxy-1-Naphthaldehyde), Metal Complexes, Azo-Schiff Ligand, Proteus Mirailis, 4-Nitroaniline.

Abstract: Synthesis and characterization of a new azo-Schiff base ligand (1-(4-((E)-(3-hydroxy-4-((E)-((4-nitrophenyl)imino)methyl)naphthalen-2-yl)diazenyl)phenyl)ethan-1-one) (HL), derived from ((E)-3-((4-acetylphenyl)diazenyl)-2-hydroxy-1-naphthaldehyde)) with 4-nitroaniline in a one to one molar ratio. Subsequently, the interaction of HL with various metal ions (CoII, NiII and CuII) in the ratio of one ligand to one metal, produced monomeric coordination connections. These compounds have been fully characterised using analytical methods and spectroscopic techniques, involve elemental microanalysis,^{1}H and ^{13}C-nuclear magnetic resonance, Fourier transform infrared spectroscopy, electronic and mass spectroscopy, as well as magnetic susceptibility and Conductivity measurements. The analytical data verified the formation of coordination compounds exhibiting four and six-coordinate geometries. The antibacterial evaluation for the ligand and its metal complexes against bacterial strains and fungus species showed a significant enhancement in the effectiveness of the antimicrobial ligand when forming complexes with metal ions. In addition, the study evaluated the antibacterial activity of synthesised compounds with a number of bacteria and fungi. The complex formation significantly enhanced the antibacterial efficacy of the ligand.

1 INTRODUCTION

Compounds that include both azomethine (–HC=N-) and azo (N=N) groups are called Azo-Schiff bases; the Schiff bases are formed by the condensation of ketones or aldehydes with primary amines, by Hugo Schiff in 1864 initially reported [1]. which are synthesised from the combining of the azo-dye compound (–N=N–) together with the Schiff base containing an azomethine linkage (–C=N–) [2]. Ligand of Schiff base produce stable complexes with different metals, so they have significant importance [3]. It has attracted significant interest from both synthetic and biological viewpoints [4]. Schiff bases can be used for multiple applications, such as preparation and purification of amino or carbonyl compounds and safeguarding these groups sensitive reactions or throughout complex [5]. In addition to biological roles, they serve as pigments and dyes [6], intermediary compounds, inhibiting corrosion [7] and polymeric stabilisers [8]. The reaction formation of the Schiff base is widespread due to the simplicity of its synthesis, high yield, and simple separation [9]. The conjugation of two active parts within a single molecule will cause an increase of the physicochemical and biological properties of this group of chelators and their corresponding chelated complexes [10]. Derivatives of Azo-Schiff base-containing compounds remain constantly a focus of study because of their numerous uses, particularly in the pharmaceutical and chemical fields [11]. Previous research has shown that nitrogen atoms in the conjugated bridge (C=N) of compounds derived from Schiff bases have a considerable coordination capacity with transition metal ions [12]. Compounds derived from Azo-Schiff have been thoroughly examined for optical storage of data, nonlinear optics, and photoswitching, demonstrating photoresponsive behaviour [13]. They contain an extensive range of applications in both biological activity and synthesis of organic molecules. As an example, the plastic, leather, and fabric production. Metal ions coordinate with most of these Azo-Schiff bases to form stable complexes. These complexes are used in many fields, especially medicine, such as corrosion prevention, metal recovery, and nuclear

waste treatment [14]. In recent years, we have documented the production of azo chemicals and the complexes they create [15]-[17]. This study deals with the synthesis of a Schiff base containing heterocyclic and azo compounds. Examining the biological activities of the synthesised compounds and the coordination properties of HL when interacting with metal ions is another goal of this effort. The synthesis of the ligand is based on a two-step procedure, including the preparation of the azo moiety ((E)-3-((4-acetylphenyl)diazenyl)-2-hydroxy-1-naphthaldehyde) (L). The reaction of L with 4-nitroaniline generated the ligand of Schiff base (HL). Furthermore, the interaction of HL with the cobalt(II), nickel(II), and copper(II) ions resulted in the isolation of a number of paramagnetic coordination compounds. This study also investigated the antibacterial properties of these synthesised chemicals.

2 MATERIALS AND EXPERIMENTAL PROCEDURES

The ligand's nuclear magnetic resonance spectra, which include (^{1}H and ^{13}C) spectrum, Utilising dimethyl sulfoxide-d_6 as the solvent, the results have been obtained with a Bruker 400 MHz spectrometer. The measurement frequencies had been 400 MHz for ^{1}H and 100 MHz to obtain ^{13}C, using tetramethylsilane as the internal standard. Fourier transform infrared spectroscopy The spectrum has been obtained utilising KBr granules and collected by using the FTIR-600 infrared Fourier spectrometer throughout a spectrum range of 4000 to 200 cm^{-1}. Analyses utilising positive ion electrospray mass spectrometry were performed with a Sciex ESImass spectrometer. We used a Stuart SMP4 electrothermal device to determine the melting points of compounds. The spectrum of UV-visible has been obtained within the wavelength range of 1000 to 200 nm utilising a Shimadzu UV-160A spectrophotometer. At room temperature, using dimethyl sulfoxide, the solutions were prepared at a concentration of 10^{-3} mol L^{-1} and analysed in a one-centimetre quartz cuvette. The Eutech Instruments Cyber Can CON 510 digital conductivity meter was employed to determine the conductivity of the solution in DMSO with concentrations between 10^{-1} and 10^{-5}. At the Heraeus Vario EL and Shimadzu AA-7000 atomic absorption, elemental (C, H, N) and metal content were utilised for the analysis. Utilising a Metrohm 686 Titro processor and a 665 Dosim unit, the quantification for chloride ions in compounds was conducted by potentiometric titration. Finally, using a magnetic balance from Johnson Matthey, the magnetic characteristics have been assessed at 30°C.

3 SYNTHESIS

3.1 Synthesis of Azo Schiff Ligand

A two-step procedure was employed for the synthesis of the azo Schiff ligand as follows;

3.1.1 Synthesis of Azo ((E)-3-((4-Acetylphenyl) Diazenyl)-2-Hydroxy-1-Naphthaldehyde) (L)

The synthesis of L performed according to the described procedure [17]-[20] as following; a round-bottomed flask (250 ml) containing 1.35g (10mmol) of 4-aminoacetophenone and 0.69g (10mmol) of sodium nitrite, 20ml of a 1:1 ethanol-water mixture had been added. At temperature range (0 to 5°C), the solution was cooled by using an ice bath. Subsequently, in a beaker containing 10 ml of cold distilled water, 3 ml of hydrochloric acid (36%) was placed, followed by the addition dropwise with stirring over one hour. Diazonium salt solution is the product of this process, then reacts with a coolant mix that has 0.8g (20mmol) of sodium hydroxide and 1.72g (10mmol) of 2-hydroxynaphthalene-1-carbaldehyde in 20 ml of ethanol. for two hours, the reaction mixture had been allowed to stir. Subsequent to the reaction, the precipitate was filtered at pH 4, washed with cold water until its pH became 6-7, and then allowed to dry. The precipitate was filtered and was orange-red in colour. The resulting product was washed with 5ml of cold ethanol then let too dry at room temperature. The yield: 2.293g (72.10%), having a melting point among 136-138°C. ^{1}H-nuclear magnetic (400 MHz, DMSO-d_6,ppm), $H_{(a)}$ at δ 9.67 ppm. 8.54; 8.45 ppm (s, 1H), which equivalent to $H_{(b)}$ and $H_{(c)}$ protons, respectively. $H_{(g.g-)}$ at 8.33 (d, J = 9.1 Hz, 2H). $H_{(h,h-)}$ displays at 7.79 (d, J = 7.9 Hz, 2H). $H_{(d,f)}$ at 7.48, 7.39 (t, J = 7.5 Hz, 2H). at 7.01 (d, J = 9.2 Hz, 2H); 6.76 (d, J = 9.6 Hz, 1H) belongs to $H_{(j,k)}$. $H_{(i)}$ at 2.60 ppm. Finally, at 15.27 ppm belongs to the impact of tautomerism. The ^{13}C-nuclear magnetic resonance (100 MHz, DMSO-d6, ppm). Resonances at δ_c = 197.11 and 193.27 ppm were assigned to: (ketonic C_a); (aldehydic C_b), respectively. The Signal of phenolic carbon (C_c) was detected at 177.23 ppm. Resonances assigned for N-(C_n), was observed at 164.44 ppm, when the other N-

(C_q) chemical shifts appeared at 147.06 ppm. The two signals displayed at 143.02; 138.89 were related to (C_w), (C_t), respectively. The peaks which related to (C_e), (C_x) and (C_k) showed with 134,54; 132.14; 130.62 ppm frequency respectively. The two groups of Carbon nucleuses of ($C_{g,g}$-); ($C_{h,h}$-), assigned at 129.75 and 124.71 ppm. Resonance of (C_u); (C_d) signals appears at 128.61; 122.71 ppm, respectively. The assignments of (C_f) and (C_j) resonance appeared at 117.70 and 119.20 ppm, respectively. The methyl group (C_m), appeared as a one peak at 27.15 ppm.

3.1.2 Synthesis of Azo-Schiff Base Ligand (1-(4-((E)-(3-Hydroxy-4-((E)-((4-Nitrophenyl)Imino)Methyl)Naphthalen-2-Yl)Diazenyl) Phenyl) Ethan-1-One) (HL)

Initially, a mixture containing 4-nitroaniline 0.433g (3.14mmol) in 10ml of ethanol, were added three drops of glacial acetic acid, was mixed with ((E)-3-((4-acetylphenyl)diazenyl)-2-hydroxy-1-naphthaldehyde) 1.0g (3.14mmol), It was initially dissolved in a 20 ml solution of a one-to-one mixture of ethanol and benzene. For 6 h, the mixture was heated at ca. 75°C and subsequently filtered while still hot. The red produce has been filtrated and also washed with 5 ml of EtOH. The final result was dried in the air. yield of ligand (HL) was 0.801g (58.09%), having a melting point among 170-172°C (see Figure 1). ^{1}H-nuclear magnetic resonance (400 MHz, dimethyl sulfoxide-d_6,ppm), at δ 9.68 ppm (s, 1H attributed to $H_{(a)}$. at 8.54; 8.45 ppm (s, 1H), which equivalent to $H_{(b)}$ and $H_{(c)}$, respectively. $H_{(g.g-)}$ at 8.35 (d, J = 9.1 Hz, 2H). $H_{(l,l-)}$ at 8.32 (d, J = 8.7 Hz, 2H) , 8.09 (d, J = 8.1 Hz, 2H) belongs to $H_{(h,h-)}$. $H_{(m,m-)}$ displays at 7.72 (d, J = 7.9 Hz, 2H). $H_{(d)}$ appear at 7.48 (t, J = 7.5 Hz, 1H). $H_{(f)}$ appear at 7.39 (t, J = 7.5 Hz, 1H). at 7.00 (d, J = 9.2 Hz, 2H); 6.77 (d, J = 9.6 Hz, 1H) belongs to $H_{(j,k)}$. $H_{(i)}$ showed at 2.60 ppm. The ^{13}C-nuclear magnetic resonance (100 MHz, DMSO-d_6, ppm). Resonances at δc = 197.11 and 177.23 ppm assigned to (ketonic C_v); (iminic C_b), respectively. (C_p) was detected at 172.74 ppm. (C_n), was observed at 150.10 ppm, (C_q) appeared at 147.06 ppm. At 138.92 ppm was related to (C_r). (C_s) showed with 134.54 ppm, at 133.55; 128.94 ppm belongs to ($C_{x,w}$). (C_c) displayed at 127.46 ppm. ($C_{g,g}$-); ($C_{m,m}$-); ($C_{l,l}$-); ($C_{h,h}$-), assigned at 127.34, 126.20, 125.72 and 124.57 ppm. (C_j) appears at 122.82 ppm. (C_k) appeared at 122.31 ppm, (C_t) appeared at 121.63 ppm.($C_{f,d}$) displayed at 121.25; 117.70 ppm. (C_u) detected at 109.62 ppm. The methyl group, (C_i) appeared as a one peak at 27.15 ppm.

3.2 Synthesis of Complexes

The synthesised complexes process was a similar process used to synthesise the Co(II) complex. The procedure is as follows: It has been placed 0.3g (0.68 mmol) from HL had been dissolv in 10 ml of mixed solution (5:5) (ethanol benzene), then 10 ml of ethanol solution KOH 0.038g (0.68mmol) has been added in a 100ml round-bottomed flask. We stirred the mix. Subsequently, the slow addition of a solution of Cobalt(II)chloride hexahydrate 0.16g (0.68mmol) in 5ml of EtOH was performed. For 2 hours, the reaction mix was subjected to reflux heating. Following the heat process, the substance underwent filtration, washed to remove any remaining unreacted material used a cold ethanol and allowed to air dry. 0.231g (61.48%) was the yield of the Co(II) complex, with a melting point above 300°C. The synthesis process has been described in Figure 2. See Table 1 for further information on yields, colors, quantity of metal salts utilised, and m.p. of the compounds resulting.

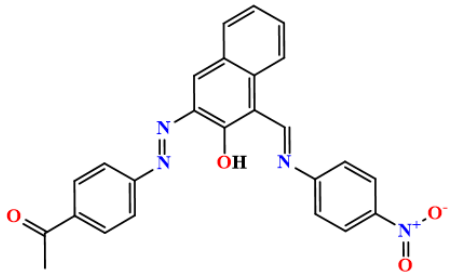


Figure 1: Chemical structure of Azo-Schiff ligand.

4 MICROBIOLOGICAL EVALUATION

The susceptibility of bacteria and fungi to the generated chemicals has been assessed utilising the Kirby-Bauer disk diffusion method. Organism colonies were suspended in an 85% sodium chloride solution to achieve a turbidity comparable to 0.5 McFarland standard. over the surface of Mueller Hinton agar in a Petri dish, this suspension was evenly spread. Wells on the agar were created with uniform spacing and concentration. In each well, 100 ml of the test sample diluted to 1 mg/ml DMSO was

Table 1: Yield, colors, metal salts quantites and m.p. of compounds.

compounds	Weight of metal salt(g)	Weight of complex(g)	Colour	m.p.ºC	Yield (%)
[Co(L)H_2O]Cl	0.16	0.37	Brown	>300*	61.48
[Ni(L)H_2O]Cl	0.16	0.37	Green	>300*	52.83
[Cu(L)Cl(H_2O)$_2$]	0.16	0.39	Dark Brown	>300*	63.32

Table 2: Microanalysis and physical characteristics of compounds.

compounds	Molecular Formula	M.Wt	Micro analysis found, (calculated)%				
			C	H	N	M	Cl
[Co(L)H_2O]Cl	$C_{25}H_{19}ClCoN_4O_5$	549.83	(54.61) 54.15	(3.48) 3.11	(10.19) 10.00	(10.72) 10.24	(6.45) 6.23
[Ni(L)H_2O]Cl	$C_{25}H_{19}ClNiN_4O_5$	549.59	(54.64) 54. 15	(3.48) 3.29	(10.19) 10.08	(10.68) 10. 19	(6.45) 6. 12
[Cu(L)Cl(H_2O)$_2$]	$C_{25}H_{21}ClCuN_4O_6$	572.46	(52.45) 52.22	(3.70) 3.36	(9.79) 9.23	(11.10) 11.00	(6.19) 6.03

Table 3: FT-IR data of the most prominent peaks (cm^{-1}).

Compounds	ν (C=O) ν (C=N)	ν (C=C)	ν N=N	ν C-O ν C-N	ν (H_2O) ν (M-OH_2)	ν (M-O) phenoli .	ν M-N	ν M-Cl
[Co(L)H_2O]Cl	1678 1618	1539 1504	1475	1382 1263	3452 752	661	474 420	-
[Ni(L)H_2O]Cl	1674 1618	1593 1539 1500	1454	1382 1263	3412 746	621	482 451	-
[Cu(L)Cl(H_2O)$_2$]	1674 1618	1597 1541 1502	1465	1359 1265	3439 742	684	499 418	287

added. After incubating, zones of inhibition were evaluated for 24 hours at 37°C and compared to reference values [21]. Control experiments using DMSO solutions confirmed the absence of intrinsic anti-microbial activity. Any of the tested bacteria or fungus.

5 RESULTS AND DISCUSSION

The Azo-Schiff Base ligand (1-(4-((E)-(3-hydroxy-4-((E)-((4-nitrophenyl)imino)methyl)naphthalen-2-yl) diazenyl) phenyl) ethan-1-one)) (HL). 4-nitroaniline and ((E)-3-((4-acetylphenyl)diazenyl)-2-hydroxy-1-naphthaldehyde) reacting in EtOH at a one-to-one ratio. As a tridentate species the ligand functions, supplying the azo's nitrogen atom, hydroxyl and nitrogen imine as donor atoms. The interaction of the ligand with the chlorides of metal of cobalt(II), nickel(II), and copper(II), occurs in a mole ratio of one to one (Ligand to Metal) resulted in the isolation of four and six-coordinate monomeric compound of the universal formula [M(L)H_2O]Cl with cobalt(II), nickel(II), [M(L)Cl(H_2O)$_2$] with copper(II) ion Figure 2. The isolated compounds exhibit stability in the air, exist as solids, and are soluble in DMSO and DMF. However, it is not soluble in other common organic solvents. Based on their physico-chemical data, complexes' coordination geometries and complexation behavior were assumed.

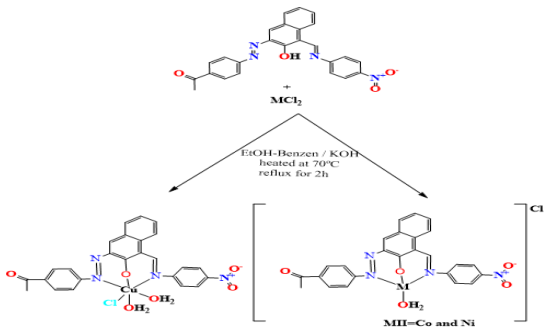


Figure 2: General route for the synthesis of HL complexes.

Results shown in Table 2 are well suited to the proposed formula. Conductance measurements of the compounds in dimethylsulfoxide solutions showed that the complexes are electrolytic and non-electrolytic. The entity of Azo-Schiff Base ligand was confirmed by C, H, N, M Table 2, Fourier transform infrared spectroscopy Table 3, Electron spectroscopy Table 4.

5.1 FT-IR and NMR Data

In Table 3 the main IR bands of the complexes with their duties have been listed. The peak is observable in the ligand spectrum at 3408 cm^{-1} due to ν(OH) stretching vibration of the phenol hydroxyl group [22]. at 1624 and 1487 cm^{-1}. The noted bands correspond to stretching of the imine group ν(C=N) and the stretching of the azo group ν(N=N), respectively [23]. The complex spectrum had a noticeable extent between 1618 and 1614 cm^{-1}.which is related to ν (C=N). Interaction between the metal ion and the ν(C=N) imine group is explained by the bands that form upon complexation [22]. Band observed at 1487 cm^{-1} in HL, which belongs to the azo group ν(N=N) [20], [22]. It was displaced and appeared at 1475, 1454 and 1465 cm^{-1} in the five complexes in order. The occurrence might have been associated with the interaction of the nitrogen atom in complexation. In addition, the spectra of the metal complex exhibited novel bands in the region of (684-621) and (499-418) cm^{-1} that hadn't been apparent in the ligand spectra attributed to ν(M-O), ν(M-N), and ν(M-Cl), respectively [22], [23]. Finally, the complexes of cobalt(II), nickel(II), and copper(II) showed peaks at (3452), (3412) and (3439) cm^{-1}, respectively. were bound to aqua molecules. At 752, 746 and 742cm^{-1}, complex bands 1,2 and 3 can be found. These are linked to water-coordinated ν(M-O) for 1,2 and 3 [24], [25]. The identification of peaks in the nuclear magnetic resonance spectra according to the numbering method illustrated in (Fig. 1). The spectrum ^{1}H- NMR of ligand with dimethyl sulfoxide-d_6 as a solvent, is seen in (Fig. 3). The chemical shift at δ 9.68 ppm (s, 1H), attributed to the phenolic proton $H_{(a)}$. The chemical shift that showed as a singlet signal at 8.54; 8.45 ppm (s, 1H), which equivalent to $H_{(b)}$ and $H_{(c)}$ protons, respectively. The spectrum shows doublet signal refers to $H_{(g.g-)}$ at 8.35 (d, J = 9.1 Hz, 2H). The signal reveals as doublet at 8.32 (d, J = 8.7 Hz, 2H) attributed to $H_{(l,l-)}$ when the frequency value 8.09 (d, J = 8.1 Hz, 2H) belongs to $H_{(h,h-)}$ proton. The doublet signal of attributed to $H_{(m,m-)}$ displays at 7.72 (d, J = 7.9 Hz, 2H). The value of $H_{(d)}$ appear as a triplet signal at 7.48 (t, J = 7.5 Hz, 1H). The value of $H_{(f)}$ appear as a triplet signal at 7.39 (t, J = 7.5 Hz, 1H). when another doublets signals displayed at 7.00 (d, J = 9.2 Hz, 2H); 6.77 (d, J = 9.6 Hz, 1H) belongs to $H_{(j,k)}$ protons. The aliphatic region revealed a singlet peak along with a set of three singlet peak belongs to $H_{(i)}$ protons appeared at 2.60 ppm. The spectrum displayed peaks at 2.51 and 3.37 ppm, corresponding to the DMSO-d_6 solvent as well as the quantity of water molecules in the solvent, respectively. Figure 4 shows the ^{13}C nuclear magnetic resonance spectrum in DMSO-d_6, which shows the correct number of carbon atoms in a molecule. Resonances at δc = 197.11 and 177.23 ppm were assigned to :(ketonic C_v); (iminic C_b), respectively. The Signal of phenolic carbon (C_p) was detected at 172.74 ppm. Resonances assigned for N- (C_n), was observed at 150.10 ppm, when the other N- (C_q) chemical shifts appeared at 147.06 ppm. The signals displayed at 138.92 ppm was related to (C_r). The peak which related to (C_s), showed with 134.54 ppm frequency, when the other two peaks which revealed at 133.55; 128.94 ppm belongs to carbon nucleuses ($C_{x,w}$). The peak displayed at 127.46 ppm attributed to (C_c). The four couples set groups of Carbon nucleuses of ($C_{g,g-}$); ($C_{m,m-}$); ($C_{l,l-}$); ($C_{h,h-}$), assigned at 127.34, 126.20, 125.72 and 124.57 ppm. Resonance of (C_j) signals appears at 122.82 ppm. The assignment of (C_k) resonance appeared at 122.31 ppm, when the resonance of (C_t) appeared at 121.63 ppm.The two carbon atoms ($C_{f,d}$) displayed at 121.25; 117.70 ppm. The signal which related to (C_u) detected at 109.62 ppm. The methyl group, (C_i) appeared as a one peak at 27.15 ppm, when the solvent signals of the DMSO-d_6 resonances appeared at 40.17 ppm [26]-[29].

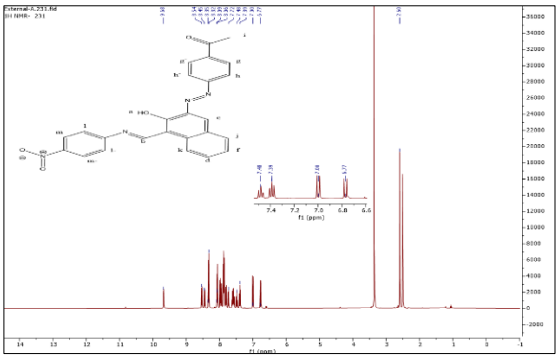


Figure 3: ^{1}H-NMR spectrum in DMSO-d_6 solutions of Azo-Schiff ligand.

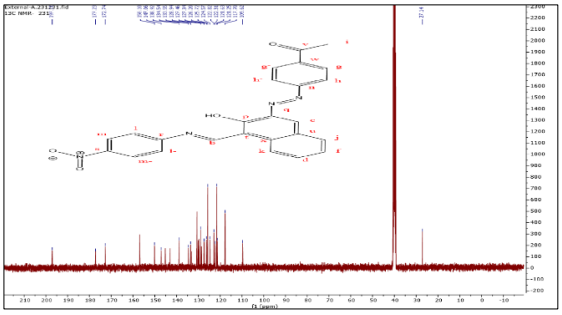


Figure 4: ^{13}C-NMR spectrum in DMSO-d_6 solutions of Azo-Schiff ligand.

5.2 Mass Spectrum

Mass spectrum HL was obtained by electron scattering positive mass spectroscopy, spectrum in Figure 5 showed the presence of a parent ion molecule (M-H)+ at m/z=437.55amu (1%) determined for $C_{25}H_{18}N_4O_4$, which requires 438.13 amu. The fragmentation pattern ligand showen in Figure 6.

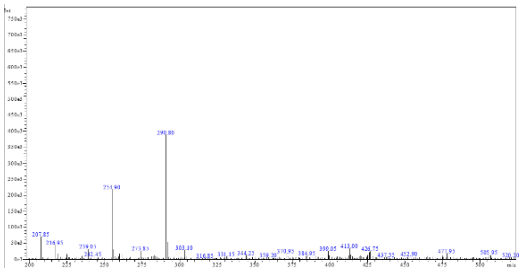


Figure 5: The electrospray (+) mass spectrum of ligand.

5.3 Electronic Spectra and Magnetic Moment

Magnetic moment and UV-visible is summarised in Table 4. Electron spectra of the complexes showed characteristic peaks in the range 257-298nm, indicating transitions π→π* and n→π*. Further, Charge transfer phenomena allow for the observed peaks in an array between 314-481nm [30], [31]. For the Co(II) complex, the spectrum exhibits bands at 676 nm of the d-d region that correspond with the transition $^4A_2(F) \rightarrow {}^4T_1(p)$. A four-coordinate complex suggested by these bands indicates a tetrahedral geometry around the ion of Co(II). The value of magnetic moment: 4.64 BM, agrees with a tetrahedral arrangement around the Co ion [30], [32]. The Ni(II) complex exhibits peaks at 400 nm and 522 nm, specified for $^1A_1g \rightarrow {}^1B_1g$ and $^1A_1g \rightarrow {}^1A_2g$, indicate the geometry of around the Ni atoms was square planar. Finally, the Cu(II) complex has a peak at 498 nm, ascribed to the $^2B_1g \rightarrow {}^2A_2g$ transition, which suggests a distorted octahedral geometry surrounding the ion Cu. The magnetic moment value for ion Cu(II): 1.83 BM is consistent with this structural interpretation [30], [32].

5.4 Biological Activity

By using *Tetracycline* as a reference medication. were utilised four bacterial strains to evaluate the antibacterial activity of the synthesised ligand along with their metal complexes: *(Escherichia coli and Proteus mirabilis (G−))* and also (*Staphylococcus aureus and Bacillus cereus (G+))*. Control experiments separately using dimethyl sulfoxide showed the absence of any intrinsic antibacterial effects [33]-[35]. Table 5 shows the inhibiting area's diameters have been in comparison with those of the antibiotic syntriaxone. The main results were:

1) HL shows antibacterial activity against (Escherichia coli and Proteus mirabilis (G−)), (Staphylococcus aureus and Bacillus cereus (G+)).
2) The Co(II) complex exhibited greater potency against the tested strains.
3) The metal complexes of HL exhibited significant antibacterial activity comparable to *Tetracycline*, this might result in potential biological uses of the synthesised compounds.

Antifungal testing has been performed on the yeast *(Candida albicans)*, utilising fluconazole as the reference medication. DMSO controls did not exhibit antifungal activity [36]-[39]. The observed antifungal activity The values for the tested substances have been shown in Table 6. The subsequent findings have been discerned:

1) Against Candida albicans. All substances exhibited antifungal activity.
2) Complexity substantially enhanced the antifungal activity of the free ligand, possibly as a result of the chelate process.
3) *Fluconazole* has shown greater effectiveness than complexes against *Candida albicans.*

Table 6: The Demonstrates of antifungal inhibition (mm) zones of ligand and the compounds.

Compounds	*Candida albicaus*
DMSO	-
Fluconazole	9
HL	10
$[Co(L)H_2O]Cl$	18
$[Ni(L)H_2O]Cl$	15
$[Cu(L)Cl(H_2O)_2]$	17

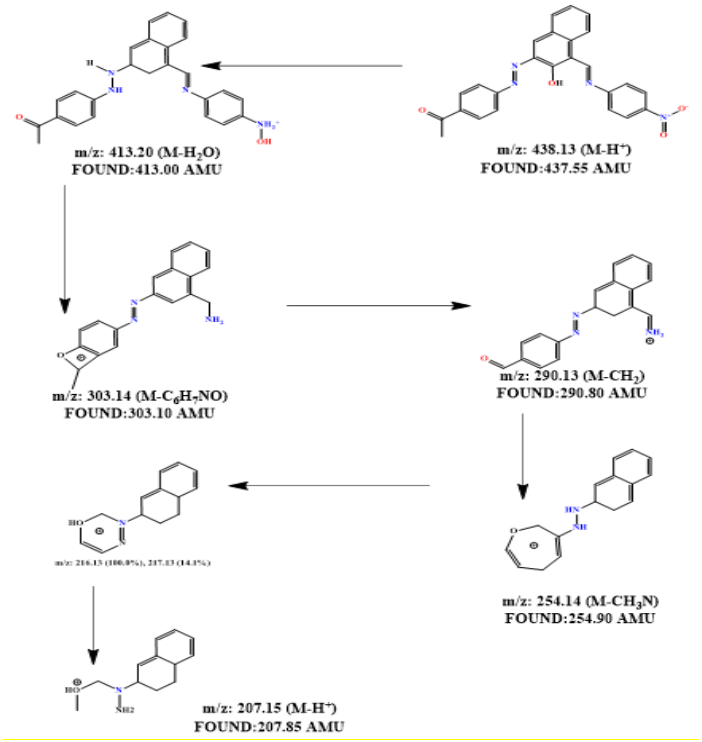


Figure 6: The fragmentation pattern ligand.

Table 4: Displays Uv-vis data for complexes in DMSO solutions.

Complex	ʎnm	Molar extinction coefficient εmax (dm^3 mol^{-1} cm^{-1})	Assignment	Suggested Geometry
$[Co(L)H_2O]Cl$	279 389 481 676	1680 1780 1190 1	Intra-ligand π → π*, n→π* C.T C.T $^4A_2(F) \rightarrow {}^4T_1(p)$	Tetrahedral
$[Ni(L)H_2O]Cl$	257 314 400 522	1820 241 190 105	Intra-ligand π → π*, n→π* C.T $^1A_1g \rightarrow {}^1B_1g$ $^1A_1g \rightarrow {}^1A_2g$	Square planar
$[Cu(L)Cl(H_2O)_2]$	298 320 498	1790 256 80	Intra-ligand π → π*, n→π* C.T $^2B_1g \rightarrow {}^2B_2g$	Distorted octahedral

Table 5: It shows the areas of antibacterial activity (mm) for HL and compounds.

Compounds	*Bacillus cereus (G+)*	*Staphylococcus aureus (G+)*	*Proteus mirabilis (G−)*	*Escherichiacoli (G−)*
DMSO	-	-	-	-
Tetracycline	30	27	14	14
HL	14	18	10	16
$[Co(L)H_2O]Cl$	21	16	12	22
$[Ni(L)H_2O]Cl$	16	21	17	17
$[Cu(L)Cl(H_2O)_2]$	16	20	16	18

6 CONCLUSIONS

The Azo-Schiff ligand (HL) along with its metal complexes with cobalt(II), nickel(II), and copper(II) have been reported. The ligand HL was synthesised from reaction of the 4-nitroaniline with ((E)-3-((4-acetylphenyl)diazenyl)-2-hydroxy-1-naphthaldehyde) in a mole ratio one-to-one. The interaction of ligand with metal ions at a ratio (one-to-one) ligand-to-metal generated isolated compounds, they have been structurally characterised via several Physical and chemical techniques involve elemental microanalysis, 1H and 13C-nuclear magnetic resonance, Fourier transform infrared spectroscopy, electronic and mass spectroscopy, as well as magnetic susceptibility, Conductivity measurements and Biological Activity. The ligand Prepared had yield (58.09%), having a melting point among 170-172°C, As for the complexes resulting from the reaction of the ligand with metal chlorides, the melting point was higher than 300□C for all complexes. The FTIR of ligand displayed characteristic peaks for phenolic, carbonyl, imine and azo groups. Complexation resulted in carbonyl peak shifts, indicating back-bonding, with additional bands related to the ν(M-N), ν(M-O) and ν(M-OH2) observed in the complex spectra. Mass spectrum of ligand showed the presence of a parent ion molecule $(M+H)^+$ at m/z = 437.55amu , measured for $C_{25}H_{18}N_4O_4$, which requires 438.13 amu. The UV/Vis indicates a tetrahedral geometry around the ion of Co(II), Square planar geometry around the Ni(II) complex and the Cu(II) complex which suggests a distorted octahedral structure. The value of magnetic moment is 4.64 and 1.83 BM for Co and Cu, respectively, this explanation agrees to the electron spectra of the complexes. At last, anti-bacterial and anti-fungal assays demonstrated enhanced bioactivity for the complexes produce a comparison to free ligand, emphasising the plus-side outcomes of complex formation.

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Synthesis Cytotoxic Effect and DFT Investigation of some Benzoyl Thiourea Compounds

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Keywords: Thiourea Benzamide, LUMO, HOMO, DFT, Breast Cancer.

Abstract: Benzoyl thiourea derivatives have garnered significant interest due to their diverse biological and pharmacological properties. A series of benzoyl thiourea derivatives (L1–L5) were synthesized using 4-nitrobenzoyl chloride and 4-methoxybenzoyl chloride as starting acylating agents, reacting with various amines, including 3,4-dimethylaniline, benzene-1,2-diamine, and ethane-1,2-diamine. The chemical structures of the synthesized compounds were confirmed through various spectroscopic techniques, including electron ionization mass spectrometry (EI-MS), Fourier-transform infrared spectroscopy (FT-IR), ^1H and ^13C nuclear magnetic resonance (NMR) spectroscopy, as well as melting point analysis. To support and rationalize the experimental findings, a comprehensive theoretical investigation was performed using Density Functional Theory (DFT) with the B3LYP functional and 6-31G++(d,p) basis set. The computational study involved the determination of frontier molecular orbital energies (HOMO and LUMO), Mulliken atomic charges, and selected structural parameters. Furthermore, the analysis identified key donor atoms in each molecule, providing insights into their electronic behavior. The in vitro cytotoxic potential of the synthesized compounds (L1–L5) was assessed against MCF-7 human breast cancer cell lines using the MTT assay. The results revealed that the compounds exhibited low cytotoxic efficacy under the tested conditions.

1 INTRODUCTION

Thioureas and urea are exceptional chemicals in organic chemistry [1]. Their properties allow them to serve as versatile building blocks in the development of various compounds, including agricultural chemicals and dyes [2]. Thioureas have seen promise in materials science, especially in the production of medical and pharmaceutical chemicals and biological [3]. Many different chemicals can be made from thiourea because it has two active main amino groups. It can also be used to make other chemicals sectors of the pharmaceutical business, owing to its biological properties. It can fight many types of cancer if found early, so these actions include both antiparasitic and anticancer effects [4].

Thiourea derivatives demonstrate notable efficacy in the treatment of breast, prostate, and lung cancer. They also demonstrate anti-tuberculosis, antibacterial, and analgesic effects. Researchers recognize benzoyl thiourea derivatives for their extensive antioxidant, antifungal, antibacterial, antitumor, anti-inflammatory, and antidiabetic properties [5], [6]. Thiourea consists of three distinct functional groups: imino, thiol and amine. Because there are two NH_2 groups on either side of the C=S bond, there are more possible derivatives of Thiourea [7], [8]. They have two strong donating groups, called carbonyl and thiourea, these groups provide both hard and soft donor sites for metal coordination through nitrogen (N), oxygen (O), and sulfur (S) atoms [9]. Carbonyl thiourea can bind anions well because it has two imine groups (-NH) on both the urea and thiourea parts [10]. thiourea to replicate the natural binding mechanisms present in live cells, and thiocarbonyl groups (C=S) in carbonyl thiourea may make the product more acidic, which makes it easier for anions to bind to a similar compound that has a carbonyl group (C=O) [11]. Compounds with thiocarbonyl groups are stable because hydrogen bonds form inside the molecules and molecules interact with each other [12], sulfur (S), nitrogen (N), and oxygen (O) atoms that act as both soft and hard bases, therefore it's very important for coordinating with metal ions [13], [14]. The aim of the study is to prepare five compounds of benzoyl thiourea derivatives (L1-L5) and evaluate the biological activity of the prepared compounds as anti-breast cancer cells and conduct a

theoretical study of the prepared compounds using computational chemistry to analyze some electronic properties.

2 MANUSCRIPT PREPARATION

4-methoxy benzoyl chloride, 4-nitrobenzoyl chloride, Potassium thiocyanate, benzene-1,2-diamine, 3,4-di methyl aniline, ethane-1,2-diamine, acetone, ethanol, methanol, ethyl acetate, diethyl ether, chloroform and hexane were purchased from Merck and Sigma-Aldrich. The purity level of the compounds was very high (around 97–99%).

2.1 Instrumentation

Melting Point was measure by equipment from Thermo Scientific. Using the KBr method, infrared spectra were collected in the 400–4000 cm^{-1} range using FT-IR 84005-SHIMADZU device. The compounds' ^{1}H-NMR and ^{13}C-NMR spectra were captured using deuterated DMSO solvents on a Bruker 400MHz spectrometer. The EI-MS spectra of the compounds was analyzed using Agilent Technologies 5975C spectrometer .

2.2 Synthesis of the Compounds (L1-L5).

The preparation methods were followed according to the literature with some modifications [15], [16].

2.2.1 3-(3,4-Dimethylphenyl)-1-(4-Nitrobenzoyl) Thiourea L1

A solution (10 mmol) containing 1.85g of 4-nitro benzoyl chloride in 15 mL of acetone was added progressively to a solution containing 0.97 g (10 mmol) of potassium thiocyanate in 10 mL of acetone. The mixture was refluxed for 1 h with stirring, and the product was filtered, then added while hot to a solution that was made from 1.21g (10 mmol) of 3,4-di methyl aniline in 20 mL acetone. The mixture was refluxed with a stirrer for 4 h, and followed by thin layer chromatographic (TLC) analysis (ethyl acetate: hexane, 3:7). The yield was cooled, filtered, dried, and recrystallized using hot absolute ethanol. The product was a light yellow solid. Yield= 44%, m.p: 187-189°C .IR data, *ν*max, cm^{-1}: 3217 (NH stretch), 3055 (CH-Ar), 1664 (C=O amide), 1590(C=C) and 1522 (NO_2), 1140 (C=S).^{1}HNMR(400MHz,DMSOd_6),δppm:12.35(s,1H,H4),11.93(s,1H,H3),8.34(d,2H,H1),8.17(d,2H,H2),7.46(d,1H,H9),7.41(s,1H,H5),7.18(d, 1H, H8), 2.23(s,6H,H6).^{13}CNMR(100MHz,DMSOd_6),δppm178.93(C9),167.25(C7),150.22(C2),138.54(C11),137.11(C5),135.99(C12),135.06(16),130.72(C1+C3),130.01(C13),125.60(C14),123.79(C4+C6),122.02(C15),19.90(C18),19.44(C17),(EI-MS,m/z (%)):329.1[M+, 13].

2.2.2 1-(3,4-Dimethylphenyl)-3-(4-Methoxybenzoyl)Thiourea L 2

A similar procedure was used as in compound L1 except instead of 4-nitro benzoyl chloride, 1.35 ml of (10 mmol) was employe 4-methoxy benzoyl chloride. The product was a white solid crystal, Yield=64%, m.p:144-164°C. IR data, νmax, cm-1: 3275 (NH stretch), 2965(CH-Ar),1666(C=O amide),1587(C=C), and,1137(C=S).^{1}HNMR(400 MHz, DMSO-d_6), δppm12.67 (s,1H, H5), 11.36 (s,1H, H4) ,8.03 (d, 2H, H2),7.44 (m, 2H, H8+H9), 7.43 (s,1H, H6) ,7.06(d, 2H, H1), 3.86(s, 3H, H3), 2.23(s,6H,H7), ^{13}CNMR(100MHz, DMSOd_6), δppm:179.43(C9), 168.06 (C7),163.70(C2) ,137.06(C11),136.12(C5),134.86(C12),131.45(C1+C3),129.98(C16),125.63(C13),124.31(14),122.02(C15)114.27 (C6+C4)56.08 (C22)19.90 (C17)19.43 (C18), (EI- MS, m/z (%)):314.3 [M+, 26].

2.2.3 1-(4-Nitrobenzoyl)-3-[2-({[(4-Nitrophenyl) Formamido] Methanethioyl} Amino)Phenyl] Thiourea L 3

A solution (10 mmol) containing 1.85g of 4-nitro benzoyl chloride in 15 mL of acetone was added progressively to a solution containing 0.97 g (10 mmol) of potassium thiocyanate in 10 mL of acetone. The mixture was refluxed for 1 h with stirring, and the product was filtered, then added while hot to a solution that was made benzene-1,2-diamine containing 0.54g of (5mmol) in 20 mL acetone. The mixture was refluxed with a stirrer for 6 h, and followed by TLC analysis (ethyl acetate: hexane, 3:7). The yield was cooled, filtered, dried, and recrystallized using hot absolute ethanol. The product was a light yellow solid. Yield=33%, m.p: 198-200°C. IR data, νmax, cm^{-1} :3362 and 3119 (NH stretch), 3061 (CH-Ar), 1661(C=O amide), 1596, (C=C), 1510 (NO_2), 1143(C=S). ^{1}HNMR (400MHz, DMSOd_6), δppm:12.31(s, 2H, H3), 12.16(s, 2H, H4), 8.40(d, 4H, H6), 8.10(d, 4H, H5), 7.94(d, 2H, H2),7.44(d,2H,H1).^{13}CNMR(100MHz,DMSOd_6),δppm:180.61(C9+C27),167.35(C7+C25),150.32(C3+C21),138.36(C17+C18),133.86(C6+C24),130.70(C2+

C4+C20+C22),127.76(C13+C16),127.23(C14+C15) , 123.87(C1+C5+C19+C23).

2.2.4 3-(4-Methoxybenzoyl)-1-[2-({[(4-Methoxyphenyl) Formamido] Methanethioyl} Amino) Phenyl] Thiourea L 4

The same steps as in compound L3 were followed, but instead of 4-nitro benzoyl chloride , 1.35 ml of (10 mmol) 4-methoxy benzoyl chloride. The product was a white solid. Yield=20%, m.p 211-213°C. IR data , νmax, cm^{-1}: 3277 and 3207 (NH stretch),2958 (aliphatic-CH), 1654(C= O amide), 1598 (C= C), and 1149 (C= S). ^{1}HNMR (400 MHz, DMSO-d6), δppm: 12.57 (s,2H,H3),11.56 (s,2H,H4) ,7.89 (d,4H,H6),7.39 (m,4H,H1+H2), 7.04(d,4H,H5) ,3.85 (s,6H,H7),^{13}CNMR (100 MHz, DMSO-d6) ,δppm:181.09(C27+C9),168.01(C25+C7),163.71(C20+C3),134.03(C17+C18),131.48(C2+C4+C20+C22) ,127.5(C6+C24),127.24(C13+C16),124.20(C14+C15) ,114.21 (C1+C5+C19+C23) ,56.08(C32+C34).

2.2.5 3-(4-Methoxybenzoyl)-1-[2-({[(4-Methoxyphenyl) Formamido]Methanethioyl}Amino) Ethyl]Thiourea L 5

The same steps as in compound L3 were followed, but instead of 4-nitro benzoyl chloride , 1.35 ml of (10 mmol) 4-methoxy benzoyl chloride was used, and instead of benzene-1,2-diamine by 0.33ml (5 mmol) of ethane-1,2-diamine. The product was a white solid. Yield=29%, m.p: 177–179 °C. IR data, νmax,cm^{-1}:3243and3179(NH stretch), 2940(aliphatic CH),1668(C=Oamide),1606.29(C=C)and1166.72(C=S). ^{1}HNMR (400MHz, DMSO-d_6), δppm: 11.20(s, 2H, H_2), 11.04 (s, 2H, H3), 7.97(d, 4H, H5), 7.04(d, 4H, H4),3.97(d, 6H, H6), 3.84 (d, 4H, H1), ^{13}CNMR (100MHz,DMSOd_6),δppm:181.49(C4+C6),167.51(C8+C11),163.53(C15+C20),131.29(C14+C16+C19+C21),124.41(C9+C12),114.19(C13+C17+C18+C22),56.03(C28+C30),43.79(C1+C2).

2.3 Anti-Cancer Activity

Cell lines and culture. MDA-MB-231 (a human breast cancer cell line) was purchased from National Cell Bank of Iran (Pasteur Institute, Iran). Cells were grown in RPMI-1640 medium (Gibco) with 10% FBS (Gibco) supplemented with antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin), respectively. Cells were maintained at 37 °C under humidified air containing 5% CO2 and were passaged using trypsin/EDTA (Gibco) and phosphate- buffered saline (PBS) solution [15].

2.4 Computational Study

Geometry Optimization of the prepared compounds (L1-L5) was performed by configuring them using the semi-empirical method, then calculate employed by the DFT (density function method at the (B3LYP) level and using the basis set 6-31G++ (d ,p) [17] using the Gaussian 09 program.

2.5 Results and Discussion

Production of the compounds (L1-L5) The reaction between Potassium thiocyanate and 4-nitrobenzoyl chloride and 4-methoxybenzoyl chloride at a 1:1 mole ratio produced benzoyl iso thiocyanate derivatives, as shown in Figure 1, while the reaction between the product with the 3,4-dimethylaniline at a1:1 mole ratio resulted in thiourea benzamide derivative compounds L1 and L2, as presented in Figure 1. In addition, the reaction between the product with the benzene-1,2-diamine and ethane-1,2-diamine at a 2:1 mole ratio resulted in thiourea benzamide derivativ compounds L3, L4 and L5 , as presented in Figure 1 [16].

2.6 Characterization Methods

Figure 2 showed the ^{1}HNMR spectra of compounds, multiple signals at 7.01–8.40 ppm, which are associated with aromatic protons [18].The presence of the amide group (-NH) was confirmed by a single peak observed at 11.93, 11.36, 12.16, 11.56 and 11.04ppm for compounds (L1–L5), respectively[19] ,while the proton signal of the (-NH) group appeared as a singlet at 12.35, 12.67, 12.31, 12.57, and 11.20 ppm .The spectra of the compounds L2, L4 and L5 showed a single peak at 3.86 ,3.85 and 3.84 ppm respectively, which could be attributed to the presence of (O-CH_3) protons in the final compounds. The spectra of the compounds L1 and L2 showed a single peak at 2.23 ppm, which could be attributed to the presence of (Ar-CH_3) protons in the final compounds. The spectra of the compound L5 showed a single peak at 3.97 ppm, which could be attributed to the presence of (Aliphatic –CH_2) protons.

^{13}CNMR spectra were observed at 178.93, 179.43, 180.61, 180.09 and 181.49 ppm, which correspond to the carbons of the C=S group in, compounds (L1-L5)

respectively (Fig. 3). However, the signals at 167.25, 168.06, 167.35, 168.01 and 167.51 ppm were assigned to the carbon of the carbonyl group (C=O) [20], [18] in compounds (L1-L5) respectively. The aromatic carbons were manifested in the range of 114.19 -163.71 ppm. However, the corresponding peak related to the(O-CH_3) aliphatic carbons appeared at 56.08 ,56.08 and 56.03 ppm in compounds L2, L4 and L5 respectively, and the corresponding peak related to the(CH_2) aliphatic carbons appeared at 43.79 ppm in the. compounds L5. the corresponding peak related to the (Ar-CH3) appeared at the range (19.43 and 19.90)ppm in compounds L1 and L2 respectively.

Figure 4 presents the infrared spectra of the synthesized compounds (L1–L5), with band assignments based on literature [20], [21]. The spectra exhibited bands of varying intensities (weak, medium, and strong). Weak peaks at 3217, 3275 (L1), 3277, 3207 (L3), and 3243–3179 cm^{-1} (L2, L4, L5) were assigned to N-H stretching vibrations. Aromatic C-H stretching (symmetric/asymmetric) appeared at 3055 cm^{-1} (L1) and 3061 cm^{-1} (L3). Aliphatic C-H stretches were observed at 2965, 2901 cm^{-1} (L2), 2958 cm^{-1} (L4), and 2940 cm^{-1} (L5), displaying medium-to-strong intensity.

Weak C=O stretches were detected for all compounds (L1–L5), while aromatic C=C vibrations showed average intensity at 1590 cm^{-1} (L1), 1587 cm^{-1} (L2), 1596 cm^{-1} (L3), 1598 cm^{-1} (L4), and 1606 cm^{-1} (L5). NO_2 stretches produced strong bands at 1522–1331 cm^{-1} (L1) and 1510–1329 cm^{-1} (L3). The C=S group resonated strongly at 1140 cm^{-1} (L1), 1137 cm^{-1} (L2), 1143 cm^{-1} (L3), 1149 cm^{-1} (L4), and 1166 cm^{-1} (L5).

In EI-MS spectra, fragments at m/z 150, 135, 150, 135, 135 corresponded to ions $(C_7H_4NO_3)^+$ (L1, L3) and $(C_8H_7O_2)^+$ (L2, L4, L5). Shared base ions reflect structural similarities between L1/L3 and L2/L4/L5. Fragment diversity confirms successful synthesis [22].

Figure 5 presents the EI-MS spectra of compounds L1–L5. Compound L1 showed a molecular ion peak at m/z = 329.1 ([C16H15O3N3S]+) with a relative abundance of 13%. Compound L5 showed a peak at m/z = 314.40 ([C17H18O2N2S]+). In relative abundance (26%) compound L2, and The molecular ion peak of the compounds L3, L4, L5 appeared with a very low relative abundance ranging between (3-5)%, The reason is that their molecular ions are unstable and dissociate easily due to their large molar masses. The bass peaks ion of the prepared compounds (L1- L5) appeared respectively in relative abundance 100.

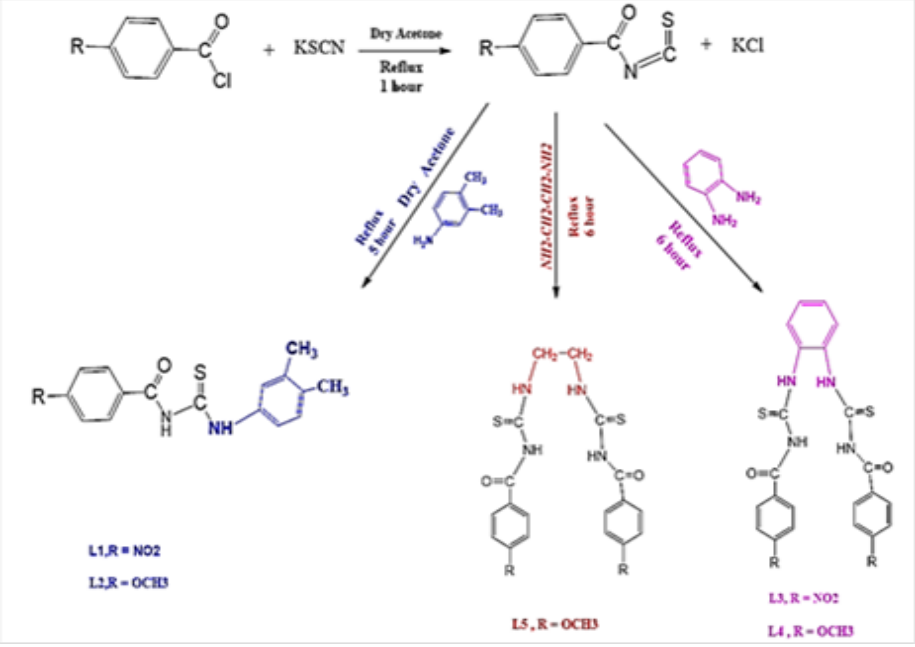


Figure 1: Preparation pathway to compounds (L1 – L5).

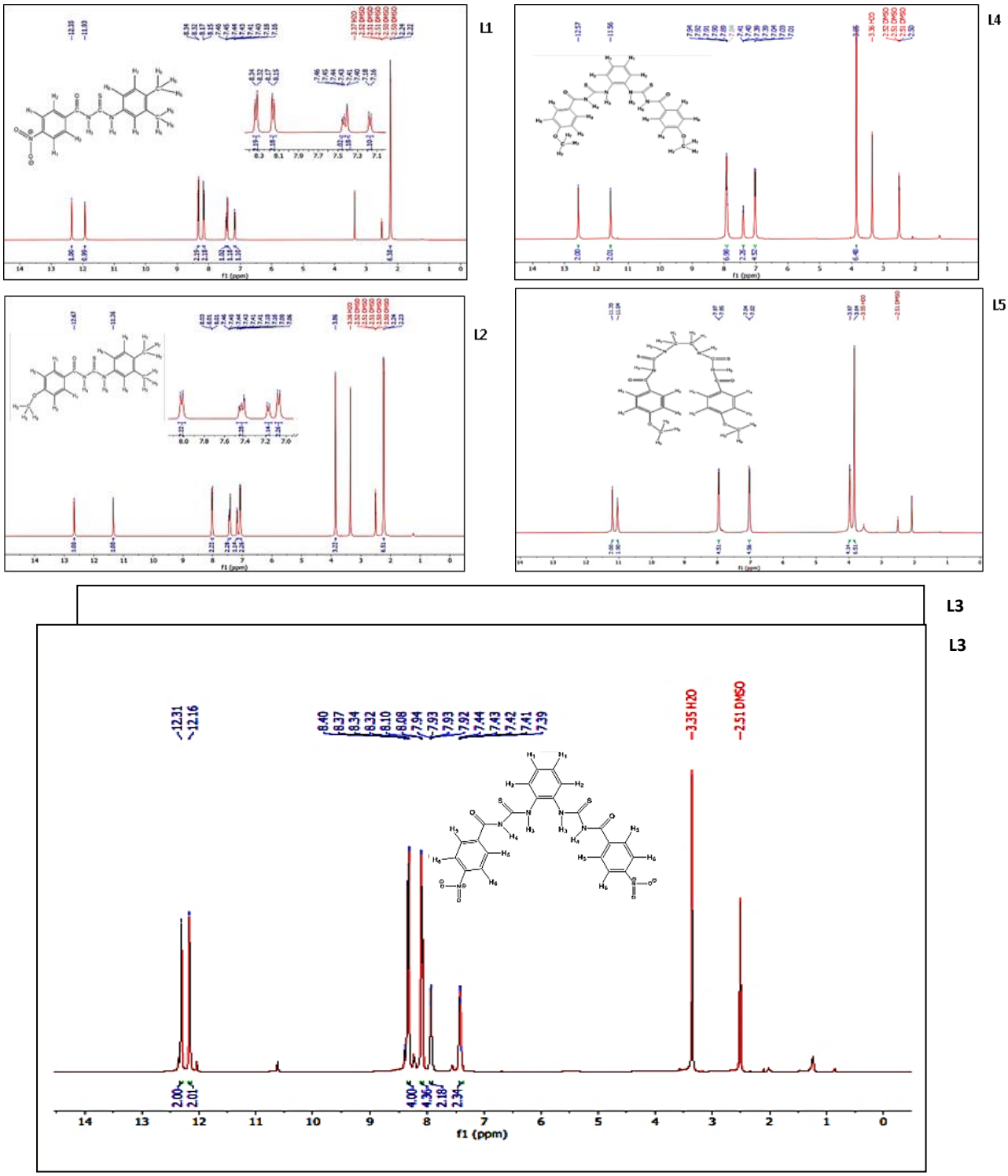


Figure 2: The 1HNMR spectra compounds (L1-L5).

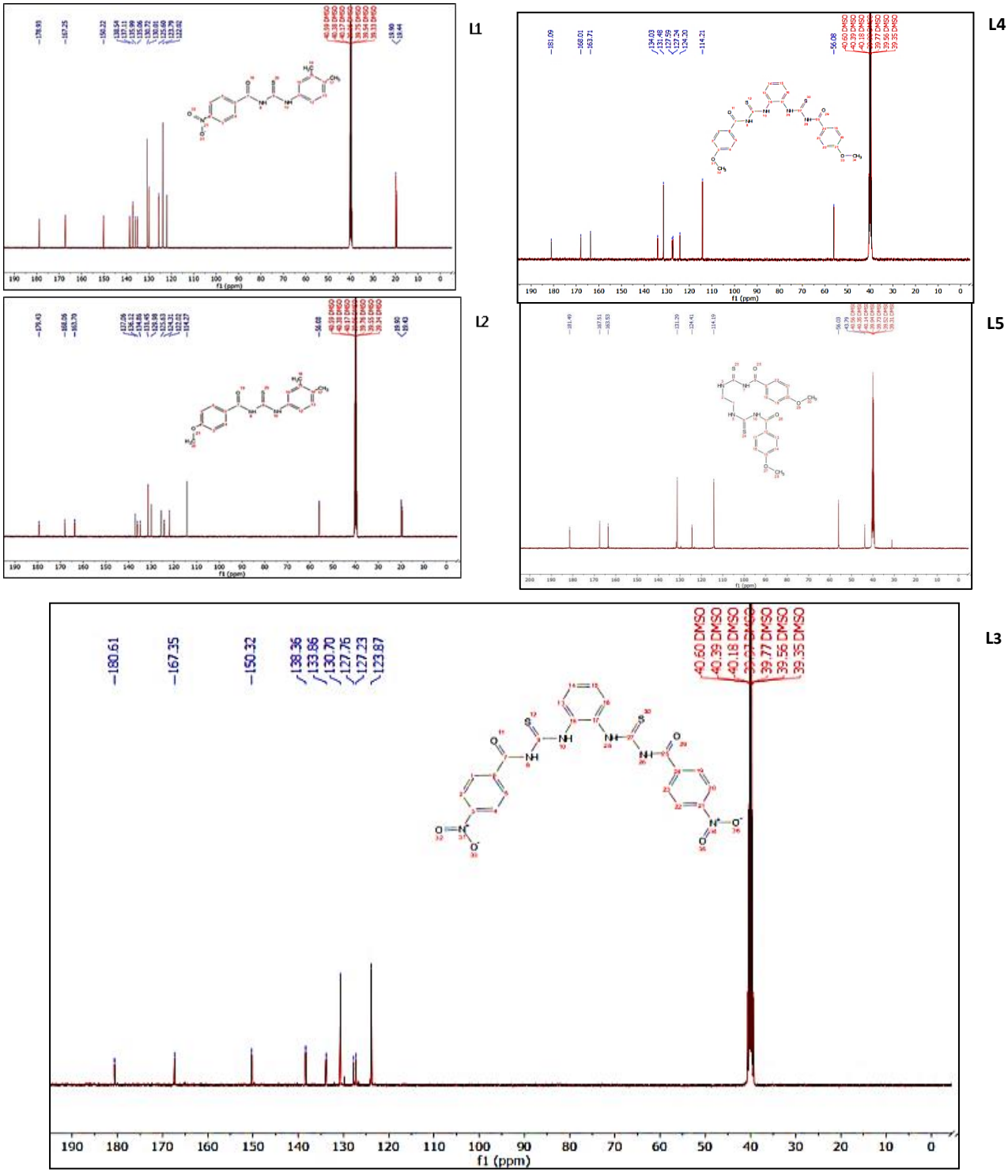


Figure 3: 13C-NMR spectra of compounds(L1-L5).

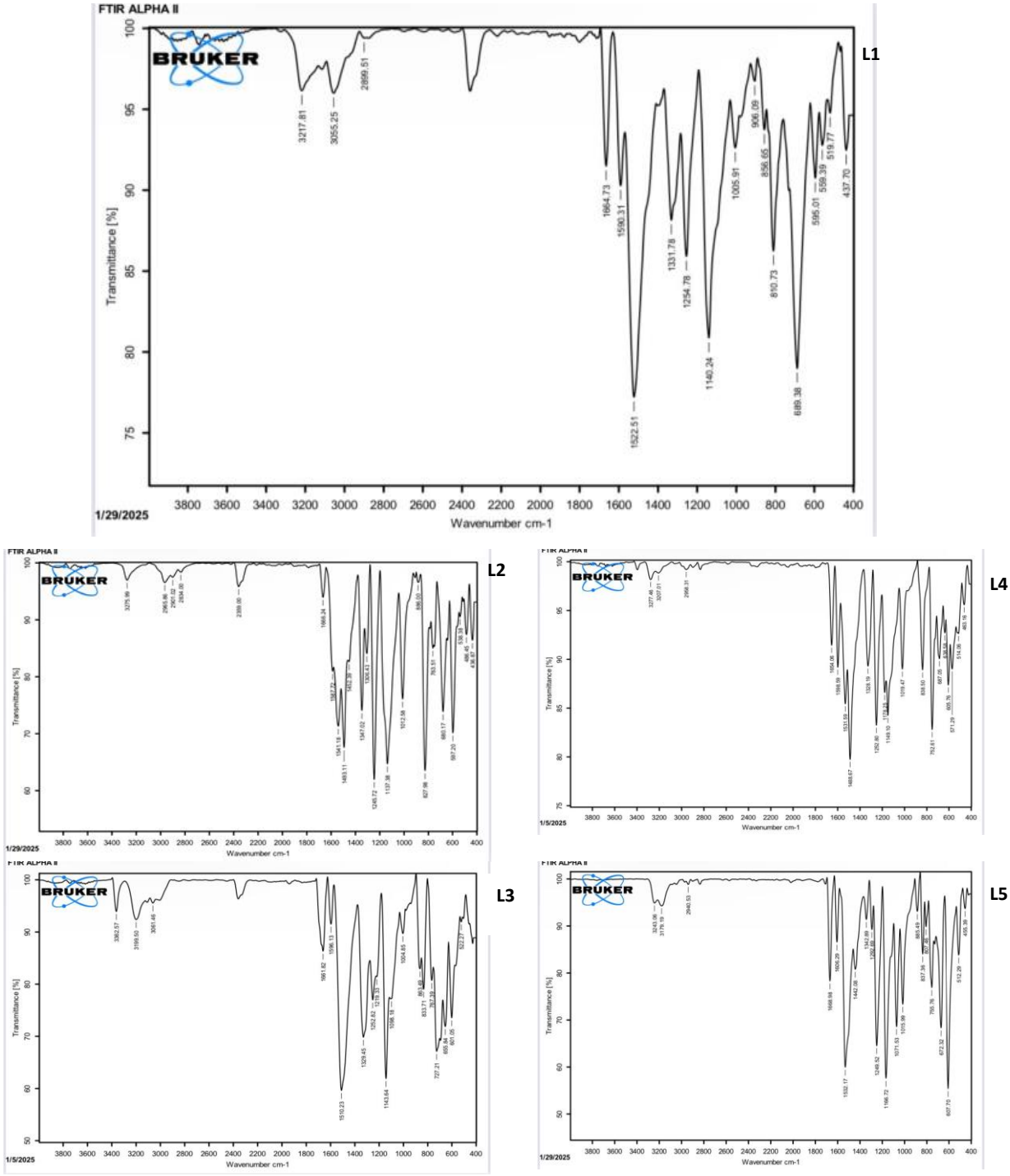


Figure 4: FT-IR spectra of compounds (L1-L5).

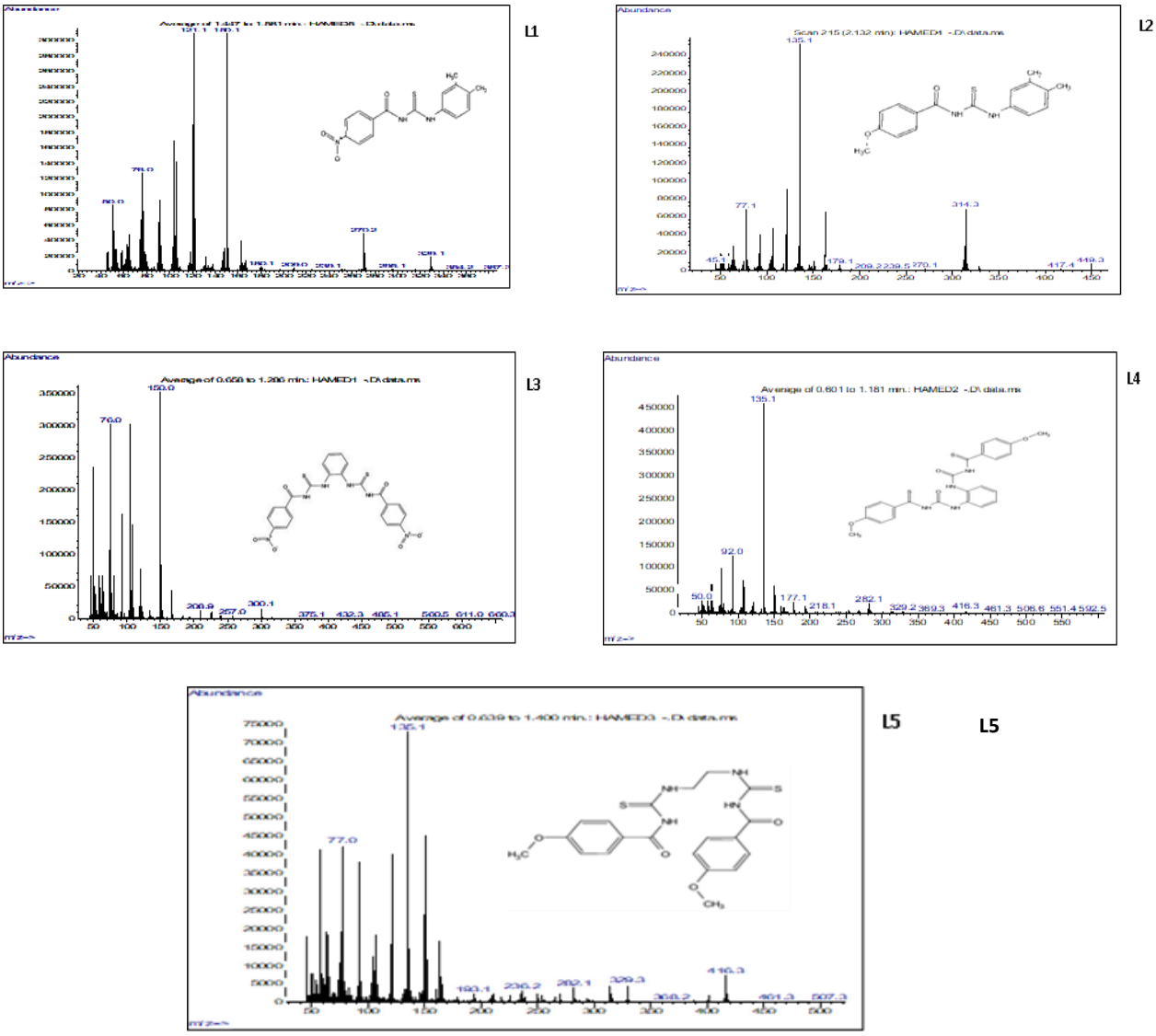


Figure 5: EI-MS spectra of compounds (L1-L5).

2.6 Anticancer Efficacy Study

The toxicity of compounds L1, L2, L3, L4, and L5 against type MCF-7 breast cancer cells was studied in *vitro*. The study was conducted using five different concentrations of (7.4, 22.2, 66.6, 200 and 600) micrograms per milliliter (µg/ml) for each compound, respectively. The inhibitory efficacy was calculated at each concentration, along with the IC_{50} values. Low efficacy in killing cancer cells was demonstrated [15], as is evident from the values shown in Table 1.

2.7 Structural Parameters of the Molecular Structure

The important structural parameters of the optimized geometries were summarized, such as calculating the bond lengths and the triple and quadruple angles of the selected benzoylthiourea derivatives studied (L1-L5). It was found that there was no noticeable change in the bond lengths despite the difference in the substitutes. As we note in Table 2, there is a convergence between the values of the bond lengths, triple and quadruple angles of the studied compounds with the practical values. This confirms that this function is correct and suitable for this type of compounds (Fig. 6).

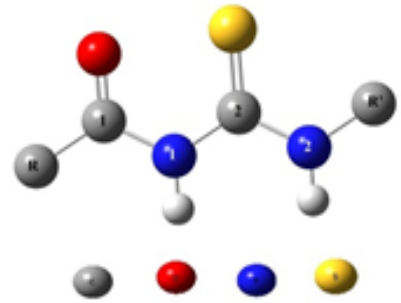


Figure 6: Structural parameters of the molecular structure.

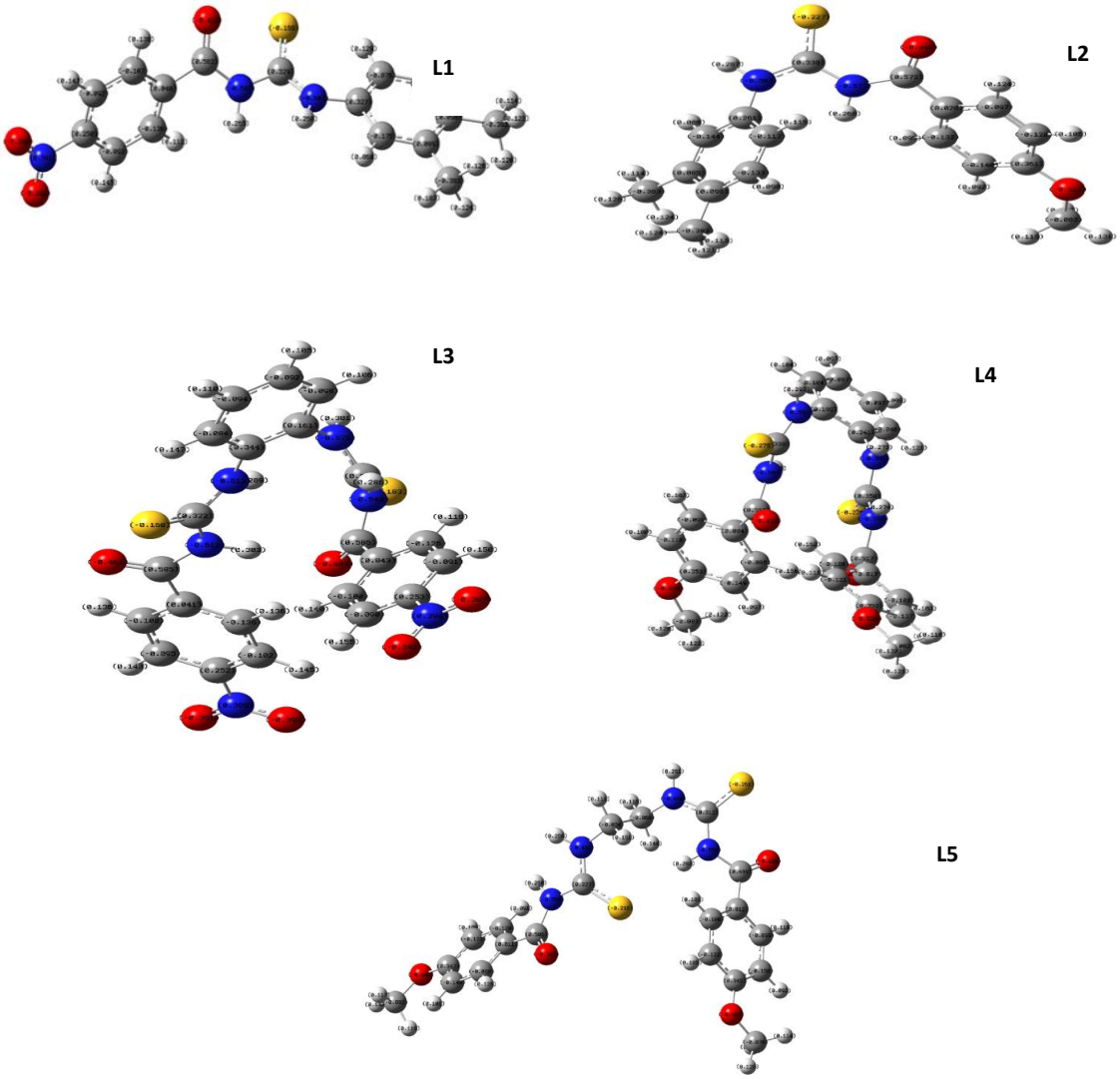


Figure 7: Mulliken charge indicator on atoms of compounds (L1-L5).

Table 1: Inhibition potential of studied compounds againt MCF-7 cell line and IC_{50} values.

Comp.No	7.4	22.22	66.66	200	600	IC_{50}(µg/mL)
L1	2.65	10.15	22.12	38.71	55.08	491.61
L2	8.94	16.52	26.74	34.55	41.52	1662.56
L3	5.83	12.65	22.12	42.12	64.32	312.13
L4	4.92	9.62	23.48	28.48	50.15	972.40
L5	7.5	10.83	21.97	38.79	49.92	702.07

Table 2: The bond lengths, triple angles, and quadruple angles of the studied compounds were calculated using the method by the DFT/B3LYP 6-31G(d,p) compounds (L1-L5).

Bond Lengths (Å)	L1	L2	L3	L4	L5	Experimental values *
C_R-C1	1.50721	1.49014	1.50654	1.49440	1.49329	1.506
C1=O	1.21466	1.19951	1.21581	1.21767	1.21367	1.221
C1-N1	1.39392	1.44943	1.39695	1.40702	1.41394	1.391
N1-C2	1.42111	1.39927	1.40845	1.40003	1.39319	1.376
C2=S	1.65365	1.65897	1.65389	1.65728	1.66785	1.663
C2-N2	1.36497	1.38529	1.37697	1.38106	1.36482	1.376
N2-$C_{R'}$	1.41556	1.43994	1.40631	1.40288	1.45730	1.338
Triple angles (◦)	L1	L2	L3	L4	L5	Experimental values *
C_R -C1-O	121.68369	126.73713	121.53657	122.31454	123.07146	121.74
O-C1-N1	123.95781	121.85704	123.08154	122.14930	122.95462	122.12
C1-N1-C2	126.69775	122.60050	125.54131	125.77605	128.29953	129.09
N1-C2-S	122.21285	122.18932	123.48392	123.78686	124.62692	118.26
S-C2-N2	128.48475	118.15765	127.23150	126.90540	123.65244	127.94
Quadruple angles (◦)	L1	L 2	L3	L4	L 5	
C_R-C1-N1-C2	167.66041	-171.11323	-167.42515	-167.95513	-168.98623	
O-C1-N1-C2	-14.63761	10.49827	15.01441	14.59979	13.42432	
C1-N1-C2-N2	138.97839	-172.28886	-140.82613	-142.09091	-154.87123	
C1-N1-C2-S	-43.38889	10.11958	-165.52039	39.99027	28.02735	
N1-C2-N2-$C_{R'}$	168.65784	6.62085	15.0758	-164.84228	-178.51631	
S-C2-N2-$C_{R'}$	-8.78332	-175.69095	11.49652	12.99456	-1.38162	

*Ref: [17]

Table 3: Calculated values of total energy and LUMO-HOMO energy gap for the studied compounds(L1-L5).

No.	Total energy (eV)	Dipole moment (Debye)	HOMO energy (eV)	LUMO energy (eV)	ΔE (eV)
L1	-31934.4188	6.9287	-6.211401	-4.896844	1.31455
L2	-29892.2335	9.8211	-6.218438	-4.388818	1.82962
L3	-55026.5903	10.1441	- 6.201413	- 4.929759	1.27165
L4	-50941.9959	6.1638	- 5.92697	- 4.163634	1.76333
L5	-47481.0062	13.3499	- 6.135356	- 4.220838	1.91451

Table 4: HOMO and LUMO orbital shapes of the prepared compounds(L1-L5).

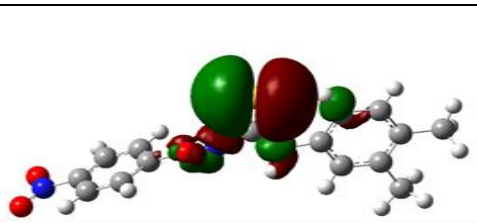
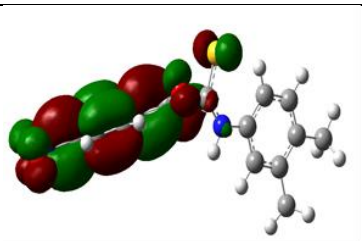
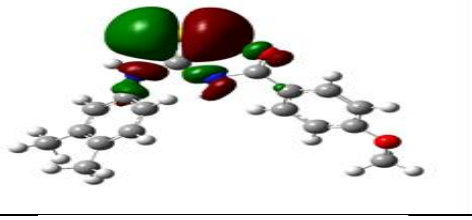
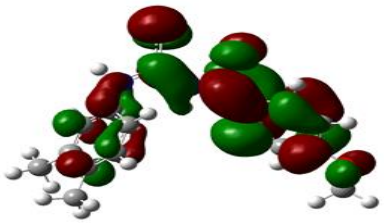
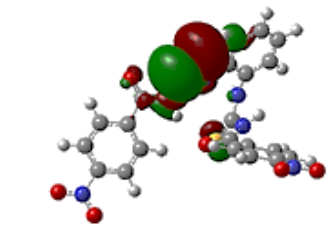
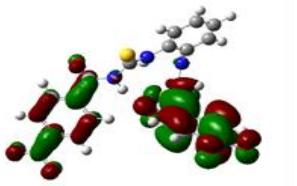
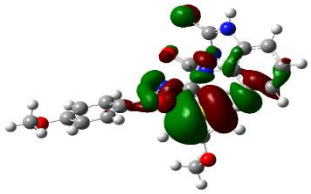
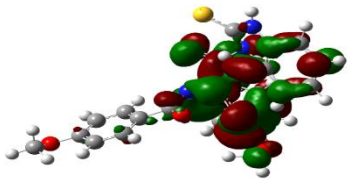
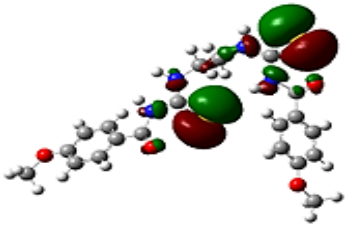
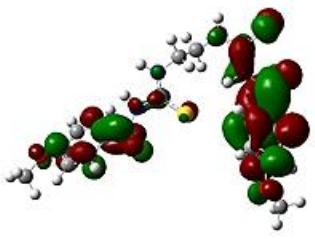
	HOMO	LUMO
L1		
L2		
L3		
L4		
L5		

Table 5: Mulliken charge values of prepared ligands calculated by DFT/B3LYP method 6-31G++(d,p).

Atoms	L1	L2	L3	L4	L5
O	-0.452	-0.449	-0.461	-0.413	-0.399
N1	-0.593	-0.577	-0.611	-0.552	-0.554
S	-0.166	-0.227	-0.168	-0.226	-0.216
N2	-0.602	-0.587	-0.613	-0.591	-0.482

2.8 Calculating of Energies

Molecular orbitals and their properties are very useful for physicists and chemists in particular, the highest occupied molecular orbital (HOMO) and the lowest empty molecular orbital (LUMO) and the energy gap between them reflect the chemical activity of the molecule. The energy values of the orbitals $\Delta E_{(LUMO-HOMO)}$, Highest Occupied Molecular Orbital (HOMO), Lowest Unoccupied Molecular Orbital (LUMO). The HOMO Orbital express the chemical activity and stability of chemical molecules [23]. In addition, the energy of the HOMO orbitals expresses the ionization potential or electron donation (donor), while the LUMO orbitals express the electron affinity or electron acceptance (accepter). The higher value of the HOMO of the molecule tends to donate electrons to a suitable acceptor molecule with low energy and empty molecular orbitals [24].

The total energy, dipole moment, HOMO and LUMO energies, and ΔE LUMO-HOMO energy gap) of the prepared molecules were calculated in the same way as themolecules were calculated in the same way as the geometric optimization, and their values are shown in dipole moment, HOMO and LUMO energies, and ΔE= LUMO-HOMO energy gap) of the prepared Table 3. From the calculated total energy values, we find that the stability of the compounds follows the following sequence L3 > L4 > L5 > L1 > L2, where the compound with the lowest energy is more stable. From looking at the energy difference values between the HOMO and LUMO orbitals shown in Table 3, we find that compound L3 has the lowest value for ΔE Lumo-Homo, which is 1.271654, which reflects its relative chemical reactivity compared to the other studied compounds, as the lower the ΔE value of the molecule, the more chemically and biologically effective it is. We also note that compound energy gap values, which indicates that these compounds are of low effectiveness. The dipole moment is an electronic measure of the polarity of molecules. When observing the values Theoretically calculated. find that the compounds, L5, L3, L2 has the highest dipole moment. When looking at the shapes of the HOMO and LUMO orbitals of the compounds studied in Table 4, we notice that the HOMO orbitals are mainly centered on sulfur, oxygen and nitrogen, which are the atoms that donate electrons. This confirms that the coordination of these molecules is through these atoms, while the LUMO is centered on the benzene molecule and part of it on the sulfur atom.

2.9 Calculating Mulliken Charge

Atomic and molecular charges can be calculated by calculations performed using computational chemistry using one of the methods developed by Robert S. Mulliken. This method, known as the Mulliken method, is calculated by analyzing the distribution of electronic charges on bonded, non-bonded and antibonded atoms. The Mulliken charge on a single atom can be determined using the lowest level of the basis sets and can be for one negative electron in the orbital or more than two negative electrons in a given orbital. Understanding the nature of atoms and making quantitative predictions about the results of experiments is a great benefit from calculating the Mullican charge [25][26]. The Mullican charge was calculated using the same calculation method used for engineering optimization, as the Mollican charge values express the electron density possessed by the atoms and through these values we can know which atoms have the highest negative potential and the highest basicity, Table 5 and Figure 7 that the two nitrogen atoms have the highest value, which ranges between (-0.428- -0.613), followed by the oxygen atoms of the carboxyl group (-0.461- -0.399), then the sulfur atoms (-0.227 - -0.166) in all molecules, so it is expected that the bonding in this type of ligands occurs within the oxygen and nitrogen atoms and sulfur due to the electron density is the highest negative potential.

3 CONCLUSIONS

In this study, a series of benzoyl thiourea derivatives (L1–L5) were successfully synthesized using 4-nitrobenzoyl chloride and 4-methoxybenzoyl chloride with selected aromatic and aliphatic amines. The chemical structures were confirmed by a combination of spectroscopic techniques, including FT-IR, ^1H NMR, ^13C NMR, and EI-MS, which verified the identity and purity of the synthesized compounds.

After preparing the compounds, identifying them and studying them biologically and theoretically, we conclude that it is easy to prepare carbonyl thiourea compounds after the correctness of the proposed structures has been confirmed using different spectroscopic techniques. If the diagnostic methods prove the correctness of the prepared structures, and the biological study of the prepared compounds

against breast cancer has proven that their biological effectiveness is weak, the computer study has shown that all the prepared compounds were chemically stable based on the values of LUOM and HOMO, and it has also been shown that the electron density is concentrated on the oxygen and sulfur atoms. the analysis identified key donor atoms in each molecule, providing insights into their electronic behavior. The in vitro cytotoxic potential of the synthesized compounds (L1–L5) was assessed against MCF-7 human breast cancer cell lines using the MTT assay. The results revealed that the compounds exhibited low cytotoxic efficacy under the tested conditions.

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Synthesis, Characterization and Biological Activity Study of a New Schiff Base-Oxime Derived Ligand and Its Complexes with Some Metal Ions

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Keywords: Oxime, Imine, Schiff Base, Complexes, Azomethine.

Abstract: A new ligand, (3Z)-3-((2-amino-4,5-dimethylphenyl)imino)butan-2-one oxime (L), was created in this research by combining 4,5-dimethyl-1,2-phenylenediamine with diacetyl monooxime in boiling CCl_4. The ligand's structure was confirmed using elemental analysis and various spectroscopic techniques including FT-IR, UV, ^{1}H NMR, ^{13}C NMR, and mass spectrometry. Co(II), Ni(II), Cu(II), and Zn(II) metal ions were complexed with a specific ligand by reacting their chloride salts with the ligand in methanol at a 1:1 molar ratio under reflux conditions. The complexes' structure was confirmed using elemental analysis, spectroscopic methods, magnetic moments, and molar conductivity investigations. The complexes exhibited tetrahedral, square planar, and octahedral geometries for Zn(II), Ni(II), Co(II), Cu(II), and, respectively. In addition to all that has been mentioned, We also obtained the biological activity of the prepared compounds against Gram-positive bacteria (Staphylococcus aureus) and Gram-negative bacteria (Escherichia coli) at different concentrations of the ligand and the prepared complexes. It was found that some of them were effective at certain concentrations, while others were not effective. In addition, the effectiveness of the cobalt complex with bacteria was examined in the presence of the antibiotic (Levofloxacin), and it was found to be effective.

1 INTRODUCTION

Hugo Schiff, a German scientist, coined the term "Schiff base" in 1864 to characterize the compounds resulting from the combination of primary amines with carbonyl compounds. Despite originating over a decade before the establishment of coordination chemistry, Schiff bases continue to hold significance as ligands. They represent a vital category of compounds distinguished by the presence of a double bond (-C=N-) between carbon and nitrogen atoms [1]. These compounds exhibit various noteworthy attributes, such as high thermal stability, flame retardancy, and degradability. As a result, they have been widely employed in catalyst development, gas separation, and drug release industries [2]. Schiff bases stand out as among the most extensively utilized organic compounds, showcasing a diverse array of biological activities. These include but are not limited to antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral, and antipyretic properties [3]. Oximes and their derivatives are important bioactive ligands widely used in coordination chemistry. Oximes are formed through the condensation of hydroxylamine with aldehydes or ketones. The oxime functional group exhibits amphoteric properties, with the azomethine group's nitrogen (C=N) being slightly basic and the hydroxyl group (OH) slightly acidic [4]. Advancements in both inorganic and bioinorganic chemistry have sparked greater interest in Schiff base complexes. This is driven by the recognition that numerous such complexes can potentially serve as models for emulating biomolecules [5]. Imine-oxime ligands have played a crucial role in coordination chemistry, exhibiting distinct chelation behaviours. They find various applications in analytical chemistry, including metal ion extraction and spectrophotometric determination. Moreover, certain imine-oxime complexes have been employed in catalyzing numerous organic reactions [6]. The biological activity of these compounds is frequently linked to their capacity to form complexes with the metals found in biosystems [7]. Additionally, certain hydrazones and oximes have found applications as herbicides, insecticides, nematocides, rodenticides, and plant growth regulators. They are also utilized as plasticizers and

stabilizers for polymers [8]. In addition, they play a significant role in applications in the fields of medicine, industry, and analysis [9]. The number of amine-imine-oxime complexes that have been reported is very low, even though a significant number of oximes and associated transition metal ion complexes have been investigated [10].

2 MATERIALS, METHODS, AND EXPERIMENTAL TECHNIQUES

2.1 Chemicals and Reagents

The synthetic work was carried out exclusively with analytical-grade substances. Among the main reagents employed were diacetyl monoxime (99%, Merck, Germany) and 4,5-dimethyl-1,2-phenylenediamine (97%, Sigma, USA). Additional salts included cesium iodide (98%, Merck, Germany), cobalt(II) chloride hexahydrate, copper(II) chloride dihydrate, nickel(II) chloride hexahydrate, and zinc(II) chloride (all ≥99%, Sigma, USA). Methanol of 99% purity (Merck, Germany) was used as the principal solvent.

2.2 Analytical and Spectroscopic Characterization

To ensure proper identification of the obtained products, a series of instrumental methods was applied. Elemental analyses (C, H, N) were performed with an Eager 300 (EA112). Infrared spectra were obtained on a SHIMADZU 8400S spectrophotometer, applying both KBr and CsI pellets. Proton and carbon NMR (^1H and ^13C) were measured on a Bruker 400 MHz instrument. UV–Vis spectra were registered using quartz cuvettes of 1 cm path length. The magnetic properties of the complexes were evaluated at room temperature with a Sherwood Scientific balance. Molar conductance was measured on a Starter 3000℃ conductivity meter [11].

2.3 Synthetic Strategy

2.3.1 Ligand Preparation

The ligand (3Z)-3-((2-amino-4,5-dimethylphenyl)imino)butan-2-one oxime (L) was obtained by condensation of 4,5-dimethyl-1,2-phenylenediamine (2.1 g, 15 mmol) with diacetylmonoxime (1.55 g, 15 mmol) in carbon tetrachloride (15 mL). The reaction was maintained under reflux with continuous stirring for 3 h. During the course of the process, the solution initially appeared dark brown and later produced a light creamy solid. The precipitate was isolated by filtration, washed sequentially with CCl4 (5 mL) and cold ethanol (3 mL), and finally dried. Yield: 1.6 g (50%); colour: creamy; m.p.: 130–134 °C.

2.3.2 Synthesis of Metal Complexes

Complexes were synthesized by reacting the ligand with the respective metal salts in methanolic solution:

- [L ZnCl]Cl (C1): Ligand L (0.3 g, 1 mmol) in 10 mL methanol was mixed with ZnCl2 (0.136 g, 1 mmol) dissolved in 5 mL methanol. The mixture was refluxed at 95 °C for 3 h. A light green solid was obtained after cooling and filtration. Yield: 0.2 g (57%); m.p.: 200–206 °C.
- [L CoCl(H2O)2]Cl (C2): Prepared analogously, substituting cobalt(II) chloride hexahydrate (0.237 g, 1 mmol) for zinc chloride. The obtained product was dark green. Yield: 0.26 g (76.4%); m.p.: 183–185 °C.
- [L CuCl(H2O)2]Cl (C3): Using copper(II) chloride dihydrate (0.17 g, 1 mmol), a dark green solid was isolated. Yield: 0.23 g (67.6%); m.p.: 155–160 °C.
- L·NiCl2 (C4): Reaction with nickel(II) chloride hexahydrate (0.23 g, 1 mmol) afforded a red compound. Yield: 0.22 g (64.7%); m.p.: 266–270 °C.

2.4 Antibacterial Screening

The newly obtained compounds were subjected to antimicrobial tests against Escherichia coli and Staphylococcus aureus. A disc diffusion method was applied. Three concentrations were prepared: 100 µg/mL (stock), 50 µg/mL (diluted in DMSO), and 25 µg/mL. The inhibitory effects were evaluated after incubation.

2.5 Culture Medium Preparation

Mueller–Hinton agar was prepared by dissolving 38 g of the medium in 1 L of distilled water, sterilized by autoclaving at 121 °C and 15 psi for 15 min. Once cooled, it was poured into sterile Petri dishes and stored under refrigeration. During testing, 100 µL of each compound solution was applied into wells, with sterile distilled water used as positive and negative controls. Incubation was carried out at 37 °C for 24 h [12], [13].

3 RESULT AND DISCUSSION

3.1 Synthesis and Characterization of the Ligand and Complexes

The Schiff base–oxime ligand (L) was obtained via condensation of diacetylmonoxime with 4,5-dimethyl-o-

phenylenediamine (Fig. 1). Its elemental analysis data matched well with the theoretically calculated values (Table 1), confirming the proposed molecular formula. Structural verification was additionally supported by a combination of spectroscopic techniques, including IR, UV-Vis, ^1H and ^13C NMR, and EI-MS. Complexes of L with Co(II), Cu(II), Ni(II), and Zn(II) were synthesized through reactions of the ligand with the corresponding hydrated metal chlorides in methanol at reflux, maintaining a 1:1 molar ratio (Fig. 2). The microanalytical and metal content results for all complexes (Table 1) further confirmed their compositions.

Table 1: Elemental analyses data and physical properties of ligands and their complexes.

No	Molecular Formula	M.wt g/mol	Yield%	Colour	m.p°C
L	$C_{12}H_{17}N_3O$	219.2	50%	Creamy	130-136
C_1	$C_{12}H_{17}Cl_2ZnN_3O$	355.5	57%	light green	200-206
C_2	$C_{12}H_{21}Cl_2CoN_3O_3$	385.1	76.4%	Dark green	183-185
C_3	$C_{12}H_{21}Cl_2CuN_3O_3$	389.7	67.6%	Dark green	155-160
C_4	$C_{12}H_{17}Cl_2Ni\ N_3O$	348.8	64.7%	Red	266-270

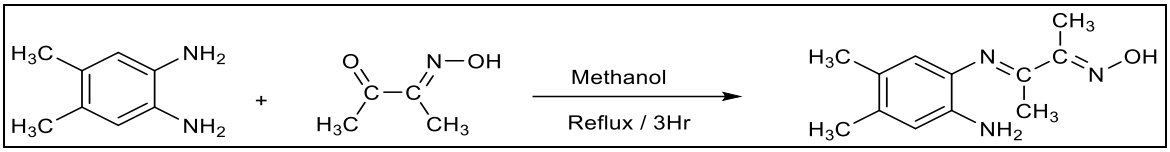


Figure 1: Preparation route of the ligands.

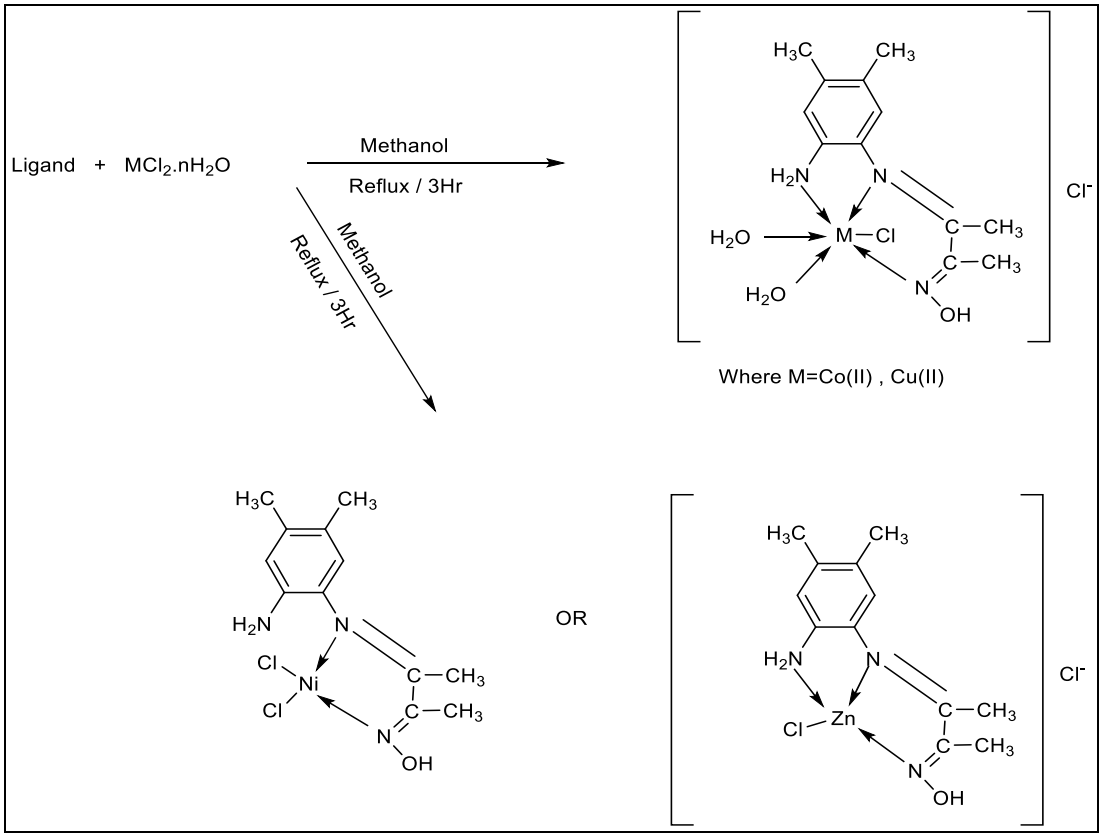


Figure 2: Preparation route of the Complexes.

3.2 Infrared Spectroscopy

The IR spectrum of ligand L displayed several diagnostic bands: ν(NH) stretching at 3379 and 3155 cm^{-1}, ν(OH) of the oxime group at 3286 cm^{-1}, and ν(C=N) of imine and oxime at 1627 and 1581 cm^{-1}, respectively. Upon complexation, the ν(C=N) signals shifted to lower frequencies (~1573 cm^{-1}), reflecting coordination of the azomethine and oxime nitrogens with the metal centers. The ν(N–O) band of the free ligand at 1134 cm^{-1} shifted to 1165–1265 cm^{-1} in the complexes, also supporting metal–ligand bonding. New absorptions at 475–425 cm^{-1} and 300–246 cm^{-1} were observed in all complexes, assignable to ν(M–N) and ν(M–Cl), respectively, and were absent in the free ligand spectrum [14], [15]. Additionally, the spectra of Co(II) and Cu(II) complexes showed features due to coordinated water molecules (e.g., 609, 1774, 3518 cm^{-1} for LCo; 864, 1797 cm^{-1} for LCu). (Fig. 3-4, Table 2).

Table 2: Infrared spectra for ligands and their complexes

No	V(N-H) Assm. Ssm.	*V(C*=N) *Imine*	*V(C*=N) Oxime	ν(N-O)	V(M-N)	ν(M-Cl)
L^1	3379m 3155m	1627s	1581m	1134m	-----	-----
C_1	3270m 3170w	1573m	1458m	1165m	475w	246m
C_2	3170w	1573w	1381w	1226m	439w	300w
C_3	3194 3156	1627	1404	1265w	432w	262w
C_4	3170w	1573s	1442w.b	1242m	425w	250w

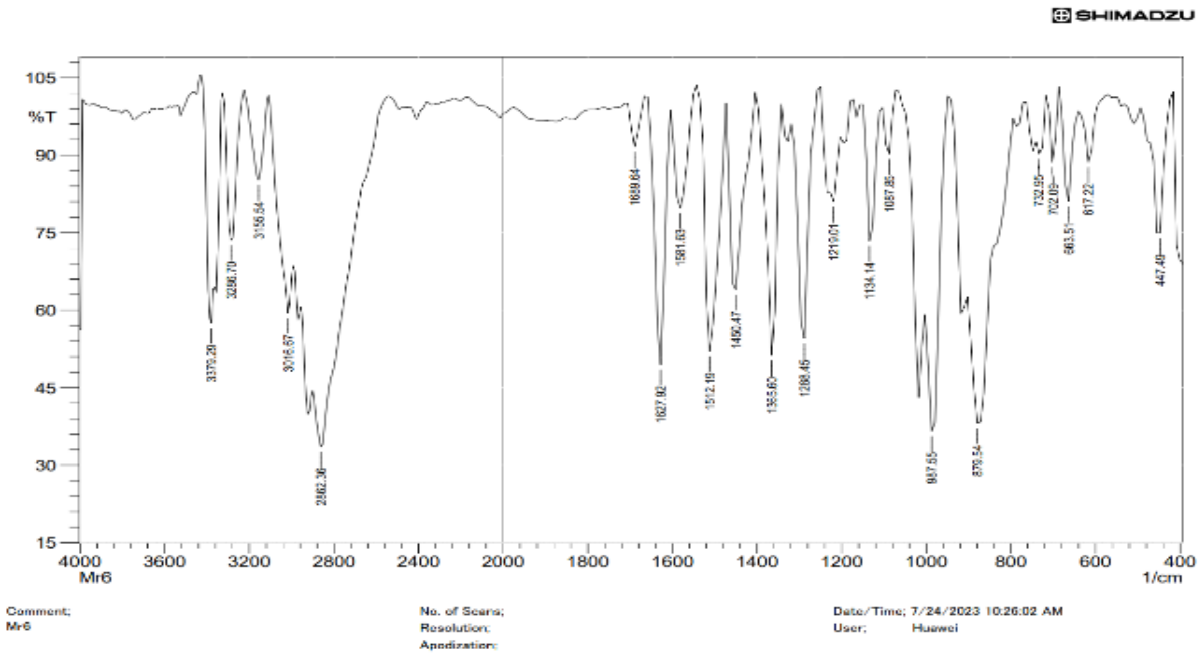


Figure 3: FT – IR spectrum of of the Ligand.

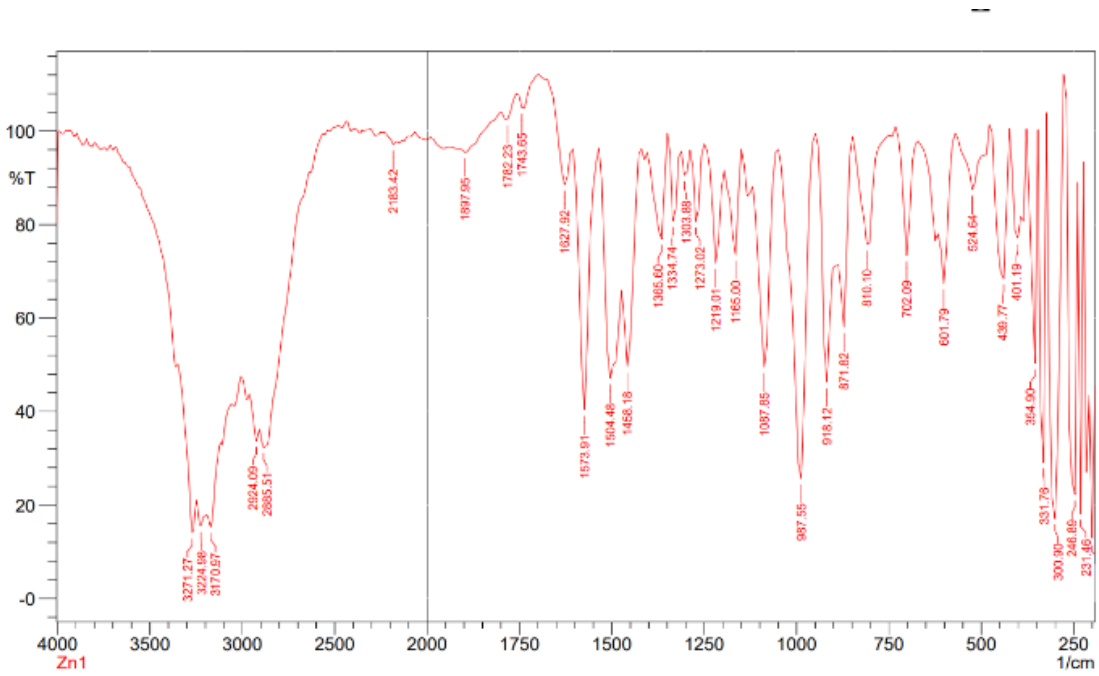


Figure 4: FT – IR spectrum of the [L ZnCl] Cl complex.

3.3 Electronic Absorption Spectra

The UV–Vis spectrum of the free ligand (Fig. 5) exhibited a strong band at 314 nm (31,847 cm^{-1}, ε = 1265 $L \cdot mol^{-1} \cdot cm^{-1}$), attributed to π→π* and n→π* transitions. In the LCu complex, a ligand-centered transition appeared at 258 nm (38,760 cm^{-1}), accompanied by a charge-transfer band at 364 nm. Weak d–d transitions at 662 and 930 nm were consistent with an octahedral geometry around Cu(II) [2], [16]. The LCo complex (Fig. 6) displayed intense bands at 256 and 272 nm (π→π* and n→π*) along with a charge transfer peak at 320 nm. A weak absorption at 500 nm was assigned to the ^4T1g → ^4A2g transition, also supporting an octahedral environment [17], [18].

For LNi, the spectrum showed π→π* and n→π* bands at 324 nm (30,864 cm^{-1}), while additional absorptions at 564 and 644 nm corresponded to ^1A1g → ^1B1g and ^1A1g → ^1A2g transitions, consistent with a square-planar geometry [18], [19]. In the LZn spectrum, intense absorptions at 256 and 318 nm were characteristic of ligand-centered and charge transfer transitions typical for tetrahedral Zn(II) complexes [17], [19]. Full spectral data are summarized in Table 3.

3.4 Conductivity and Magnetic Properties

Molar conductivity values (43–65 $S \cdot cm^2 \cdot mol^{-1}$) indicated that all complexes, except LNi, behaved as 1:1 electrolytes in solution, while Ni(II) complex was non-electrolytic [20] (Table 4). Magnetic moment measurements (Table 5) supported the proposed geometries: octahedral for Co(II) and Cu(II) (μ_eff = 2.19 BM), square planar for Ni(II) (μ_eff = 0.7 BM), and tetrahedral for Zn(II). These results are consistent with the spectral analyses [21].

3.5 NMR Spectroscopy

The ^1H NMR spectrum of ligand L in DMSO-d_6 (Fig. 7) displayed a diagnostic oxime proton at 11.40 ppm (singlet), which disappeared upon D_2O exchange. Aromatic and NH_2 protons resonated between 6.49–6.30 ppm, with NH_2 also vanishing after D_2O addition. Methyl groups were observed at 1.96, 2.29, and 2.63 ppm. In the LZn complex, these resonances shifted significantly: the NH_2 protons appeared at 4.24 ppm, and the oxime proton at 11.39 ppm, confirming coordination. The aromatic signals were shifted downfield, and methyl resonances appeared at 1.97, 2.40, and 2.61 ppm (Table 6).

The ^13C NMR spectrum of L (Fig. 8) revealed signals for C=N–OH at 164.78 ppm and C=N– at 153.49 ppm, along with aromatic carbons between 137–166 ppm. Methyl carbons appeared at 23.12, 20.21, 19.58, and 9.81 ppm [22]-[25]. In LZn, the oxime carbon signal shifted downfield to 153.16 ppm, confirming metal coordination. Other carbons showed comparable but slightly shifted signals (Table 7) [28].

Table 3: Electronic spectral data of L1 and its metal complexes.

No	λ nm	λ cm^{-1}λ	ΣMax (dm^3 mo^{-1} cm^{-1})	Assignment	Suggested geometry
L^1	314	31847	1265	Ligand Field	-----
C_1	256 318 372	39063 31447 26882	1796 1500 95	Ligand Field Charge transfer Charge transfer	Tetrahedral
C_2	256 272 320 500	39063 36765 31250 20000	1441 1114 673 130	Ligand Field Ligand Field Charge transfer $^4T_{1(g)}F \rightarrow {}^4A_{2(g)}F$	Octahedral
C_3	258 364 430 662 930	38760 27473 23256 15106 10753	2829 604 244 273 137	Ligand Field Charge transfer Charge transfer $^2B_{2(g)} \rightarrow {}^2B_{1(g)}$ $^2B_{2(g)} \rightarrow {}^2A_{1(g)}$	Octahedral
C_4	324 564 644	30864 17731 15530	1604 250 164	Charge transfer $^1A_{1(g)} \rightarrow {}^1B_{1(g)}$ $^1A_{1(g)} \rightarrow {}^1A_{2(g)}$	Square planar

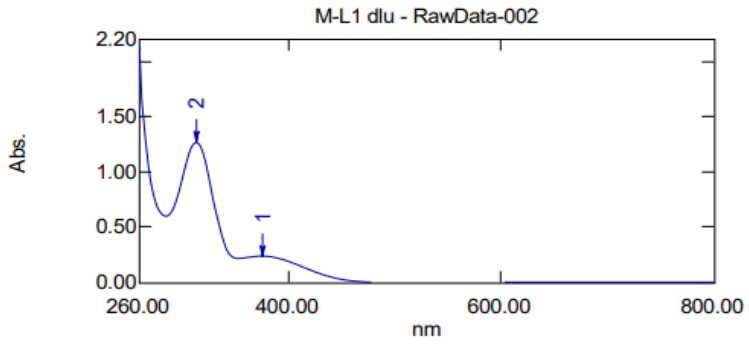


Figure 5: UV-VIS spectrum of the ligand.

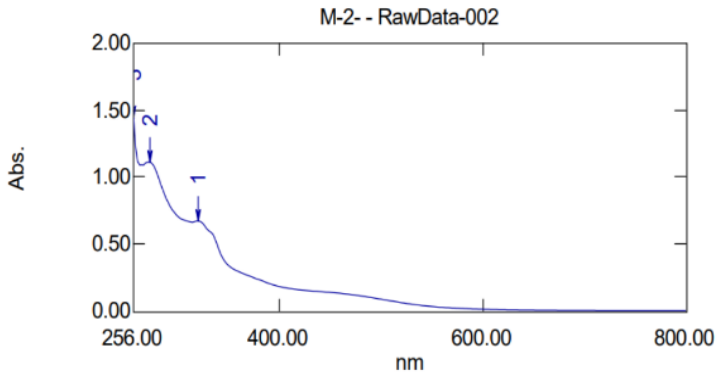


Figure 6: UV-VIS spectrum of the LCoCl (H2O)2]Cl complex.

Table 4: The molar conductance of the complexes.

COMP.	COMPLEX	Molar conductivity $S.cm^2.mole^{-1}$^
C_1	[LZnCl]Cl	43
C_2	[LCoCl $(H_2O)_2$]Cl	65
C_3	[LCuCl $(H_2O)_2$]Cl	136
C_4	$LNiCl_2$	43

Table 5: Magnetic Moment (μeff =B.M) of L1 complexes at 298K and suggested stereochemical structures.

Compound	μ_{eff}. B.M	Proposed Geometry
[LZnCl] Cl	diamagnetic	tetrahedral
[LCoCl $(H_2O)_2$] Cl	2.19	octahedral
[LCuCl $(H_2O)_2$] Cl	2.19	octahedral
$LNiCl_2$	0.7	Square planar

Table 6: 1HNMR data (δ, ppm) for the L1 ligand and L1Zn complex in DMSO-d6.

Funct. Group	δ(ppm) (Ligand)	δ(ppm) (C_1)
–C=N–OH	11.40 (s,1H)	11.39 (s, 1H)
C–H (ar)	(6.30-6.49) (d,2H)	(6.32-7.93) (d, 2H)
CH_3–C=N–OH	2.63 (s,3H)	2.61 (s, 3H)
CH_3–C=N–	1.97(s,3H).	2.40(s, 3H).
CH_3–C (ar)	2.29 (s,6H)	1.97 (d, 6H)
NH_2	4.24 (s,2H)	4.24 (s, 2H)

Table 7: ^{13}C nmr data for the ligand and L1Zn complex.

Funct. Group	δ(ppm) Ligand	δ(ppm) (C_1) Complex
–C_9=N–OH	163.92	152.28
C_7=N–	152.25	151.94
C_{13},C_5,C_4,C_{16},C_{15},C_{14}(ar)	(115.35-136)	(116.27-138.54)
C_{10}	21.88	21.90
C_1	18.97	18,99
C_2	18.34	17.88
C_8	8.56	8.59

Table 8: EI mass spectral data of L1 ligand.

Mass/charge m/z	Relative abundance
219	23
204	31
186	56
161	100
149	55
136	62
120	35
168	12
93	29
77.1	28
65	13
51	12

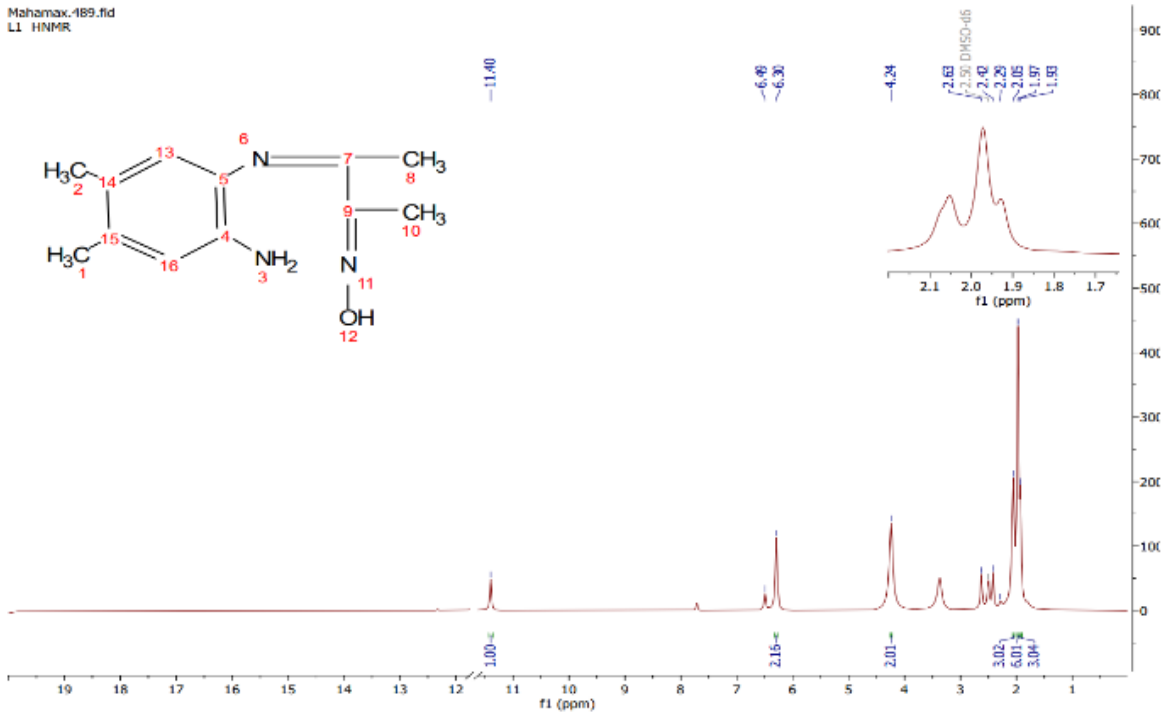


Figure 7: [1]Hnmr spectrum of the ligand.

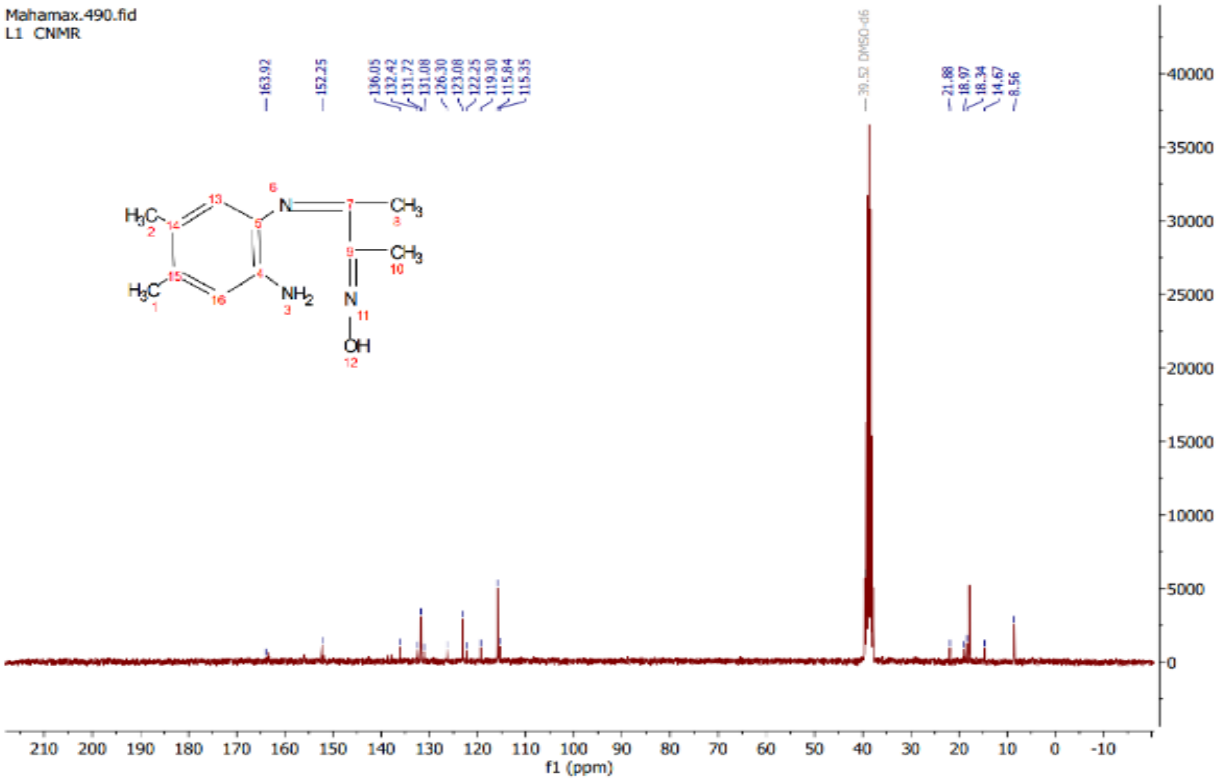


Figure 8: [13]Cnmr spectrum of the ligand.

Table 9: Biological activity and inhibition zone (mm) data for prepared ligand and their complexes.

Compound	Concentration, (µg/ml)	Staphylococcus Aureus, (G+)(mm)	Escherichia Coli, (G-)(mm)
L^1	100	25	20
	50	17	16
	25	R	R
C_1	100	18	17
	50	14	15
	25	R	R
C_2	100	34	30
	50	25	20
	25	19	15
	Levofloxacin Control	23	26
		R	R
C_3	100	21	18
	50	15	15
	25	13	14
C_4	100	R	17
	50	R	15
	25	R	14

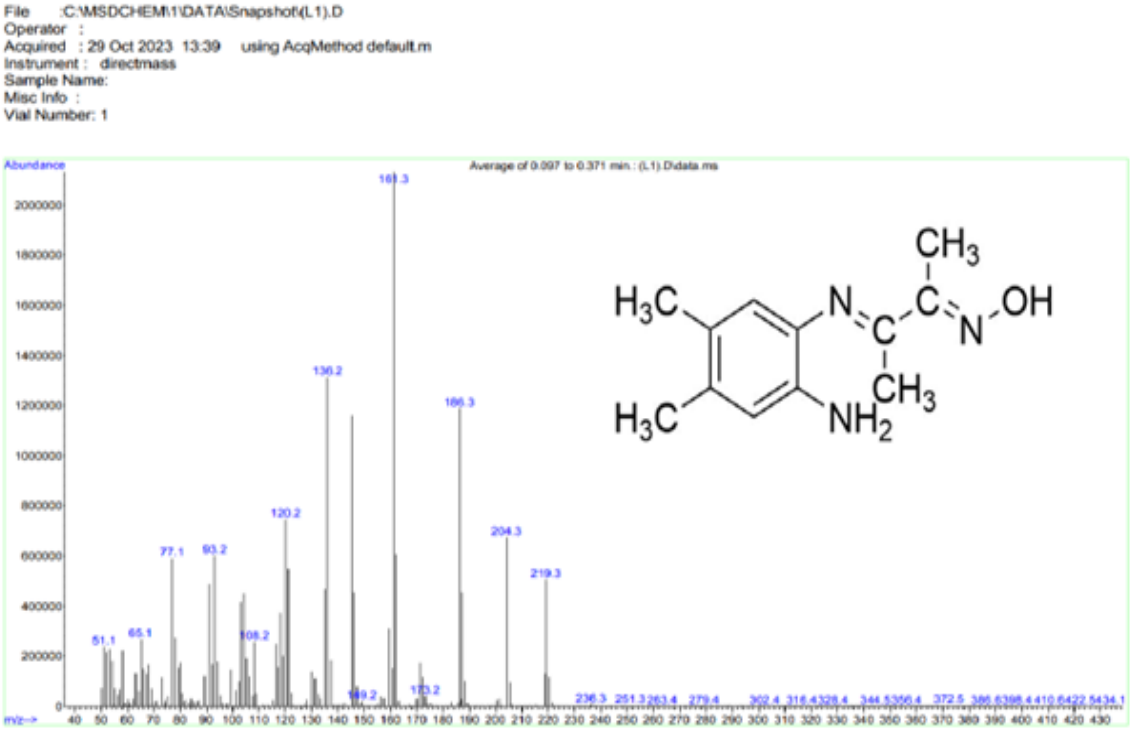


Figure 9: EI mass spectrum for the Ligand.

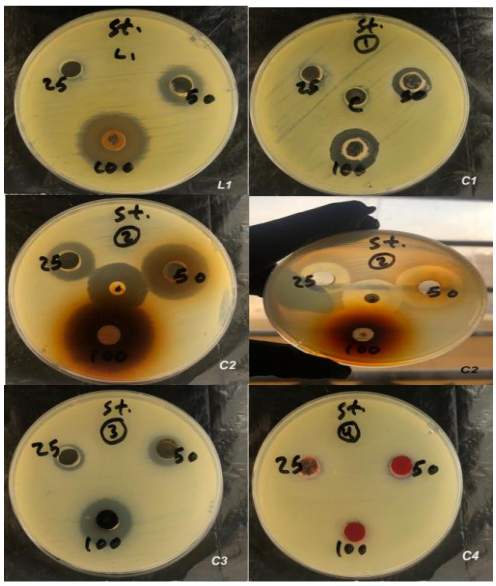


Figure 10. Evaluation of Antiviral Efficacy of the Ligand and Its Complexes [C1, C2, C3, C4] Against Staphylococcus aureus

3.6 Mass Spectrometry

The EI mass spectrum of the ligand displayed a molecular ion peak at m/z = 219 (M^+), matching the calculated molecular weight. Fragment ions are listed in Table 8 and illustrated in Figure 9. For LZn, the parent ion was observed at m/z = 355.57, again consistent with the expected formula.

3.7 Antibacterial Activity

The ligand and complexes were screened against Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative) at concentrations of 25, 50, and 100 µg/mL in DMSO. At the lowest concentration (25 µg/mL), LZn and the free ligand exhibited no inhibitory effect against S. aureus, while LCo and LCu showed measurable activity. Similarly, against E. coli, both L and LZn were inactive at 25 µg/mL, whereas all complexes except LNi were active at higher concentrations. Notably, the LNi complex showed no significant inhibition of S. aureus at any tested concentration (Table 9, Fig. 10) [29], [30].

4 CONCLUSIONS

Within the scope of this investigation, a novel Schiff base-oxime ligand was synthesized and characterized, in addition to its complexes with zinc (Zn^{2+}), cobalt (Co^{2+}), nickel (Ni^{2+}), and copper (Cu^{2+}), all of which were studied at reasonable relative ratios. Several methods, including magnetic susceptibility, molar conductivity, Fourier transform infrared spectroscopy, nuclear magnetic resonance (NMR) with ^{13}C and 1H, ultraviolet-visible spectroscopy, and mass spectroscopy, were utilized to investigate the complexes. Several of their structural characteristics were validated by the results that were obtained. The amine-oxime ligand and its metal complexes demonstrated activity that ranged from moderate to good against the two different species of bacteria that were utilized in this investigation. The geometric morphologies of the complexes were proposed, and the Zn^{2+} complex was found to be tetrahedral. On the other hand, the Co^{2+} and Cu^{2+} complexes were found to be octahedral, and the Ni^{2+} complex was found to be square planar. The article's principal objective is to catalyze ongoing study by providing readers with expansive insights into the astounding capabilities and multifarious complexity of the oxime moiety. This will be accomplished by providing readers with thorough information. Therefore, the continuous examination of these complexes may show to be a fruitful endeavour for future investigations. This prospect is supported by the fact that it is highly plausible.

ACKNOWLEDGMENTS

The authors thank the Department of Chemistry, College of Education for Pure Sciences/ University of Diyala, for providing chemicals and support.

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Synthesis and Structural Properties of Tungsten Disulfide as Transition Metal Dichalcogenides

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Keywords: Tungsten Disulfide, Sol-Gel, X-Ray Diffraction, FESEM and FTIR.

Abstract: This study involved the synthesis of tungsten disulfide nanoparticles (WS_2) by the sol-gel process. X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM), and Fourier transform infrared spectroscopy (FTIR) techniques were employed to analyze the results. XRD indicated that the nanoparticles have a hexagonal morphology, and the Scherrer equation was employed to ascertain the average crystallite size (D_{av}) of the synthesized nanoparticles, around 39 nm. FESEM images reveal that the crystalline structures exhibit heterogeneity in size and shape, with WS_2 nanosheets aggregating to create a substantial block characterized by varied interior thicknesses. The mean size distribution of WS_2 is approximately 39 nm. These findings align with the X-ray diffraction data. FTIR analysis of WS_2 nanoparticles showed that the (W-S) bond is represented by the bands at 572.71 cm^{-1} and 2924.15 $cm^{-1,}$ whereas the strong and weak absorption bands of the (S-S) bond are located at 494.4 cm^{-1} and within the range of 571.71-769.36 $cm^{-1.}$ The bands at 3740.65 cm^{-1} and 1625.21 cm^{-1} represent the (O-H) bond results.

1 INTRODUCTION

A group of thin film semiconductors known as two-dimensional transition metal dichalcogenides (TMDs) is defined by the general formula (MX_2), where M denotes transition metals (such as molybdenum, tungsten, vanadium, and zirconium) and X denotes an atom of chalcogen (such as sulfur, selenium, and tellurium). The compound consists of layers of chalcogen atoms sandwiched between the transition metal layer, as shown in Figure 1.

This family of materials has unique electronic, optical and catalytic properties, so it has a wide range of applications such as energy, environment and biomedicine [1]-[3]. Tungsten sulfide (WS_2) is a transition metal chalcogenide [4]. WS_2 occurs in the form of inorganic fullerene-like (IF-WS_2) structures and other two-dimensional structures, such as nanotubes and nanosheets [5]. These structures consist of two-dimensional molecular sheets held together by van der Waals forces. Figure 2 shows crystal structure of WS_2. Therefore, WS_2 nanoparticles have unique properties, such as lubricity, mechanical strength, and potential applications in tribology, electronics, energy storage, and other fields [6]. To prepare WS_2 nanoparticles, there are several techniques including (chemical vapor deposition, mechanical activation method, pulsed laser deposition (PLD), sol-gel method [7]-[8]. In this study, WS_2 nanoparticles were prepared by sol-reaction method, and the structural properties were studied using XRD and FESEM techniques.

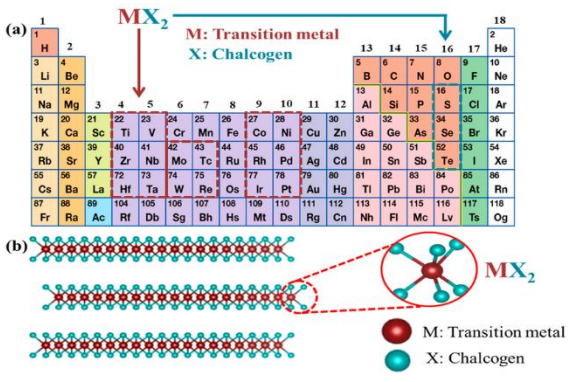


Figure 1: TMDs materials consist of 16 transition metals and three chalcogen atoms [9].

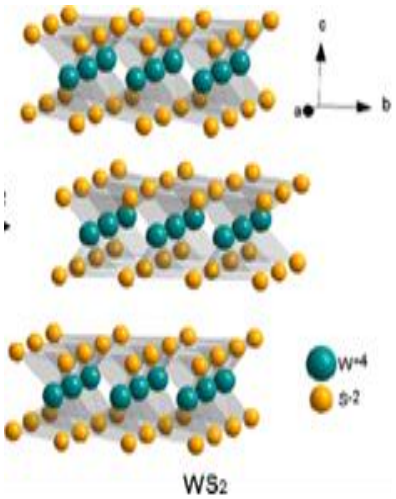


Figure 2: Crystal Structure of WS_2 [9].

2 EXPERIMENTAL DETAILS

WS_2 nanoparticles were prepared by sol-gel method by following the following steps:

1) A concentration of 0.1 M was used to dissolve sodium tungstate dehydrate, which has a molecular weight of 329.86 g/mol and a density of 2.23 g/cm^3. This substance was dissolved in deionized distilled water. There was also the utilization of thiourea (SC(NH2)2, and possessed a molecular weight of 12.76 g/mol, a purity of 99%, and a concentration of 0.1 M. There was also the addition of citric acid, which had a chemical formula of C6H8O7, a concentration of 0.5 M, and a molecular weight of 192.123 g/mol.
2) After the dissolving process was done, the acidity (pH) of the solution was found to be 1.4. It was neutralized by adding drops of ammonia solution (NH4OH) with a strength of 25%, the drops were added to the ready solution at regular times. The solution stayed on the magnetic mixer until it turned white and the pH level was equal to 7.
3) Once the solution had reached a neutral state, the temperature of the magnetic mixer was increased until the solution reached a temperature of 80 degrees Celsius. The temperature remained the same, and the solution was kept on the magnetic mixer for forty to fifty minutes. This allowed the liquid to gradually evaporate and transform into (Gel).
4) To eliminate any liquids or water molecules, the particles were annealed for two hours at 500°C in an electric furnace. Then, as illustrated in Figure 3, the particles were put into the furnace for two hours, and then for twenty-four hours until the temperature was close to room temperature, at which point (WS_2) particles were produced. Before sintering, wear (a) a solution; (b) a powder; and (c) powder nanoparticles (WS_2).

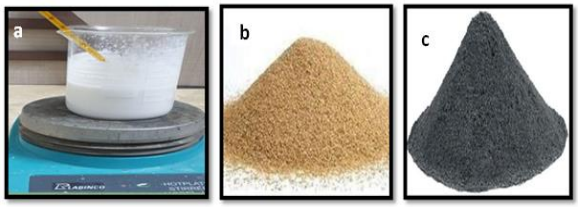


Figure 3: Steps for preparing WS_2 nanoparticles by sol-gel method wear a) show solution, b)as a powder before sintering, c) powder nano particles (WS_2) particles after sintering.

3 RESULTS AND DISCUSSIONS

3.1 Results of XRD

Figure 4 presents the XRD pattern of the synthesized WS_2 nanoparticles. The diffraction peaks 2Θ~14.33°, 28.98°, 32.52°, 33.51°, 39.56°, 44.14°, 49.82°, 58.38°, 60.40° and 76.01°, have appeared. Miller index-favored directions describe it. (002), (004), (100), (101), (103), (006), (105), (110), (112), (116), and (118), respectively. These results were found to be largely consistent with the International Data Card (ICDD card no. (00-008-0237) belonging to (WS_2) [10]. In addition, the results indicated the hexagonal structure of the nanoparticles. The large number of peaks indicates that the prepared grains are polycrystalline [11]. The distance between interplanar spacing (d_{hkl}) with the same Miller's coefficients ($_{hkl}$) was found using Brack's law and the relation (1) [8]. "d_{hkl}" was found to be close to and match the number on the WS_2 standard card (ICDD card no. 00-008-0237) [12]:

$$n\lambda = d_{hkl} \sin \Theta_B. \quad (1)$$

Where, d_{hkl} : The distance between interplanar spacing, Θ_B: Brack angle, λ: wavelength, n: order of reflection.

For the produced WS_2 nanoparticles, the crystal lattice constants (ao = bo, and co) were computed using the hexagonal structure relation (2) [13]. Table 1 displays these values.

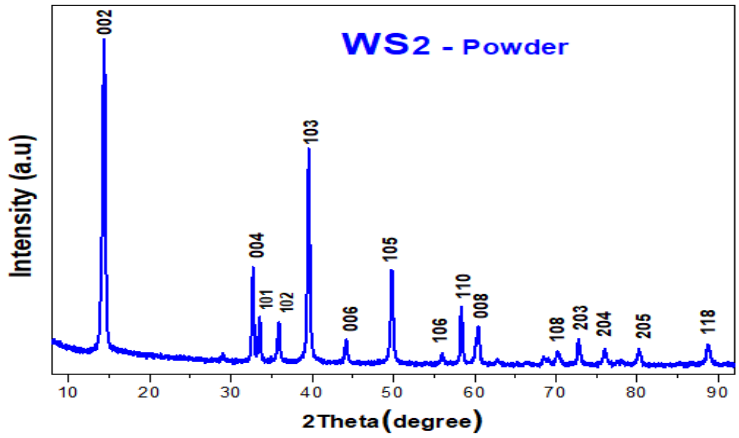


Figure 4: XRD of WS_2 nanoparticles.

$$\frac{1}{d^2{}_h} = \frac{4}{3}\left(\frac{h^2+hk+k^2}{a_o^2}\right) + \frac{l^2}{c_o^2}. \qquad (2)$$

The Miller coefficients *h*, *k*, and *l* were used to calculate the lattice constants ao and co, which were obtained from the planes (002) and (103), respectively. The lattice constant values were found to be consistent with those in the WS_2 standard card (ICDD No. 08-0237). These data are presented in Table 1. The Scherrer method was employed to determine the average crystallite size (D_{av}) of the nanoparticles that were prepared, as outlined in (3) [14]-[16].

$$D_{av} = \kappa\lambda / (\beta \cos \theta_B). \qquad (3)$$

The symbol β represents the full width at half maximum (FWHM), whereas θ_B represents the Bragg's diffraction angle. The symbol κ represents the shape factor, and λ represents the wavelength of the X-ray that is diffractioning the sample. As can be seen in Table 1, the crystallite size of the nanoparticles that were formed was determined to be about 22 nanometers. The dislocation density (δ) is a measurement that is used to determine the amount of defects present in the crystal. The lowest dislocation density value that was discovered in this work provided evidence that the sol-gel method was successful in producing WS_2 nanoparticles that had demonstrated good crystallization. Formula 4 was utilized in order to calculate the dislocation density [17], which may be found in Table 1.

$$\delta = 1 / D^2_{av} \qquad (4)$$

A definition of specific surface area (SSA) is the area represented in mass units (m^2/g). It ascertains material quality and constitutes a significant attribute of nanoparticles. It also provides information about superficial encounters [17]. Table 1 presents the SSA values obtained by 5 [17]:

$$SSA = S_V / \rho . \qquad (5)$$

To find the surface density (SV), we need to take the number of K_{SV} (which is 6 for a sphere) and D_{av}. The material density for WS_2 nanoparticles is ρ, which is 7.5 g/cm^3, i.e., SV = . Rearranging (5) yields the following (6):

$$SSA = 6 \times 10^3 / (D_{av}\, \rho) \qquad (6)$$

3.2 Analysis of FESEM

The WS_2 nanoparticles synthesized with sol-gel were analyzed using FESEM. The crystals in Figure 5a and 5b are of different sizes and shapes, and the WS_2 nano sheets can be stacked to make a big block with different thicknesses inside. The findings here are in agreement with those of studies [18]-[20]. The size range of WS_2 is shown in Figure 5b. It is about 42 nm on average.

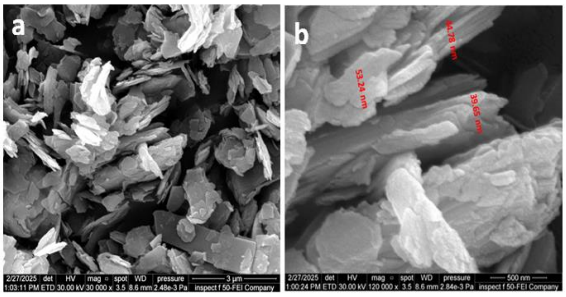


Figure 5: FESEM image of WS_2 nanoparticles: a and b.

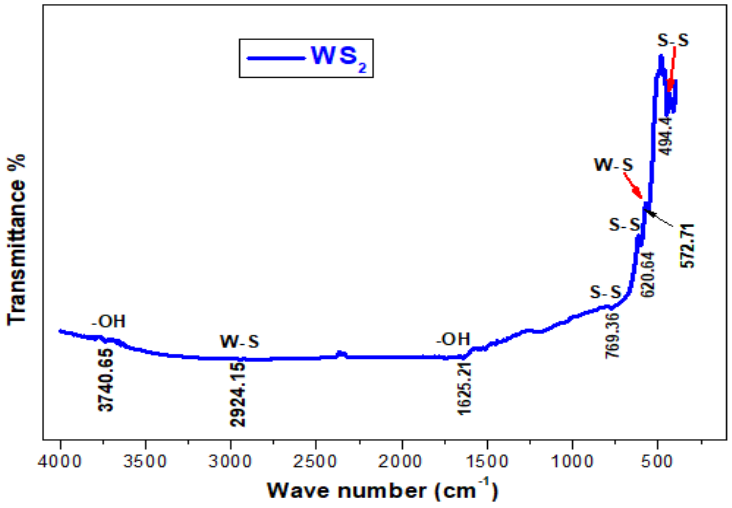


Figure 6: FTIR measurements for WS2 nanoparticles.

Table 1: Hexagonal structure relation.

θ°	d-spacing (Å)	FWHM (rad)	average crystallite size (D_{av}) (nm)	Micro Strain $*10^{-3}$	Dislocation density (δ) nm^{-2}	SSA ($m^2.g^{-1}$)
14.33	6.1666	0.3464	23	0.45775	0.00189	35
28.98	3.0808	0.5196	16	0.37711	0.00390	50
32.52	3.0792	0.3464	24	0.41297	0.00174	33
33.51	2.6735	0.3464	24	0.41297	0.00174	33
39.56	2.2779	0.5196	16	0.28549	0.00390	50
44.17	2.0504	0.3464	25	0.40511	0.00160	32
49.82	1.8303	0.3464	25	0.33445	0.00160	32
58.38	1.5806	0.5196	18	0.181	0.003086	45
60.40	1.5324	0.5196	18	0.44459	0.003086	15
76.01	1.2519	0.3464	29	0.23658	0.001189	28

3.3 FTIR Measurements

Fourier transform infrared (FTIR) measurements were performed for WS_2 nanoparticles prepared by the sol-gel method in the range of 400-4000 cm^{-1}. The transmittance spectrum was measured as a function of wavenumber. The transmittance spectrum was also measured as a function of wavenumber. Figure 6 shows relatively strong absorption bands at wavelengths of 494.4, 572.71, 620.64, 769.36, 1625.21, 2925.15, and 3740.65 cm^{-1}. These results are consistent with studies [21]-[25] which showed that the strong and weak absorption bands of the (S-S) bond are located at 494.4 cm^{-1} as well as between 571.71-769.36 cm^{-1}, While the bands located at 572.71 and 2924.15 cm^{-1} are due to the (W-S) bond. While the bands located at 1625.21 and 3740.65 cm^{-1} are due to the (O-H) bond.

4 CONCLUSIONS

The sol-gel method was used to make transition metal dichalcogenides (WS2) nanoparticles by mixing sodium tungstate dehydrate [Na2WO4.2H2O] with thiourea (SC(NH2)2). The XRD study gave us nanoparticles with an average particle size of (39 nm). These results were the same as the particle size we got from the FESEM images, which was about (42 nm).

These findings align with the X-ray diffraction data. FTIR analysis of WS2 nanoparticles showed that the (W-S) bond is represented by the bands at 572.71 cm^{-1} and 2924.15 cm-1, whereas the strong and weak absorption bands of the (S-S) bond are located at 494.4 cm-1 and within the range of 571.71-769.36 cm^{-1}. The bands at 3740.65 cm^{-1} and 1625.21 cm^{-1} represent the (O-H) bond results.

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Preparation and Characterization of Corn Oil Alkyd Resin Polymer under the Influence of a Catalyst and Study of its Polymerization Kinetics

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Keywords: Alkyd Resins, Corn Oil Polymerization, Effect of Catalyst Polymerization, FTIR, Activation Energy (Ea).

Abstract: This work involved the preparation of the polymer (alkyd resin) in two steps: the first was the preparation of monoglyceride (MG) by glycerolysis method through the reaction of glycerin and corn oil using (Pb_3O_4) as a catalyst at temperature of 150 ℃. The second, was by condensation polymerization of the alkyd resin by polymerizing the MG prepared from the first step with a dicarboxylic acid (succinic acid (SA) in this case) in the presence of a catalyst ($CaCO_3$) and over a range of temperatures. ˚C (210, 200, 190, 180, 170). The prepared polymer was studied and characterized using Infrared spectroscopy. Adhesiveness tests of the prepared polymers showed their solubility in water and other organic solvents, and that upon evaporation, their adhesion properties were restored. This makes these polymers potentially usable in various industries, such as the dye and paint industries. Using water as a solvent is economically superior to other organic solvents. The kinetics of polymerization reactions were studied by tracking the decrease in the acid value (AV) and the amount of water removed from the reaction medium. The energy of activation (Ea) and the parameter frequency coefficient (A) had also calculated by Arrhenius equation. The energy of activation depends on the decrease in AV found to be (Ea= -9.0456 kJ) and that depends on the amount of water removed is -8.1992 kJ . The thermodynamic functions ΔH, ΔS, and ΔG were also calculated.

1 INTRODUCTION

Alkyd resins are defined as modified polyester with adding fatty acids. They were manufactured by using polyols, like glycerol, dicarboxylic acid or carboxylic acid anhydride [1]-[2], such as phthalic acid anhydride. They can also be defined as a type of cross-linked polyester and thermosetting plastics. 90% of them are used in liquid form for coating dishes. They are characterized by their hardness and dimensional stability even at high temperatures. They are easy to color, and are characterized by transparency, adhesive strength, and heat resistance. They are also characterized by their inability to be affected by strong acids and bases, and their low resistance to moisture. They are used as pigments in paints and the printing ink industry [3]. Alkyd resins are considered important materials for coating resins. Traditionally binders are used to bind surface coatings today, alkyd resins are used as coating compounds because of their unique properties such as hardness, durability, and abrasion resistance [4]-[5]. They can also be defined as polycondensation products between polycarboxylic acids and polyhydroxyl alcohols modified with fatty acids [6]-[7]. Alkyd resins are one of the most indispensable raw materials in the coatings and decorative paint industries. Their widespread use is due to their inexpensive raw materials and ease of manufacture. Alkyd resins are not only used in decorative paints, but are also used in air-curing paints, machine tool coatings, inks, matte and semi-gloss varnishes for wooden furniture, and materials designed to protect surfaces from environmental influences [8]. The presence of oil and glycerin as components of alkyd resins gives them the quality of being environmentally friendly compared to petroleum polymers made traditionally that pollute the environmental leading to high degradation. They received attention due to their importance as inherent non-toxic nature and biodegradability [9]. Usually, oils or fatty acids used in the manufacture of alkyd

resins are derived from natural sources such as flax, sunflower, coconut, bitter almond, soybean, corn, palm, cotton and other natural oils that have long chains for the manufacture of polymeric resins such as alkyds depending on the nature of the unsaturated fatty acids that present. The fatty acids included in the composition of alkyds give them flexibility, which makes them used in the manufacture of flexible coatings [10].

2 EXPERIMENTAL PART

During the synthesis and characterization of corn oil-based polymers, the following equipment was used to control reaction parameters, measure properties, and ensure accuracy:

A) Equipment used.
1) FTIR spectrometer.
2) Magnetic stirrer bath heater.
3) Electronic thermometer to control the polymerization temperature.
4) Sensitive balance for sample weight.
5) Ostwald capillary viscometer.

B) Materials used.
1) Corn oil.
2) Succinic acid (SA) from CDH.
3) Red lead oxide (Pb3O4) from CDH.
4) Calcium carbonate (CaCO3) from CDH.
5) Methanol (BDH).
6) Sodium hydroxide (NaOH) from Fluka.
7) Nitrogen gas (N2) from Iraq.

3 METHOD

The synthesis of corn oil-based polymers was carried out in two sequential steps.

Step 1. Glycerolysis reaction: In the following step, 66 ml of glycerol is added, along with (33 ml) of commercial corn oil and the catalyst of 0.3 g of red lead oxide (Pb_3O_4). These are placed in a 250 mL glass beaker and stirred at 150 °C for 5 hours, according to Figures 1 and 2, a separating funnel were used to separate the products that left over night to get a clear separated two layers of monoglycerides (the lower part of the funnel contents) and the upper part, which IR tested reveling unreacted materials. The lower part, which represents the monomer formed, then be taken for the polymerization reaction [11], and its IR spectra shown in Figures 3-6, represents corn oil, glycerin, the monomer monoglysride (MG) and the prepared polymer.

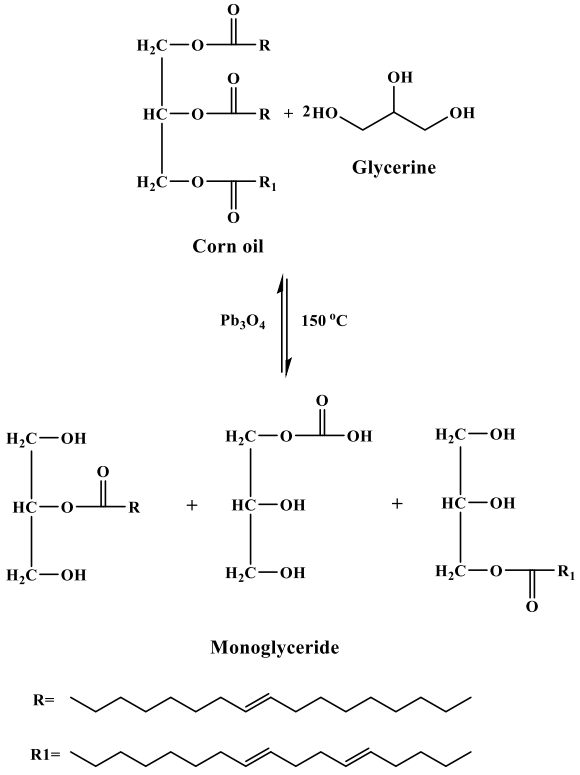


Figure 1: Stepwise glycerolysis reaction.

Step 2. Polymerization: in this step, the separated monoglyceride (monomer) from the separating funnel reacts. Then a 20 g of succinic acid (SA) as a monomer added to 50 ml of monoglysride as a seconed monomer (MG) and 0.3 g of the catalyst (red lead oxide (Pb3O4). These are placed in a three-hole glass flask, at different temperatures (170, 180, 190, 200, 210 °C), with a 10 °C interval for each experiment. Continuous stirring is carried out using a control unit (electronic thermometer) connected to the heater. High-temperature-resistant glass wool is used to maintain the reaction temperature. The polymerization process is carried out in the absence of oxygen in the presence of nitrogen gas (N_2) (an inert atmosphere). The benefit of an inert atmosphere is to prevent the reaction mixture from oxidation and to prevent the burning and blackening of the formed polymer [12] as shown in the following reaction Figure 2.

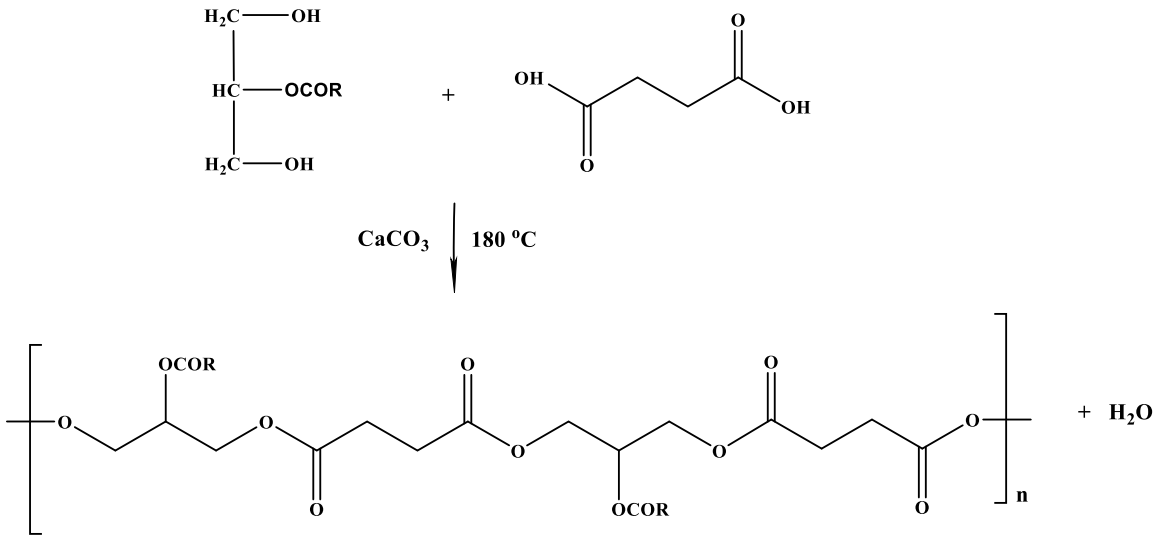


Figure 2: Stepwise glycerolysis reaction.

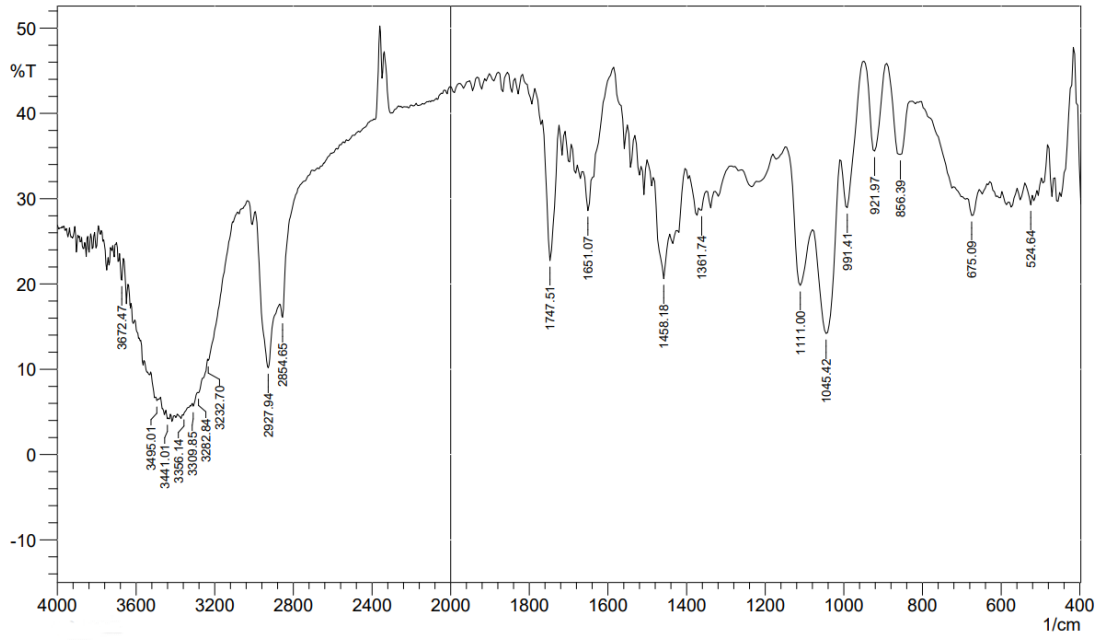


Figure 3: IR spectrum of the corn oil.

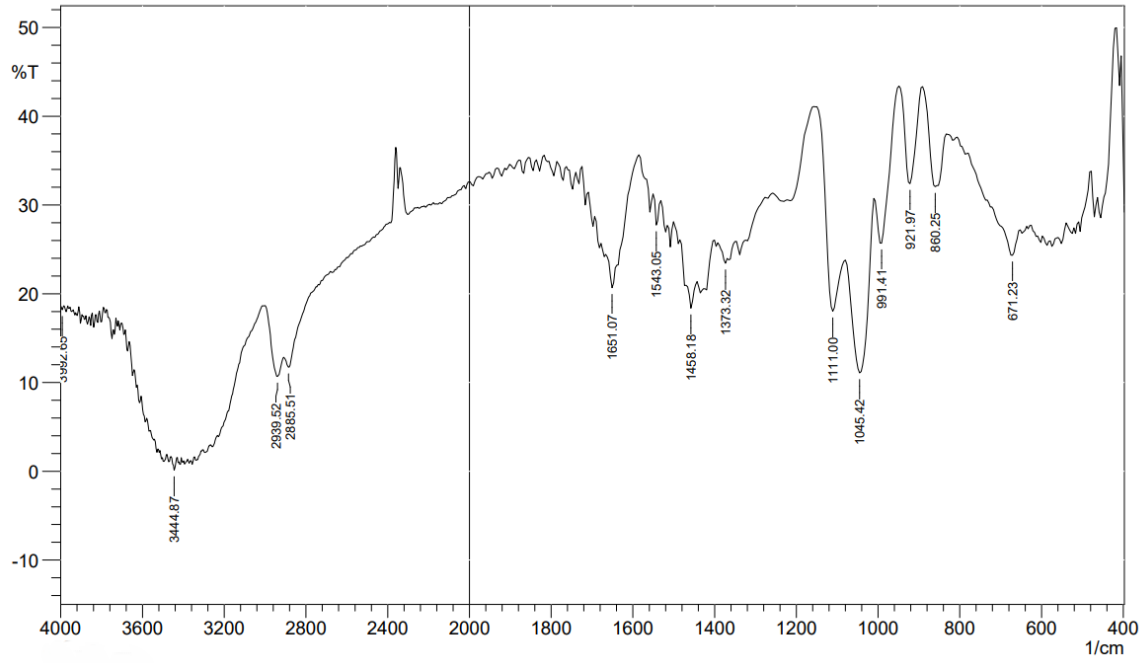


Figure 4: IR spectrum of Glycerin.

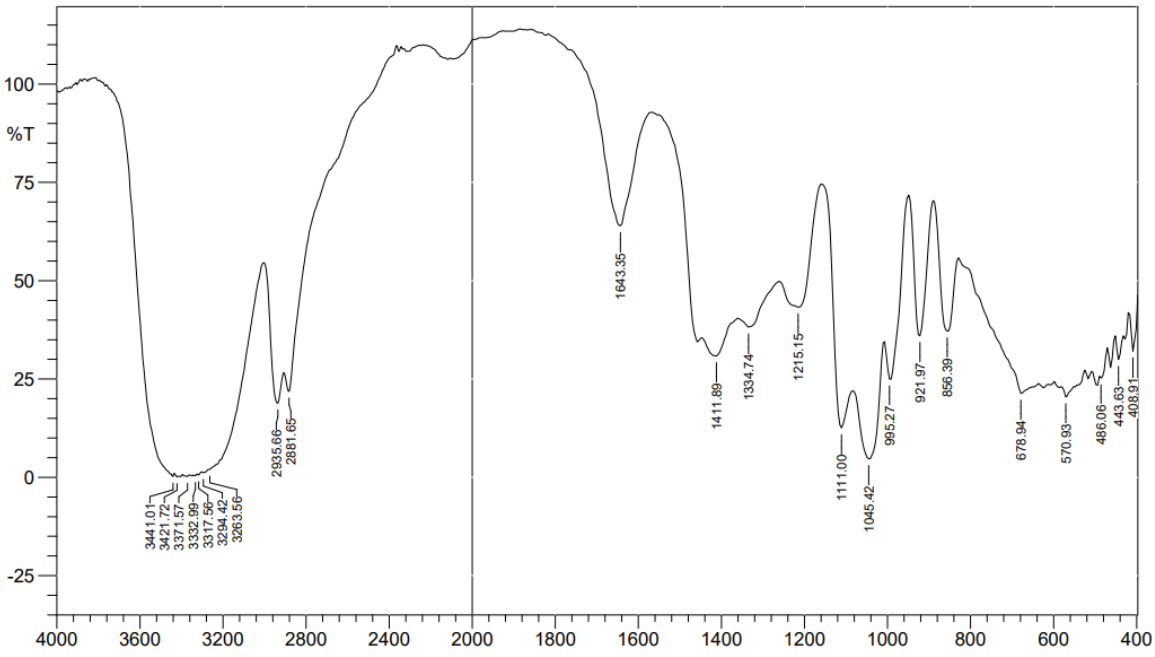


Figure 5: IR spectrum of monoglyciride (MG).

4 RESULTS AND DISCUSSION

4.1 Polymer Identification

FTIR Analysis: The prepared polymer was characterized using FTIR techniques, where absorption bands at 3421 cm^{-1} were related to vibrational stretching frequency of the (O-H) group bond, two stretching bands for asymmetric stretch at 2939 cm^{-1} and symmetric stretch at 2885 cm^{-1} were related to the normal aliphatic bond as (C-H). The band at the frequency 1662 cm^{-1} which related to the vibrational stretching of (C=O) bond, and two absorption bands at frequencies (1041 cm^{-1}) and (1238 cm^{-1}) were related to the (C-O) bond [13] As shown in Figure 6.

4.2 Reaction Kinetics by Tracking the Decrease in the Acid Value of the Polymer

The reaction was conducted at different temperatures and with an interval of 20 minutes between readings of the prepared polymer. The acid value decrease was determined by the (1):

$$Acid\ value = \frac{V_{NaOH} \times N_{NaOH} \times 40}{Weight\ of\ Polymer}. \quad (1)$$

Where: ((Acid value represents the acid value, ((V represents the volume of sodium hydroxide coming down from the burette, and N)) represents the concentration of sodium hydroxide (0.1M), (40) represents the molecular weight of (NaOH), and (wp) represents (1g) of the weight of the polymer drawn during the polymerization process. The mobility of the polymer was calculated by decreasing the acid concentration (SA) in the presence of the catalyst ($CaCO_3$) [14]-[15]. As shown in the following Figure 7.

4.3 Reaction Kinetics by Tracking the Amount of Water Removed from the Polymer

The reaction was conducted at different temperatures, with a 20-minute interval between readings, and using the catalyst (CaCO3), as shown in Figure 8.

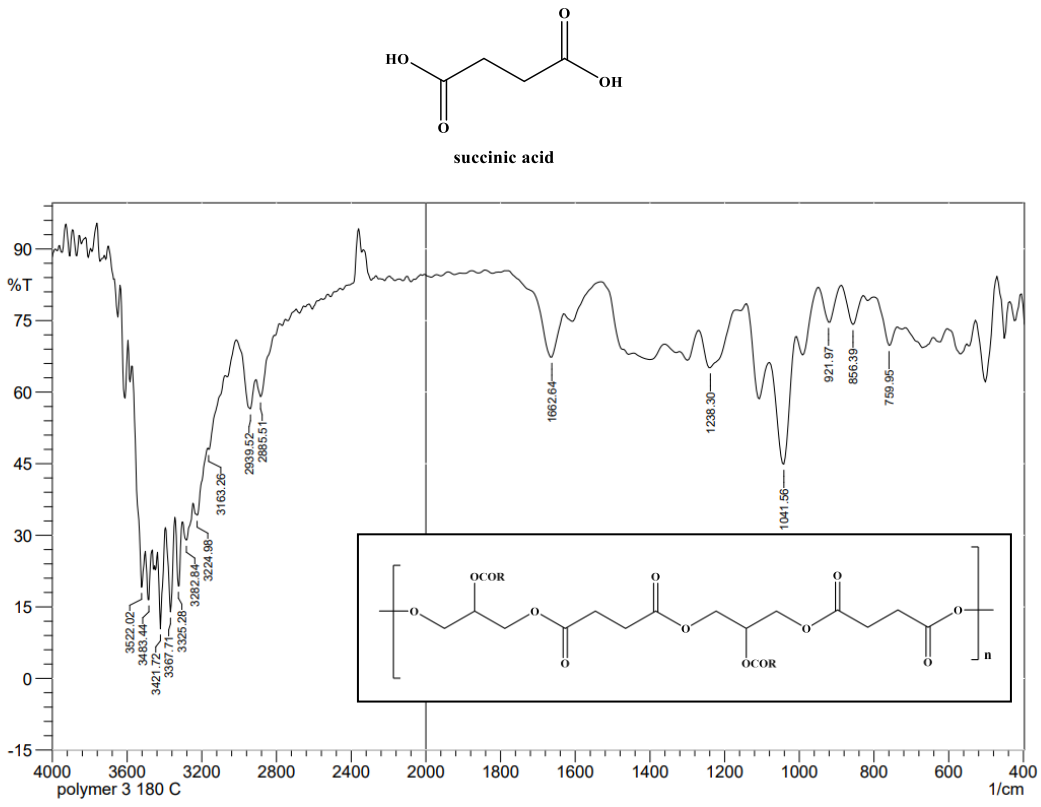


Figure 6: IR spectrum of the polymer prepared from the reaction of (SA) and (MG) in the presence of the catalyst (CaCO3).

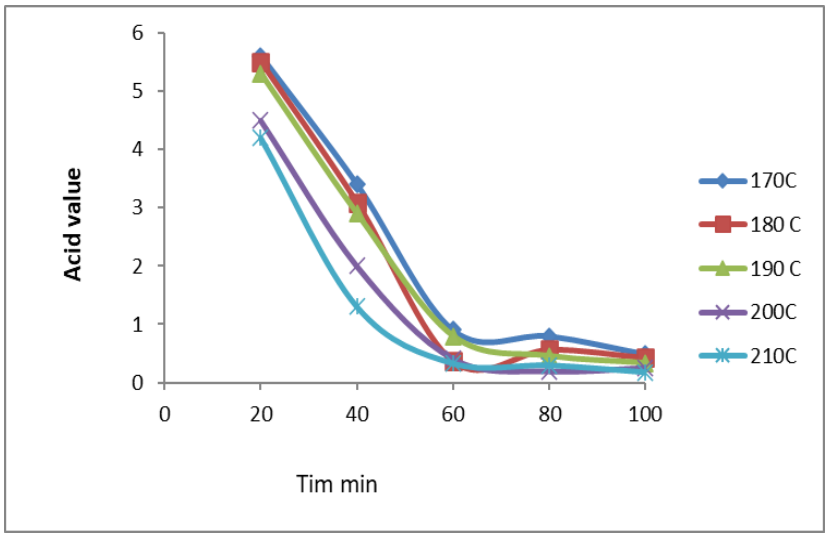


Figure 7: Reaction rate of polymerization at temperatures (200, 180, 170, 190, 210°C) in the presence of (CaCO3).

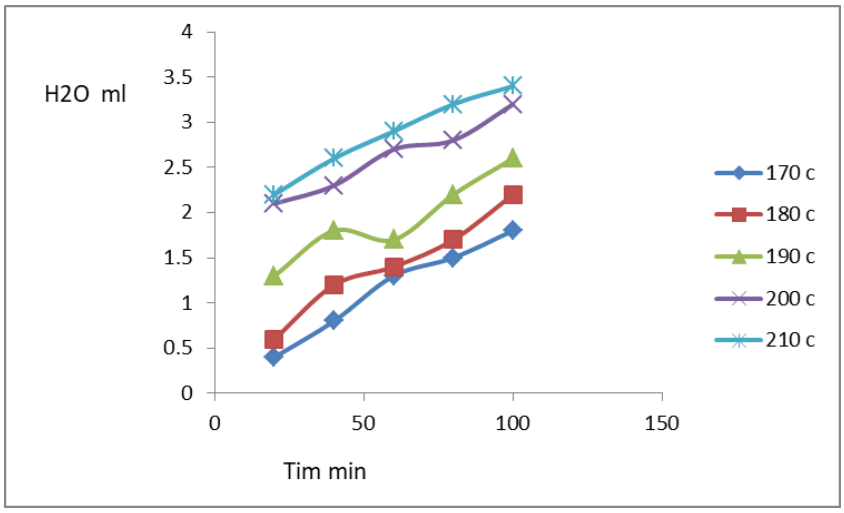


Figure 8: The rate of polymer preparation from the amount of eliminated water versus time at temperatures of 170, 180, 190, 200, 210°C in the presence of (CaCO3).

4.4 Calculating Activation Energy

The results were processed using the following Arrhenius (2):

$$k = Ae^{-Ea/RT}. \quad (2)$$

Taking the natural logarithm (ln) of both sides of the (3), it becomes:

$$lnk = lnA - \frac{E_a}{RT} \quad (3)$$

The activation energy was calculated through the curves represented in Figures 9 and 10, where (k) represents the reaction rate and was obtained by tracking the decrease in acid concentration with time as well as by tracking the amount of water removed with time. Ea represents the activation energy (E_A) in (kJ) units and a is a factor of frequency in s^{-1} units and T represents the absolute temperature in units of Kelvin (K). It was calculated from the following relationship.

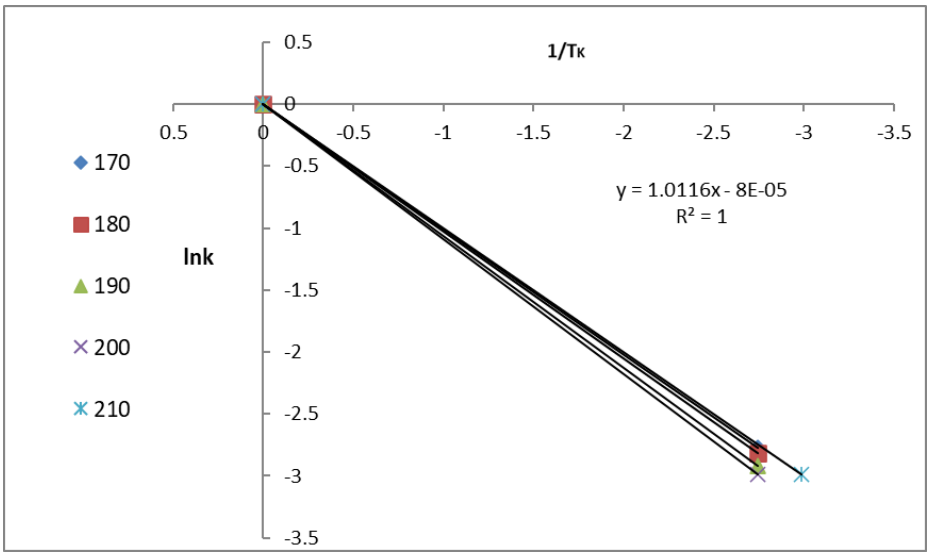


Figure 9: Graphical representation of 1/T(K) vis lnk of the polymerization reaction based on decrease of acid value in the presence of (CaCO3) as a catalyst.

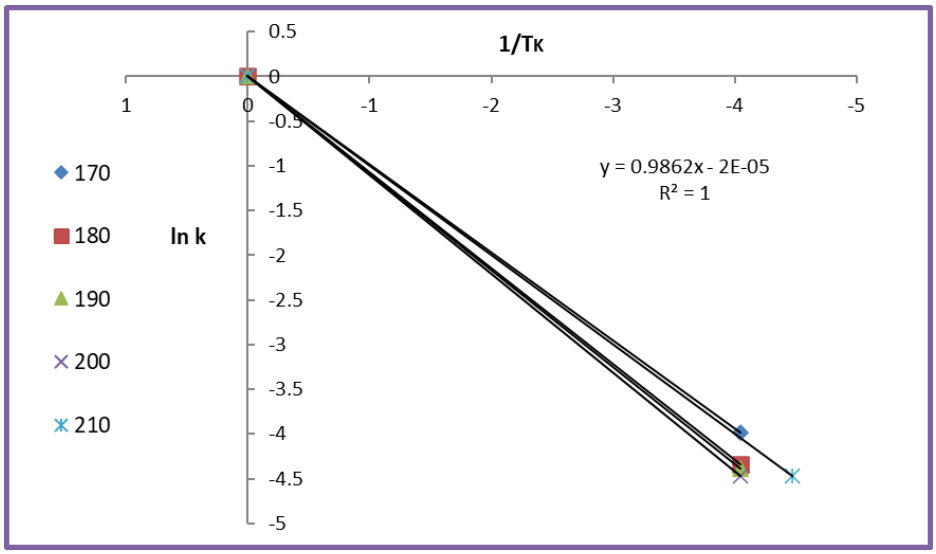


Figure 10: The graphic representation of 1/T vis lnk for the polymerization reaction based on the quantity of water elimination in the presence of CaCO3.

Table 1: The functions (ΔH, ΔS, ΔG) of the polymer in the presence of (CaCO3) as a catalyst.

T ˚C	TK	ΔH kJ.mol^{-1}	ΔG kJ.mol^{-1}	ΔS kJ.mol^{-1}. K^{-1}
170	443	-0.009	10.110	-0.00000332
180	453		10.458	
190	463		10.828	
200	473		11.463	
210	483		11.994	

4.5 Calculating the Thermodynamic Functions (ΔH°, ΔS°, ΔG°)

The thermodynamic functions of the first polymer P1 were calculated in the presence of the catalyst (CaCO3) and based on the decrease in acid value and using the equation of Van't-Hoff for the graph from the calculation of the Arrhenius equation for the Figures 9 and 10. The value of (ΔH) is calculated from graph slope and the value of the entropy change (ΔS) is calculated from graph intercept when applying the Van't-Hoff. As shown in Figure 9.

$$\frac{\Delta S^\circ}{R} + lnk = \frac{-\Delta H^\circ}{TR}. \quad (4)$$

$$Slope = \frac{-\Delta H^\circ}{R}. \quad (5)$$

$$Intercept = \frac{\Delta S^\circ}{R}. \quad (6)$$

Where R is the constant of universal gas (8.314 J/mol.K), T is the absolute temperature in Kelvin (K), and k is the rate constant of reaction. The free energy of the reaction (ΔG) was calculated from the following (7):

$$\Delta G^\circ = -RTlnk. \quad (7)$$

As shown in the following Table 1.

5 CONCLUSIONS

The study demonstrated that temperature has a significant impact on the reaction rate during polymer preparation, underscoring the importance of thermal control in polymerization processes. Water-soluble polymers can be obtained, which has positive economic and environmental benefits by reducing reliance on harmful organic solvents. The results showed that catalysts (Pb3O4, CaCO3) have an effective impact on accelerating polymerization reactions, making them important in improving process efficiency.

The decrease in acid value and the amount of water removed from the polymerization process can be relied upon as important indicators for studying polymer kinetics. It was demonstrated that the choice of monomer used in the reaction has a significant impact on the physical and application properties of the resulting polymer, necessitating careful monomer selection to achieve the desired performance in targeted applications.

The thermodynamic functions (ΔH, ΔG, ΔS) of the polymer can be calculated in the presence of catalysts (CaCO3) by tracking the decrease in the acidity value.

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SECTION 5

APPLIED BIOLOGY AND BIOTECHNOLOGY INNOVATIONS

Proceedings of the 13th International Conference on Applied Innovations in IT (ICAIIT)

JUNE 2025

Study of the Relationship between Heme Oxygenase-1, Interleukins (6, 12) and Coenzyme Q10 in Iraqi Pediatric Patients with Chronic Myeloid Leukemia

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Keywords: HO-1, Chronic Myeloid Leukaemia (CML), Interleukin (6, 12), Coenzyme Q10.

Abstract: A global disorder impacted by both genetic and environmental factors, chronic myeloid leukaemia (CML) is associated with increasing mortality and morbidity rates and has social and economic consequences. This particular kind of cancer affects the bone marrow, a spongy substance found in the center of most bones that produces the majority of the body's blood cells, Mossler was the first to describe the bone marrow biopsy technique for the diagnosis of leukaemia. Wilhelm Epstein coined the word "acute leukaemia" to distinguish the rapidly growing and fatal leukaemia from the more indolent chronic leukaemia, myeloid cells, the malignant cell in acute myeloid leukaemia, were identified by Otto Naegeli, who divided leukaemia into myeloid leukaemia and lymphoid leukaemia. The present study was conducted to determine the level of heme oxygenase-1 enzyme and to study and estimate the level of some biochemical parameters such as: interleukins (interleukin-6, interleukin-12) as well as the enzyme coenzyme COQ10 in the serum of patients with chronic myelogenous leukaemia in paediatric patients. The study included (60) patients who visited the Central Children's Hospital in Baghdad Governorate and who were previously diagnosed with chronic myelogenous leukaemia through a complete blood count and bone marrow examination in addition to other tests used to diagnose patients with leukaemia, and (30) apparently healthy people of both sexes in the same age group and considered them a control group. The results showed no significant increase ($P<0.05$) in the levels of heme oxygenase-1, interleukin-12 and coenzyme Q10 when compared to the control group, but there was a significant difference at the probability level ($P<0.05$) in interleukin-6 when compared to the control group in patients with chronic myeloid leukaemia in children.

1 INTRODUCTION

Cancer is a disease in which a group of cells lose the laws of normal cell division and develop uncontrollably, so that cancer cells interact with signals that activate the normal cell cycle, leading to abnormal cell growth and promoting altered cells. [1]. Many diseases are characterized by unchecked or uncontrolled cell growth; the terms "cancer" and "tumors" are often used interchangeably. Cancer is one of the leading causes of death and disability worldwide [2]. Multifactorial cancer is characterized by a number of genetic alterations influenced by both the body and society, Typical hallmarks of this type of cancer include cell death resistance, unregulated gene expression, unchecked replication, and metastasis (spread to other tissues) [3], [4].

The bulk of the body's blood cells are transported by the sponge-like bone marrow, which is located in the middle of most bones and is affected by leukaemia, a kind of cancer. Bone marrow blood-forming stem cells, often called immature blood cells, are the first cells that undergo mutations to become leukaemia-type cells [5]. Abnormal cells in the bone marrow, regardless of the type of leukaemia, prevent the formation of other cells, leading to a variety of negative consequences [6]. Severe anaemia occurs as a result of decreased red blood cell production, and the risk of infection increases when the neutrophil count in the blood is low. As the incidence of thrombocytopenia increases, clotting factors decrease, and the risk of bleeding (low platelet count) increases. Cancer cells infiltrating various organs can lead to enlargement and fibrosis of the liver, spleen, and lymph nodes. When cancer cells invade the brain, they can lead to meningitis, ventricular dilatation, increased

intracranial pressure, nausea, vomiting, papilledema, neck stiffness, loss of appetite, hypoglycaemia, general weakness, muscle atrophy, and sometimes death if the patient does not receive appropriate treatment. All of these are symptoms of hypermetabolic disease, which deprives cells of nutrients [6].

2 TYPES OF LEUKEMIA

The kind of injured stem cells determines whether the leukemia is lymphocytic or myeloid. When people refer to blast leukemia or stem cell leukemia, they usually indicate a lymphocytic or myeloid sickness. Leukemia's go by a variety of names depending on the specific kind of cells that are affected, which may be perplexing for patients,

There are four main types of leukemia, and they differ in their treatment methods and response to treatment [6], [7].

1) Acute Lymphoblastic Leukemia (ALL) is a malignancy that impacts lymphocytes, which are progenitor cells. It may occur in locations other than the bone marrow, such as peripheral blood. Research suggests that an aberrant immune response to infections is believed to originate with cellular genes. Lymphocytic leukemia, a kind of malignancy, primarily targets cells that differentiate into B and T lymphocytes [8];
2) Acute myeloid leukemia (AML) has an irregular proliferation of myeloid cells. The number of divisions is more than 20% to diagnose chronic myeloid leukemia in the bone marrow. It is also known as the cancer that affects the stem cells in the bone marrow that will later become granulocytes, erythrocytes and megakaryocytic blood cells [9];
3) Chronic myeloid leukemia (CML): the malignant genetic condition of blood-forming stem cells known as chronic myeloid leukemia leads to an increase in myeloid cells, red blood cells, and platelets in the peripheral blood. The cancerous cells are the myeloid cells in the blood, which include all types of cells found in the blood except for lymphocytes [10];
4) Chronic lymphoblastic leukemia (CLL): there is a wide range of symptoms associated with CLL, a kind of leukemia that mostly affects the elderly. The cells affected by cancer are B and T lymphocytes in the blood. Specific genetic changes that impede clonal programmed cell death lead to the transmission of leukemia [11].

3 BIOCHEMICAL VARIABLES

3.1 Heme Oxygenase-1 Enzyme (HO-1)

Heme oxygenase-1 is a stress protein, metabolic enzyme, host immune stress inducer and anti-inflammatory. HO activity is represented by two major isoforms, HO-1, HO-2), HO-3.

Encoded by two distinct genes (HMOX1 and HMOX2) respectively, heme oxygenase-1 is a microsomal enzyme with a key antioxidant and anti-inflammatory role in heme degradation.

By lowering biliverdin and carbon monoxide generation, biliverdin reductase also lowers bilirubin and free ferritin Fe+2 synthesis [12]. Byproduct of biliverdin is anti-inflammatory and antioxidant bilirubin. Recent studies suggest that bilirubin enhances health by means of hepatic mechanisms [13].

HO-1 is a heat- and shock-resistant enzyme, and scientists call this family of proteins Heat Shock Proteins (HSPS).

HO-1 enzyme with a molecular weight of 32 kDa contains 288 amino acid residues encoded by the HMOX1 gene. HO-1 activity depends on NADPH-Cytochrome P450 reductase [14].

Some studies have confirmed that HO-1 inhibitors prevent platelet aggregation, increase fibrin dissolution and phagocytosis, and thus prevent tissue damage and clotting. In addition, hemin is an activator of neuroglobulin, a protein involved in the transport and storage of oxygen in nerve cells, which increases the intracellular partial pressure of oxygen in nerve cells and is necessary to protect nerve cells from hypoxia [15].

3.2 The Presence of Heme Oxygenase-1 Enzyme

HO-1 is a protein with a molecular weight of 32 kDa that belongs to the stress protein family, which is found in high concentrations in the liver, spleen and bone marrow. The enzyme can be stimulated by a variety of environmental stimuli, including ultraviolet radiation, heavy metals, glycolipids, growth factors, hydrogen peroxide, nitric oxide, inflammatory cytokines, hyperoxidation and hypoxia [16].

HO-1 is located in the membranes of the endoplasmic reticulum, and studies have also shown its presence in liver mitochondria [17].

3.3 The Importance of Heme Oxygenase-1 Enzyme

Under physiological conditions, it has been observed that most tissues have low levels of HO-1 enzyme except for the cells of the endoreticular system, in which heme is present at high levels, due to the removal of aged red blood cells. In addition, almost all stressful conditions, such as hypoxia, low oxygen, exposure to heavy metals, mycotoxins, inflammatory factors, and ultraviolet radiation, stimulate the rate of HO-1 enzyme [18].

Cells are unable to engage their defense mechanisms in the absence of functional HO-1, according to the results. By reducing ROS formation during heme breakdown, the HO-1 enzyme protects cells. The HO-1 enzyme pathway generates protective byproducts; increasing their synthesis is one possibility. Hepoxigenase-1 Is Not Without Its Drawbacks [19].

The HO-1 enzyme may be detrimental to human health because to the excess bilirubin, ferritin, and carbon monoxide it creates. A greater consumption of NADPH, an essential molecule for several physiological processes, would occur from an increase in HO-1 enzyme activity. The oxidative pentose phosphate pathway is mainly responsible for replenishing NADPH and making it more resistant to oxidative stress destruction [20].

3.4 Interleukin-6 (IL-6)

The gene encoding interleukin-6 Cytokines, tiny proteins with molecular weights between fifteen and twenty kDa, influence autocrine, endocrine, and non-endocrine signaling [21], [22]. Several cytokines have a role in the formation and operation of the immune system, including IL-6 family cytokines, which are linked to hepatic acute phase protein production and B cell activation [23]. Interleukin-6 (IL-6) is a cytokine that exemplifies homeostasis. [24]. When homeostasis is disturbed, IL-6 is rapidly released, after being disrupted by infection or tissue damage, and contributes to host defense in such conditions by activating acute phase and immune responses. However, excessive, prolonged and unregulated The production of IL-6 negatively affects acute systemic inflammatory response syndrome and chronic immune diseases.

Recent research suggest that IL-6 inhibition may be beneficial in managing many disorders, including both acute and chronic systemic inflammatory diseases [25].

3.5 Interleukin-12 (IL-12)

Since its discovery in 1953, researchers have characterized the endogenous pyrogen, today known as IL-1. Since then, they have been working to change patients' immune systems in order to fight malignant tumors using exogenous cytokines. In the beginning, the difficulties of consistently producing an adequate amount were the fundamental challenges of cytokine-based immunotherapy.

Antigen-presenting cells, such as macrophages and dendritic cells, are in charge of producing IL-12, a complex cytokine that regulates T lymphocyte recruitment and effector function. It is well recognized as a potent cytokine that induces inflammation. IL-12 is made up of both P40 and P35 subunits. As a result, IL-12 is responsible for a significant improvement in immune responses to malignancies [26], [27].

The IL-12 cytokine family includes a large number of different cytokines. These cytokines include IL-12, IL-23, IL-27, IL-35, and IL-39, which was newly found. Recent research has shed light on the diverse functions of several members of the IL-12 family, including effector and immune regulatory roles. These investigations have also shed insight on the role of IL-12 cytokines in modulating innate and adaptive immune responses in cancer. These cytokines may provide useful choices for improving immune-modulatory treatment methods. The fact that it is thought to be an important effector cytokine has significant implications for anticancer treatments as well as immunotherapies involving natural killer cells and Th1 helper cells. Interleukin-12 (IL-12) activates STAT4 and increases IFN-γ production, facilitating Th1 helper cell differentiation via T-bet transcription. Consequently, interleukin-12 is a key player in the fight against cancer [28].

3.6 Coenzyme Q10

First described as a small lipophilic molecule common in cell membranes in 1955, coenzyme Q10's function as an electron carrier in the mitochondrial electron transport chain was shown in 1957. For two decades, its purpose remained unknown until 1986 when the benefits of Coenzyme Q10 treatment in Kearns-Sayre disease were reported [29].

It is named quinone ubiquinone because it is ubiquitous in all cells and its chemical structure consists of two benzoquinone rings with a variable number of isoprenyl units. Its reduced form is known as ubiquinol and the reduced form is

ubiquinone. Through a series of oxidation-reduction reactions, they generate each other (Q Cycle).

Substances found in cell membranes include coenzyme Q10, which is also known as CO Q10 or ubiquinone. The isoprenoid side chain and benzoquinone ring define this small, lipophilic chemical. Complex Q, an enzyme complex located in the mitochondrial interstitial matrix, facilitates its production in humans. Benzoquinone ring and isoprenes 10 are both produced via the cholesterol biosynthetic route; benzoic acid comes from 4-hydroxybenzoic acid, while isoprenes 10 come from mevalonic acid. Electron transport to Complex III is facilitated by the quinone ring, a functional group. Coenzyme Q10 (ubiquinone) may be reduced to ubiquinol by means of a reversible reaction. The polyisoprenoid chain forms hydrophobic membranes at its lipophilic terminus [30].

As part of the electron transport chain (ETC), ubiquinone is present in every cell membrane and helps electrons go from complexes I and II to complex III. The lysosomes at 1.86 μg/mg and the mitochondrial membrane at 2.62 μg/mg were found to have the greatest quantities of ubiquinone (COQ10) in rat liver, respectively. The electron transport pathway inside mitochondria relies heavily on ubiquinone [31]. Consumption of COQ10 has shown epigenetic effects on genes linked to signaling, transport, transcriptional regulation, pathogenic mutations, phosphorylation, and genetic development, suggesting that it may have a role in controlling gene expression. Coenzyme Q10 is an essential antioxidant and performs its primary role in the electron transport chain in addition to stabilizing intracellular membranes, such as the plasma membrane, which in turn prevents phospholipid peroxidation.

Furthermore, Ubiquinone and semiquinone participate in the recycling of other antioxidant molecules, diminishing ascorbate and α-tocopherol levels while assisting in the regulation of cellular redox status. Recent research has associated ubiquinol with the safeguarding of plasma low-density lipoproteins (LDL) from oxidation, an essential anti-atherosclerotic mechanism. The mechanism of CO Q10's pro-oxidant effect remains incompletely understood; however, it plays a function in signaling associated with gene regulation. Its supplementary functions include modulating pore permeability, thereby affecting apoptosis [32].

Coenzyme Q10, synthesized by the human body, is the only lipid-soluble antioxidant. Coenzyme Q10 is synthesized in humans from tyrosine or phenylalanine (benzoquinone ring and mevalonic acid) by a series of enzymes (Complex Q) located in the interstitial membrane of mitochondria and the endoplasmic reticulum, with the majority produced endogenously. The procurement from foreign sources is common in various animal proteins (lamb, beef, chicken, and fish) and cereals (barley and wheat), among others. The recommended daily dosage is 3-5 mg [33].

4 MATERIALS AND METHODS OF WORK

Research samples were collected for patients with leukemia from Baghdad Karkh Health Department/Central Children's Hospital in Baghdad Governorate, and the control group for the period from November to May 2024 AD.

The blood samples included 60 patients with leukemia, comprising 30 males and 30 females, aged between 1 and 14 years. Their infection with leukemia was confirmed through diagnostic processes via blood tests, and through their clinical symptoms, diagnosed by the doctor and found in the patients' files and records. The special questionnaire form was relied upon and the required information for each patient was recorded in it.

The control group samples included 30 healthy and disease-free samples, including 15 male samples and 15 female samples. Their ages also ranged from 1 - 14 years.

Diagnostic tests: the levels of heme oxygenase-1, interleukin 6 and 12, and coenzyme Q10 were measured in the serum of pediatric patients with chronic leukemia and compared with the serum of the control group using the diagnostic kit prepared by Sunlong Biotech (China) by the Sandwich-ELISA method. This ELISA uses the "sandwich-ELISA" method. The microelisa plate provided in this kit is pre-coated with an antibody specific for the variant. Standards or samples are added to the appropriate wells of the microelisa plate, where they bind to the specific antibody. Next, a horseradish peroxidase (HRP)-conjugated antibody specific for the variant under study is added to each well of the plate, incubated, and free components are washed off. TMB substrate solution is added to each well containing only the variant under study. The HRP-conjugated antibody will appear blue, turning yellow after the stop solution is added. Finally, the optical density (OD) is measured using a spectrophotometer at a wavelength of 450 nm.

5 RESULTS AND DISCUSSION

5.1 Heme Oxygenase-1 Levels

The (mean ± standard deviation) of the (heme oxygenase-1) level in the serum of the leukemia patients group and the control group are shown in the Table 1.

Table 1: Mean ± standard deviation of heme oxygenase-1 enzyme level.

Groups Parameter	Mean ± SD		p-value
	Patients	Control	
Heme oxgenase-1	5.086 ± 5.353	3.963 ± 3.836	0.305

The study found no significant difference in predicted heme oxygenase-1 enzyme levels between patients with chronic myeloid leukemia and the control group ($p < 0.05$). This study's results contradict the previous one's [34].

Heme oxygenase-1 (HO-1) has recently been identified as a potential regulator of granulocyte production in stress-induced circumstances such as chemotherapy, in addition to its numerous other functions in cell proliferation and differentiation. According to studies, overexpression of heme oxygenase-1 (HO-1) is associated with enhanced cancer cell proliferation and treatment resistance [34], [35].

The promoter region includes polymorphisms that impact the expression of the HO-1 gene (HMOX1), such as the length polymorphism (GT)n and the single-nucleotide polymorphism (SNP) A(-413T). Variants with shorter GT repeat sequences and 413-A related to HO-1 stimulation are more likely to be identified. According to the previous studies, patients with acute lymphoblastic leukemia showed a greater incidence of short alleles than controls. The fact that short alleles enhance the likelihood of treatment failure lends credence to the theory that HO-1 plays a role in chemotherapy resistance. According to the research, HO-1 might be a feasible target to examine if previous therapies for ALL have failedin total [35].

Recent research indicates that immunodeficiency increases the chance of recurrence in people with acute myeloid leukemia (AML). The research discovered that heme oxygenase-1 (HO-1) is required for both medication resistance and AML cell proliferation. The same study also showed that HO-1 inhibits the development of Human Leukocyte Antigen-C (HLA-C), allowing natural killer cells to avoid immune system detection then [36]. Innate immunity, which includes natural killer cells, is critical for combating cancer, especially when acquired immunity is inadequate. The HO-1/HLA-C axis may lead these cells to function differently in myeloid leukemia. When used to treat acute myeloid leukemia, anti-HO-1 medicines are considered to greatly boost natural killer cell anticancer activity (Fig. 1) [36].

The effects of HO-1 vary. HO-1 has long been recognized for its anti-inflammatory and stress-response effects in the body. HO-1 stops carcinogenesis in its tracks and helps maintain redox balance in healthy cells. Heme oxygenase-1 expression may be linked to pathological traits and clinical outcomes, according to findings from cancer biopsies. Worse survival rates are often associated with increased heme oxygenase-1 levels in tumor tissues. The study's author reached the following conclusion: the efficacy of patient diagnoses and treatments may be dependent on our capacity to understand the relationship between HO-1 and clinical data [37]. Because of its crucial role in cancer development and chemotherapy resistance, the HO-1 enzyme has been the focus of all previous research. Researchers discovered that changing the HO-1 system may boost cancer's susceptibility to treatment. The link between HO-1 regulation levels and a variety of outcomes, including cell cycle arrest, cell death, tumor survival, and progression, supports this [38].

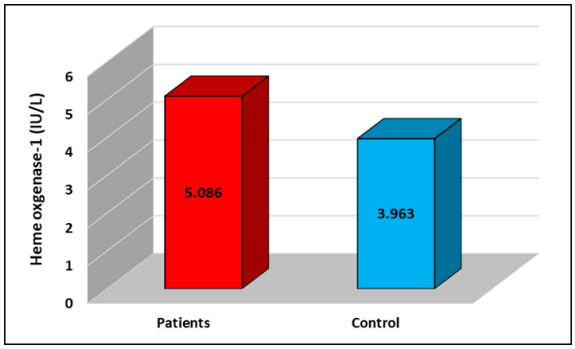


Figure 1: The level of (Heme oxygenase-1) in the sera of patients with leukemia and the control group.

In another study conducted by the researcher, the aim of the study was to draw attention to the effect of HO-1 in circulating monocytes, the presence of bone marrow-derived suppressor cells and complement receptors on a subset of monocytes in advanced cancer patients registered for treatment,

abnormal immune cells were detected in the tumor environment; it was shown in this study that HO-1 levels were clearly high in classical monocytes in all types of cancer studied, and these results are not consistent with the results of our study, where the results of HO-1 levels were analyzed for their diagnostic and predictive potential for clinical outcomes [39].

5.2 Levels of (CoEn Q10, IL-12, IL-6)

The (mean ± standard deviation) levels of CoEn Q10, Interleukin 12, and Interleukin 6 in the serum of the leukemia patients group and the control group are shown in Table 2.

Table 2: Mean ± standard deviation of levels (IL-6 IL-12, CoEnQ10) * p < 0.05.

Groups Parameter	Mean ± SD		p-value
	Patients	Control	
CoEn Q10	0.767±0.370	0.856±0.339	0.265
IL-12	4.962±1.174	5.186±1.680	0.455
IL-6	6.422±0.412	7.250±1.316	0.002*

The results shown in the table above showed that there was no significant difference at the probability level of p<0.05 for the level of the enzyme coEn Q10. These results differed from the results reached by the researcher Rasha (2024), as the coenzyme (CoQ10) is considered an essential cofactor in the electron transport chain in the mitochondria, which is useful in treating liver disorders. The polycyclic aromatic hydrocarbon (DMBA) -dimethylbenz[a]-anthracene 7,12 is what causes and promotes carcinogenesis when this study was conducted on mice with leukemia caused by DMBA)) and the analysis was evaluated Biochemical, immunological and histological examination of the liver to determine whether CoQ10 would treat or alleviate liver injury caused by DMBA-induced leukemia in a mouse model. The results of the researcher showed that CoQ10 treatment significantly reduced liver enzyme levels compared to leukemia model animals. Malondialdehyde (MDA) levels were significantly lower in the CoQ10 group compared to the DMBA group, while glutathione (GSH) and superoxide dismutase (SOD) levels increased significantly. Histological examination of the livers of DMBA-infected mice revealed loss of normal hepatic structure, which was restored after CoQ10 administration (Fig. 2) [40].

In another study, it was shown that CoQ10 provides some protection against cardiac or hepatic toxicity during cancer treatment [41]. Also, through the results shown in the table above, it was found that there was no significant difference at the probability level of p <0.05 for the level of IL-12. These results differed from the results reached by researchers when a study was conducted on patients with acute myeloid leukemia (AML). The level of IL-12 was estimated. Clearly lower values of IL-12 were observed among AML patients compared to the control group. The aim of this study was to analyze IL-12 and to determine the possibility of using IL-12 as a potential anti-cancer drug [42]. IL-12, which is mainly produced by dendritic cells, monocytes, macrophages, and B cells, activates many groups of immune cells that can recognize and destroy cancer cells, and stimulate immunity during the cancer immunity cycle. IL-12 can also be used as an immune regulator in cancer immunotherapy. It has shown great potential in inhibiting tumor growth and improving the tumor environment through many previous clinical models [43].

Cytokines and IL-12 show strong anticancer activity but suffer from a narrow therapeutic window due to the activation of immune cells outside the tumor (Fig. 3) [44].

The above results showed a significant difference at the probability level of p<0.05 for IL-6 (Fig. 4). This study agrees with many researchers and shows the existence of associations between the level of IL-6 and types of leukemia [45]. In many studies, cytokines secreted by cells infected with acute myeloid leukemia in an endogenous or exogenous manner affect the proliferation of acute myeloid leukemia cells, where IL-6 can be used as a biomarker to target myeloid leukemia cells [46].

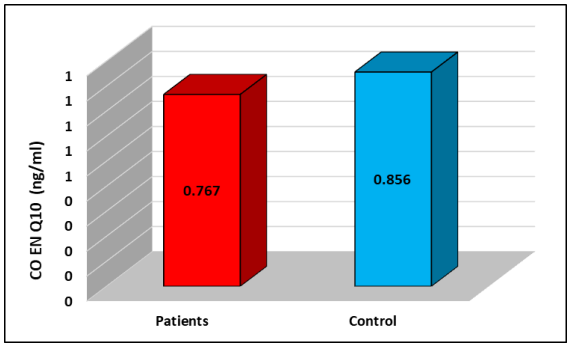


Figure 2: The level of (CoEn Q10) in the sera of patients with leukemia and the control group.

In another study conducted on infants, a statistically significant association was found between pediatric patients with polycythemia and the control group, where IL-6 values for the polycythemia group in children were higher than the control group [47]. IL-6 and IL-10 provide good predictive value for the diagnosis of severe infection (SI) in children with SI. Resulting from leukemia [48].

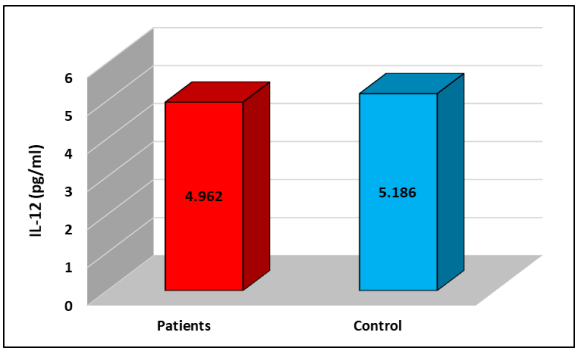


Figure 3: The level of (IL-12) in the sera of patients with leukemia and the control group.

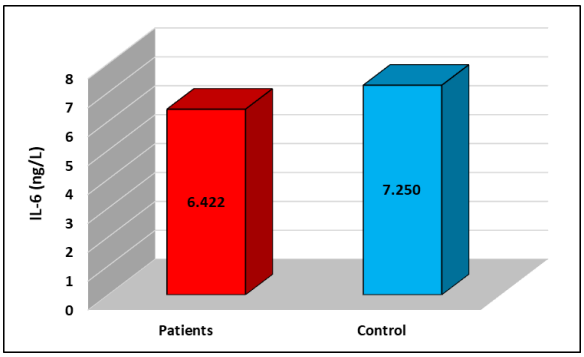


Figure 4: Level of (IL-6) in the sera of patients with leukemia and the control group.

5.2 Coefficient of Correlation

The correlation between heme oxygenase-1 (HO-1) and the above-mentioned biochemical variables was studied for chronic myeloid leukemia patients in children, to clarify the nature of the relationship between (HO-1) and other biochemical measurements (Fig. 5-7).

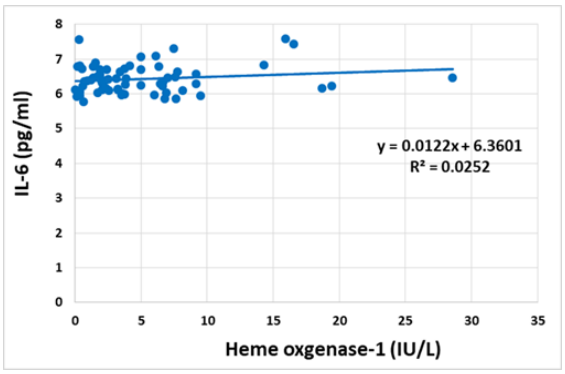


Figure 5: The correlation between heme oxygenase-1 and IL-6 level.

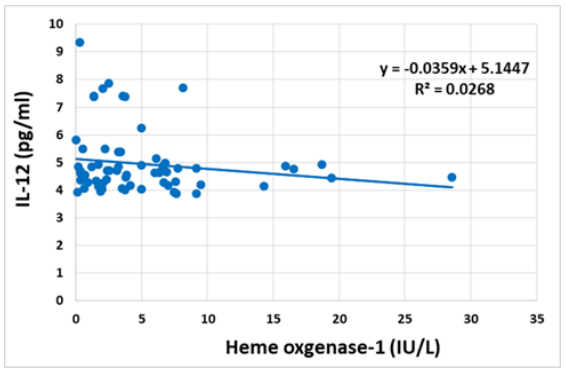


Figure 6: Correlation between heme oxygenase-1 and IL-12 level.

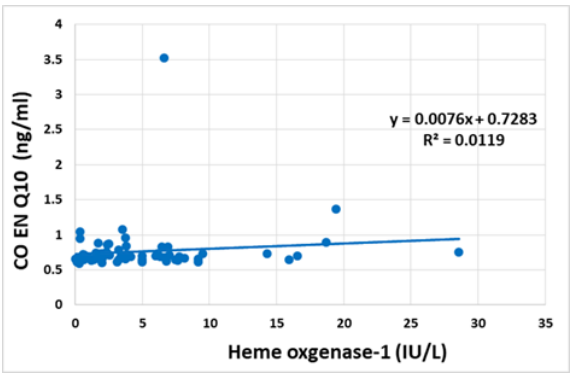


Figure 7: The correlation between heme oxygenase-1 and COENQ10 level.

6 CONCLUSIONS

Based on the study findings, interleukin-6 (IL-6) emerges as a potential biomarker for childhood chronic myeloid leukemia (CML), showing a statistically significant association that may be useful for diagnosis or disease monitoring. The analysis also indicated no significant gender-based differences among CML patients, suggesting that the disease progression and biomarker expression levels are not influenced by sex in the pediatric population. Furthermore, the results revealed no significant differences in serum levels between heme oxygenase-1 (HO-1), interleukin-12 (IL-12), and Coenzyme Q10 (CoEQ10), indicating that these factors may not independently distinguish CML pathology in children. However, a notable and statistically significant difference was observed between HO-1 and IL-6, implying a possible immuno-inflammatory interaction between oxidative stress markers and pro-inflammatory cytokines in the CML microenvironment. This could point toward underlying mechanisms linking oxidative imbalance and immune dysregulation in the pathogenesis of childhood CML.

These findings warrant further investigation into IL-6 as a clinical indicator, and into the biological roles of HO-1 and IL-6 in disease progression. Larger cohort studies and longitudinal monitoring are recommended to validate these relationships and potentially inform targeted therapies or diagnostic frameworks.

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Evaluation of Interferon-Gamma, Vitamin E, and Electrolytes (Sodium, Potassium, Chloride) Levels as Potential Biomarkers in Iraqi Breast Cancer Women

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Keywords: Breast Cancer, Interferon-Gamma, Vitamin E, Sodium, Potassium, Chloride.

Abstract: The primary objective of this study is to evaluate the potential of interferon-gamma, vitamin E, and electrolyte levels (sodium, potassium, and chloride) as biomarkers for breast cancer diagnosis. Patients and Methods: This study was conducted at the Oncology Teaching Hospital, Medical City, Baghdad, and included 130 female participants who ranged in age from 30 to 71 years. The blood samples were collected between May 13, 2024, and August 11, 2024.Methods: The levels of interferon-gamma, vitamin E, and electrolytes (sodium, potassium, chloride) were measured in the serum of the participants using appropriate laboratory techniques. The data obtained were then analyzed to identify any significant differences among the three groups. The results demonstrated that interferon-gamma levels were significantly elevated in both the newly diagnosed group (G1) and the chemotherapy-treated group (G2) when compared to the control group (C). Additionally, a decrease in vitamin E levels was observed in the newly diagnosed group (G1) compared to the treated group (G2) and the control group (C). The electrolyte analysis revealed a significant reduction in sodium and chloride levels in the newly diagnosed group (G1) compared to the other two groups, while potassium levels were significantly elevated in the newly diagnosed group (G1) compared to both the treated and control groups Furthermore, the ROC analysis showed that interferon-gamma (IFN-γ) demonstrated high sensitivity (93.33%) and specificity (62.50%), making it a strong marker for cancer detection. Sodium (Na) exhibited exceptional diagnostic performance with 100% sensitivity and specificity. Similarly, chloride (Cl) showed excellent results, with 100% sensitivity and 88.89% specificity. In contrast, vitamin E exhibited lower sensitivity (51.11%) but demonstrated high specificity (92.50%). The findings suggest that interferon-gamma, vitamin E, and electrolyte levels, specifically sodium, potassium, and chloride, could be significant biomarkers for differentiating between newly diagnosed breast cancer patients and healthy individuals.

1 INTRODUCTION

Cancer is a group of diseases that occur when cells lose control over their growth and division, leading to abnormal cell proliferation. Cancerous cells are characterized by their ability to invade adjacent tissues and destroy them, as well as their potential Cancer can spread to other parts of the body via the blood or lymphatic system, develops from the transformation of normal cells into malignant ones through a series of stages, typically progressing from a precancerous lesion to an invasive malignant tumor. These abilities are considered key characteristics of malignant tumors, while benign tumors are characterized by limited growth and an inability to invade surrounding tissues or spread to other body parts. However, in some cases, benign tumors can transform into malignant cancer [1]. In Iraq, breast cancer is the most prevalent type of cancer among women and one of the main causes of cancer-related mortality in the country. In recent years, the prevalence of breast cancer among women has significantly increased [2]. Early diagnosis and adherence to treatment are critical factors in reducing the risk of the disease progressing to advanced stages, which significantly contributes to lowering mortality rates. Moreover, adopting a

healthy lifestyle and modifying daily habits are essential elements in the prevention of diseases such as cancer [3]. Vitamin E is a fat-soluble antioxidant that plays an important role in protecting unsaturated fatty acids from oxidative stress. The most active form of this vitamin is alpha-tocopherol. Due to its role as an antioxidant, low levels of Vitamin E have been linked to an increased risk of cancer [4]. Vitamin E is one of the major fat-soluble antioxidants found in plasma and red blood cells in humans. Tocopherols are found in lipoproteins and cellular membranes, where they have the ability to scavenge free radicals such as oxygen (O) and hydroxyl (HO) radicals. However, its primary biological role is in interacting with peroxide radicals (ROO) to form tocopherol radicals, which contributes to preventing lipid peroxidation resulting from oxidative stress. Oxidized Vitamin E can be regenerated primarily by Vitamin C, along with other compounds such as glutathione (GSH) and Vitamin A [5]. Natural killer (NK) cells, T lymphocytes, macrophages, and epithelial cells all produce the soluble cytokine interferon-gamma (IFN-γ) [6]. IFN-γ is essential for cellular immunity activation and anti-tumor immunological stimulation responses through its ability to induce programmed cell death. It is considered beneficial in immunotherapy for various types of cancer. Additionally, IFN-γ inhibits angiogenesis in tumor tissues and stimulates the death of regulatory T cells [7]. About 60 years ago, IFN-γ was identified to be the sole member of the type II interferon family. White blood cells produce this substance, which was first identified as a viral inhibitor. The IFN-γ gene encodes IFN-γ, which is made up of two polypeptide chains that are not covalently bound. IFN-γ is found in human blood in three different molecular forms with different molecular weights. Of them, one is the free active form of IFN-γ, while the other two are the cytokine's mature forms [6,8]. Sodium is a naturally occurring element found in soil, water, and plants. It is essential for human health and enters the body through food and water. Sodium is abundant in foods, with the most common form being sodium chloride (table salt). Sodium is also found in water, although its concentration varies depending on the water source. Hyponatremia is a common electrolyte disturbance seen in various diseases, including cancer. It is frequently observed in patients with malignancies, including early-stage cancer, particularly breast cancer [9,10]. Potassium is the primary intracellular cation, with more than 95-98% of potassium residing inside cells. It is necessary for the digestive system, muscles, neurons, kidneys, and heart to all operate properly. Potassium is a component of key minerals required for proper daily physiological functions, playing a critical role in muscle and tissue health throughout the body. Maintaining optimal potassium levels is crucial for overall health [11]. Chloride represents two-thirds of the negatively charged ions (extracellular anions) in the serum. It plays an essential role in maintaining acid-base balance and works alongside sodium to regulate the osmotic balance of body fluids. Chloride levels in the blood increase in conditions such as increasedrespiratory rate, severe fever, aspirin poisoning, anxiety, and dehydration. Conversely, chloride levels decrease in conditions such as slow respiratory rate (e.g., in morphine poisoning, severe vomiting, chronic diarrhea, untreated diabetes mellitus, hyponatremia, and mercury diuretic use). Chemotherapy and radiation treatments can cause vomiting, leading to a loss of chloride from gastric secretions. Repeated vomiting may result in hypochloremia. Chloride levels are often related to sodium levels in the blood, and hypochloremia is common in cancer patients with hyponatremia due to kidney damage from chemotherapy or interference with AVP secretion by cancer cells [12,13].

2 MATERIALS AND METHODS

Study Design: this research was carried out at Medical City's Oncology Hospital in Baghdad, where a total of 130 blood samples were collected and categorized into three groups: 40 healthy controls (C), 45 individuals with newly diagnosed breast cancer (G1, prior to the initiation of treatment), and 45 patients who had undergone chemotherapy (G2). All blood samples were allowed to coagulate before being processed for serum separation. Inclusion Criteria: for the control group, blood samples were obtained from individuals who had no history of breast cancer, as confirmed by comprehensive medical evaluations. For the breast cancer group, blood samples were collected from individuals after confirmation of the diagnosis through standard procedures, including mammography and biopsies, such as Fine Needle Aspiration (FNA) or Core Needle Biopsy (CNB). The chemotherapy group consisted of patients who had undergone chemotherapy, with blood samples collected 4-6 weeks after the completion of chemotherapy. Exclusion Criteria: exclusion criteria included individuals with a history of benign tumors, rheumatoid arthritis (RA), type 2 diabetes mellitus

(DMII), kidney failure, liver failure, cardiovascular diseases, autoimmune disorders, or any other chronic systemic conditions that could interfere with the study results. Sample Collection and Preparation: serum was taken from samples of venous blood., which were collected from participants. After allowing the blood to coagulate, the samples were immediately subjected to centrifugation at 2300×g within 10 minutes of collection. The separated serum was then stored at -85°C until analysis. Biomarker Measurements: the serum concentrations of interferon-gamma (IFN-γ) and Vitamin E were quantified using enzyme-linked immunosorbent assay (ELISA) kits provided by Elk Biotechnology, USA, following the manufacturer's instructions. Duplicate measurements were performed for each sample, and the results were compared with a reference curve. Additionally, the levels of electrolytes (sodium, potassium, and chloride) were measured using a fully automated GE300 Genrui Electrolyte Analyzer. Ethical approval: this study adhered to the ethical principles established in accordance with the Declaration of Helsinki, the procedure was carried out following the patient's verbal and informed consent prior to sample collection. The study protocol, along with the subject information and consent form, received approval from the local ethics committee of the Baghdad Health Directorate Ministry of Health, as documented under number 4081, dated 29/1/2024.

3 STATISTICS

Statistical analysis for this study was performed using SPSS software (version 26). Data were assessed using Group means were compared using the Duncan Multiple Range Test at a significance level of ($p < 0.01$), and Analysis of Variance (ANOVA) was used to find significant differences. Additionally, MedCalc software was used to perform Receiver Operating Characteristic (ROC) curve analysis to evaluate the diagnostic performance of the assessed parameters, including sensitivity, specificity, and diagnostic The normality of the data distribution was verified using the Shapiro-Wilk test, and the data were found to follow a normal distribution.

4 RESULTS

4.1 Statistical Analysis and Findings

The findings indicated that the level of IFN-γ was significantly higher in both the G1 and G2 groups compared to the control group (C). The G1 group's vitamin E levels were considerably lower than those of the G2 group and the group C. In terms of electrolyte levels, the G1 group had significantly greater potassium levels than the G2 group and the group C, while the G1 group had significantly lower sodium and chloride levels than the G2 group and the group C. as shown in Table 1.

The different letters indicate the presence of statistically significant differences, while the same letters indicate the absence of statistically significant differences.

4.2 ROC Analysis for Parameters Under Investigation

By comparing the area under the Receiver Operating Characteristic (ROC) curve with the Area Under the Curve (AUC) for the parameters being studied, the diagnostic ability was assessed, as indicated in Table 2.

Table 1: Serum concentration of vitamin E, IFN-γ and electrolytes among studied groups.

Groups Parameters	Mean ± SD		
	C	G1	G2
Vitamin E (µg/ml)	16.83±3.83 a	13.38±4.85 b	16.22±5.13 a
IFN-γ (Pg/ml)	44.92±13.67 b	71.13±22.95 a	64.008±18.31 a
Na (mmol/L)	142.56±4.97 a	126.93±3.61 c	136.19±9.33 b
K (mmol/L)	3.84±0.31 b	4.3±0.54 a	3.83±0.48 b
CL (mmol/L)	108.28±5.31 a	96.79±3.54 c	102.28±7.24 b

Table 2: The receiver operating characteristic ROC curve analysis for the tested parameters.

Variables	Cut-off value	Sensitivity %	Specificity %	P Value	AUC
V.E	≤12.2	51.11	92.50	<0.001	0.737
IFN-γ	>42	93.33	62.50	<0.001	0.835
Na	≤131.9	100.00	100.00	<0.001	1.000
K	>4.27	62.22	93.33	<0.001	0.773
CL	≤102.9	100.00	88.89	<0.001	0.961

5 DISCUSSION

From Table 1 and the figure, it is evident that Vitamin E levels were considerably lower in the G1 group as opposed to the control group (C) and the G2 group. Our current study's findings are consistent with those of Abiakaetal., who conducted a study in Kuwait and observed a decrease in Vitamin E levels in women with breast cancer before receiving treatment [14]. Similarly, the current study's results are consistent with those of Torun et al., who reported reduced Vitamin E levels in women with breast cancer compared to healthy wome [15]. Our findings also match a study conducted in Mosul, which measured antioxidant vitamins and showed lower Vitamin E levels in women with breast cancer compared to the control group [16]. However, our findings differ from those of a study conducted in France by Gerber, Richardson, Salkeld, Chappuis, who observed elevated Vitamin E levels in women with breast cancer compared to healthy women [17]. This discrepancy may be due to their control group being selected from a hospital population in neurosurgery departments.

Vitamin E is a natural antioxidant that helps remove Reactive oxygen species and free radicals from the body in addition to its antioxidant role, Vitamin E has other important biological functions, such as maintaining cell membrane integrity, influencing DNA synthesis, and modulating cell signaling. Numerous studies have shown that antioxidants can reduce DNA damage caused by oxidation, which in turn may decrease mutations and carcinogenesis [18]. Vitamin E is crucial for immune system function, as immune activity is associated with the release of free radicals from oxygen that are involved in macrophage function. Therefore, the immune system is more sensitive than other systems to a deficiency of antioxidants in the diet [19].

Regarding IFN-γ, our study's findings are consistent with the ones of Borj et al., who conducted a study in Iran and observed elevated IFN-γ levels in the serum of women with breast cancer [20]. Our results are also in agreement with a study conducted in Baghdad by Hassan & Mohamme in 2021, which included 88 samples (58 breast cancer patients and 30 controls) and discovered that women with breast cancer had greater levels of IFN-γ than the control group. [21]. The elevated levels of IFN-γ in the early stages of breast cancer are likely a natural immune response to fight cancer cells. IFN-γ activates immune cells such as T-cells and natural killer cells, contributing to tumor suppression. However, after chemotherapy, IFN-γ levels may decrease due to the impact of chemotherapy on white blood cells and the immune system, which reduces the body's ability to produce this cytokine naturally [22]. IFN-γ is primarily produced by immune cells such as T-cells and natural killer cells and plays a key role in supporting inflammatory processes and fighting viral infections, which can influence breast cancer progression IFN-γ gene polymorphisms may raise the risk of breast cancer by impairing the immune system's capacity to identify aberrant cells [23].

Regarding sodium levels, our research aligns with the findings of Chanihoon etal [24], who conducted a study on women with breast cancer aged 25–73 years and found lower sodium levels in patients compared to healthy women. However, our results do not agree with those of Yousif et al., who reported higher sodium levels in women with breast cancer [25]. The reduction in sodium levels may be due to increased secretion of antidiuretic hormone (ADH), which causes fluid retention and dilutes sodium concentration in the blood. This phenomenon can occur due to abnormal secretion of vasopressin by cancer cells. Additionally, low sodium concentration can stimulate cancer cell proliferation. Cancer cells cause metabolic changes

and fluid imbalance, which can lead to low sodium, particularly in advanced stages or in patients with severe symptoms such as anorexia or vomiting, Certain chemotherapy treatments for breast cancer can also trigger vasopressin secretion or affect fluid and electrolyte balance, leading to reduced sodium levels [26].

From Table 1, it is evident that potassium levels were significantly higher in the G1 group compared to both the G2 group and the control group (C). Our findings are consistent with Al Dleemy study conducted in Nineveh, which found elevated potassium levels in women with breast cancer compared to healthy women [16].

However, our results differ from those of Chanihoon et al., who reported reduced potassium levels in women with breast cancer, with further declines in potassium as the disease progressed [24]. The increased potassium levels in the G1 group may be due to the movement of potassium between cells and extracellular fluid, which is regulated by the Na+/K+-ATPase pump. This pump helps maintain cell membrane functions by creating a concentration gradient across the cell membrane. Various factors, including the blood pH, can influence potassium movement. When the serum is alkaline (high pH), potassium moves into the cells, while in acidic conditions (low pH), potassium moves out of the cells [27]. Rapidly growing tumors lead to the destruction or breakdown of surrounding tissues, causing the release of potassium from damaged cells into the bloodstream. Additionally, tumor side effects may decrease kidney function in excreting potassium. Potassium channels play a role in promoting cancer cell growth and metastasis, and these channels increase in cancer cells. Elevated lactate levels in the blood are indicative of increased metabolic activity, which is linked to tumors and decreased blood pH, contributing to potassium efflux from cells and raising potassium levels in the serum [28], [29]. The chloride levels in the G1 group were significantly lower compared to both the G2 group and the control group (C), and chloride levels were also significantly lower in the G2 group compared to the control group, as shown in Table 1. Hypochloremia is a common electrolyte disturbance in breast cancer patients due to chemotherapy treatments that lead to changes in pH balance and cause electrolyte imbalances such as reduced sodium and chloride levels, often resulting from kidney damage or fluid loss due to vomiting and diarrhea [29]. This decrease may also be due to metabolic changes caused by cancer, as some cancers, including breast cancer, lead to the syndrome of inappropriate antidiuretic hormone secretion, which causes fluid retention and lowers chloride concentration in the blood. In this syndrome, excess ADH is secreted, increasing water reabsorption in the kidneys and diluting electrolyte levels such as chloride [30], [24]. The ROC results are presented in Table 2. According to the ROC analysis, varying performance levels of biological markers are revealed in distinguishing positive and negative breast cancer cases. Vitamin E showed lower sensitivity (51.11%) but high specificity (92.50%), making it effective in detecting negative cases. Vitamin E had a modest ability to correctly identify positive cases, but its high specificity suggests that it can effectively reduce the occurrence of false positives. Interferon-gamma (IFN-γ), on the other hand, demonstrated high sensitivity (93.33%) and moderate specificity (62.50%), indicating a strong ability to correctly identify positive cases, but it had a higher rate of false positives. Sodium (Na) exhibited perfect sensitivity and specificity (100%), with an AUC of 1.000, highlighting its excellent ability to distinguish between positive and negative cases. Potassium (K) showed moderate sensitivity (62.22%) and high specificity (93.33%), with an AUC of 0.773, suggesting good diagnostic potential but slightly reduced performance compared to other markers. Chloride (Cl) displayed 100% sensitivity and 88.89% specificity, with an AUC of 0.961, reflecting its strong diagnostic capability, although not as perfect as sodium. All markers showed statistically significant results ($P<0.001$), reinforcing the reliability of these findings.

6 CONCLUSIONS

The results of the current study demonstrated a significant decrease in the levels of Vitamin E (V.E) and Sodium (Na) in the G1 group (newly diagnosed breast cancer patients) compared to the C group (healthy controls). A significant increase in the levels of Interferon-gamma (IFN-γ) was observed in G1 compared to C. As for the Potassium (K) levels, G1 exhibited the highest value compared to C, while Chloride (CL) levels were higher in C compared to G1 and G2 (chemotherapy-treated women). These results indicate distinct biological and physiological effects on biochemical parameters between newly diagnosed breast cancer patients, chemotherapy-treated women, and healthy controls.

Furthermore, Na (Sodium) and CL (Chloride) showed the highest accuracy in distinguishing between positive and negative cases, achieving

100% sensitivity and specificity with high AUC values (1.000 and 0.961, respectively). On the other hand, IFN-γ demonstrated elevated sensitivity with a good AUC value (0.835). Meanwhile, V.E and K exhibited lower accuracy, with AUC values of 0.737 and 0.773, respectively.

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Physiological and Immunological Study of Biomarkers in Non-Alcoholic Fatty Liver Disease for Predicting Chronic Kidney Diseases

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Keywords: Chronic Kidney Diseases (CKD), FABP-4, Non-Alcoholic Fatty Liver Disease (NAFLD), TNF-α.

Abstract: Non-alcoholic fatty liver disease (NAFLD) is characterized by the accumulation of lipids in macrovesicular hepatic vesicles in about ≥5% of hepatocytes in patients who do not have a secondary cause for steatosis, such as alcohol consumption, hepatitis C, medications, protein malnutrition, or parenteral nutrition. The current research was designed to evaluate the association between non-alcoholic fatty liver disease and chronic kidney disease in patients with NAFLD. This study was conducted from December 2023 until March 2024 at the following main locations: AL-Nasriya Technical Hospital, Nasiriya Governorate, Iraq; Mohammed AL-Mousawi Children′s Hospital, Nasiriya Governorate, Iraq; and AL-Imam AL-Hussain Hospital, Nasiriya Governorate, Iraq. The samples consisted of sixty patients diagnosed with NAFLD and twenty-eight patients without NAFLD. Patient samples included 30 males aged between 28 and 75 years and 30 females aged between 20 and 70 years. The control group's ages ranged between 28 and 65 years. The creatinine test results showed a non-significant increase in patients with NAFLD compared to the control group; in contrast, the glomerular filtration rate (GFR) decreased significantly in the patients group compared to the control. The results also showed significant decreases in FABP-4, PAI-1, and TNF-α levels in patients with NAFLD compared to the control group. Based on the results of the current study, FABP-4 and PAI-1 are considered excellent markers for predicting chronic kidney disease (CKD), and TNF-α can also be considered a reliable marker for predicting CKD.

1 INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD): it's characterized by accumulation of the fat in macro-vesicular hepatic about ≥ 5% of hepatocytes in patients who don't have a secondary caused for steatosis as e.g. alcohol consumption, hepatitis C, medications, protein malnutrition and parenteral nutrition [1].

The alarming increase in the prevalence in NAFLD worldwide is attributed to the increased the population who have obese and diabetic people. The prevalence rate of NAFLD is higher in men compared to women and tends to be rise in women after menopause. The globally prevalence of NAFLD is beginning to raising from 25.3% in 1990-2006 to 38.0% in 2016-2019. The causes for this increasing trend are multifactorial and predominantly blamed on urbanization, unhealthy diet habits and sedentary lifestyles [2].

NAFLD and metabolic syndrome (MS) are two different entity share common clinical and physiopathological attributes with insulin resistance (IR) as the most related [3].

Although the NAFLD is a complex and multifactorial disease that involves several genetic, epigenetic and environmental factors, But, isn't fully understood yet pathogenesis for this disease due to sensitivity to the accumulation of fat via a second pathogenic insult, the liver prompts inflammation and cell death that consequently interprets into oxidative stress (OS), which finally leads to non-alcoholic steatohepatitis NASH and fibrosis [4].

Most patients with NAFLD may remain asymptomatic, but about 5-10% of them are evaluated for development of the complication cirrhosis with a high risk of death. The existence of T2DM may be the most important clinical predictor of liver-relative morbidity and mortality in the NAFLD. [5].

Prevalence of NAFLD is about 80% to 90% in obese adults, 30% to 50% in patients with diabetes mellitus (DM), 90% or more than that in the patient with hyperlipidemia, 3-10% in children, and as high as 40% to 70% between children with obesity [6], [7].

Chronic Kidney Disease: CKD is a complex, progressive chronic condition that is defined either by abnormalities in structures or functions of the kidney and lasts for ≥ 3 months. CKD has a significant effect on global health, both as a direct cause of global mortality and as an important risk factor to CVD, identifying novel modifiable risk factor for CKD is critical to reducing the burden of disease. Globally, about 697.5 million statuses in all stages of CKD were recorded, with a global prevalence of 9.1% and 1.2 million deaths from CKD in 2017 [8].

CKD tends to associated with MS, likeT2DM, obesity, and hypertension. While in countries with low and middle income, the contagious diseases and environmental toxins are also commonly associated with CKD [9], [10]. Interestingly, the cellular phenomena, metabolic pathways and molecular mediators included in NAFLD and CKD are similar to each other and include IR, ectopic fats depositions, and activations of insulin and transformation growth factor pathway [11].

Accurate assessment of renal functions in the patients with LD is of central importance, particularly in the patients with cirrhosis. Where estimated Glomerular Filtration Rate (eGFR) is one of the monitoring indicators of the kidney functions in these patients and one the guiding criteria for simultaneous liver and kidney transplantation [12]. The most common methods used for estimating the GFR in these populations are depend on creatinine, which is affected by a decrease in creatinine production, a reduction of skeletal mass, and potentially by raising serum bilirubin. Creatinine is a product of the breakdown of creatine, which is manufactured in the liver and primarily stored in muscle tissues. Therefore, serum creatinine is closely related to body mass and is therefore affected by a number of variables such as age, gender, muscle mass, and ethnicity [13].

2 MATERIALS AND METHODS

The current study was conducted through duration from December 2023 and continued until March 2024. It has been carried out at the following main location:

- AL-Nasriya Technical Hospital, Nasiriya Government, Iraq.
- Mohammed AL-Mousawi Children′s Hospital, Nasiriya Government, Iraq.
- AL-Imam AL-Hussain Hospital, Nasiriya Government, Iraq.

2.1 Subjects

In this study included 88 participants. This participant divided into 60 participants infected with NAFLD and 28 participants non-infected with NAFLD. The infected participant divided into 30 patient man their ages range between (28-75) years and 30 patient women their ages range between (20-70) years. control group their ages range between (28-65) years, the information collected from them included the following:(Name, age, height, weight, sex, address, marital status, educational level, work, type of treatment using) where patient with cardiac disease, kidney disease, hepatitis disease is excluded and them with a genetic history of diabetes were excluded as well.

2.2 Collection of Blood Samples

After obtaining official approvals from the Thi-Qar Health Department to facilitate mission to collect samples from patients with NAFLD visiting the above-mentioned hospitals, also the approval of the medical committees in the hospitals was obtained, and then the approval of the patients' consent for the purpose of taking blood samples from them.

The time for sample collection began from 8:30 a.m. to 1:00 p.m. when 5 ml of venous blood was drawn for both infected and non-infected with non-alcoholic fatty liver disease. 2 ml was transferred to tubes containing EDTA to measure the HbA1c. 3 ml of blood were placed in tubes containing the gel substance. It was discarded in a centrifuged at speed reaches to 5000 revolutions per minute for the period 5 minutes, where blood serum was separated from the other components and after they were separated and put in a tube to keep the serum at a temperature of -20 below zero until performing biochemical tests.

2.3 Parameters Measured

2.3.1 Creatinine

This reagent is designated for use in laboratory (automated method) where it allows the quantification of creatinine in human serum and

plasma or urines to screen its level, which was provided by a company Biolabo SAS.

2.3.2 Plasminogen Activator Inhibitor (PAI)

Sandwich kit is used to assay PAI levels in Human serum, which was provided by a company Sunlong Biotech.

2.3.3 Human Fatty Acid Binding Protein 4

Sandwich kit is used to assay FABP4 level in Human serum, which was provided by a company Sunlong Biotech.

2.3.4 Human Tumor Necrosis Factorα (TNF)

Sandwich kit is used to assay TNF-α level in Human serum, which was provided by a company Sunlong Biotech.

2.4 Statistical Analysis

The data of current study was statistically analysis by used of SPSS (Statistical Package of Social Science version 26), depends on using independent sample t test and LSD, Chi-square, person for correlation, and ROC curve analysis at p. value < 0.05.

3 RESULTS

The current study showed creatinine increased non-significantly in patients with NAFLD group compared to the control group, in contrast, the GFR decreased significantly in patients group compared with the control group at p. value < 0.05, as in Table 1.

The present study showed that the FABP-4, PAI-1, and TNF decreased significantly in the patient with NAFLD group compared to the control group at p-value < 0.05 as in Table 2.

The current study recorded that a Creatinine recorded a strong negative correlation with GFR. The FABP-4 scored a weak positive correlation with PAI-, and strong correlation with TNF. The PAI-1 scored weak positive correlation with TNF, at p. value < 0.05, as in Table 3.

Table 1: Evaluation of renal function test in patients with NAFLD and control groups.

Kidney function tests	Patients No. 60	Control No. 28	p. value
	Mean ± S. E		
Creatinine	0.95 ± 0.04	0.87 ± 0.05	0.230
GFR	86.9 ± 5.67	109.1 ± 10.7	0.039*

Table 2: Evaluation of immune parameters in patients with NAFLD and control groups.

	Patients No. 60	Control No. 28	p. value
	Mean ± S. E		
FABP-4	13.1 ± 0.30	21.7 ± 1.02	<0.001**
PAI-1	0.83 ± 0.02	0.92 ± 0.0.4	0.044
TNF	7.86 ± 0.37	16.7 ± 1.01	<0.001**

This study recorded that 56% of patients their creatinine concentration increased compared with control group with cut value for positive 0.6, while only 33% of patients their GFR mean within the range of control group at p. value < 0.05 with cut value for positive 47.3, in addition, the sensitivity of parameters 0.91% and 0.86 and the specificity 0.85% and 0.96% respectively at above cut value, as in Table 4 and Figure 1.

This study recorded that lees than 1% of patients their FABP-4 mean within the range of control group, regarding API-1 the study noted 28% of patients had API-1 mean more than the mean of control group, according to TNF less than 1% of patients their mean of TNF increases than mean of TNF in control group at p. value < 0.05 as in Table 5 and Figure 2.

Table 3: Person correlation between involved parameters.

		Creatinine	GFR	FABP	PAI	TNF
Creatinine	r. value		-0.809**	-0.045	-0.065	0.016
	p. value		0.000	0.734	0.620	0.904
GFR	r. value			-0.037	-0.020	-0.058
	p. value			0.778	0.882	0.660
FABP	r. value				0.388**	0.712**
	p. value				0.002	0.000
PAI	r. value					0.351**
	p. value					0.006

Table 4: Receiver operating characteristic curve for renal function test.

Variable	Cut point	Sensitivity	Specificity	Area	S. E	p. value	Asymptotic 95%C1	
							Lower bound	Upper bound
Creatinine	0.60	0.91	0.85	0.562	0.065	0.351	0.434	0.690
GFR	47.3	0.86	0.96	0.337	0.063	0.014	0.213	0.461

Table 5: Receiver operating characteristic curve for immune parameters.

Variable	Cut point	Sensitivity	Specificity	Area	S. E	p. value	Asymptotic 95% CI	
							Lower Bound	Upper Bound
FABP-4	9.43	1.000	1.000	0.954	0.020	0.000	0.916	0.992
API-1	0.805	0.964	0.817	0.711	0.063	0.001	0.588	0.834
TNF-α	3.86	1.000	1.000	0.934	0.024	0.000	0.886	0.981
Positive 28 Negative 60								

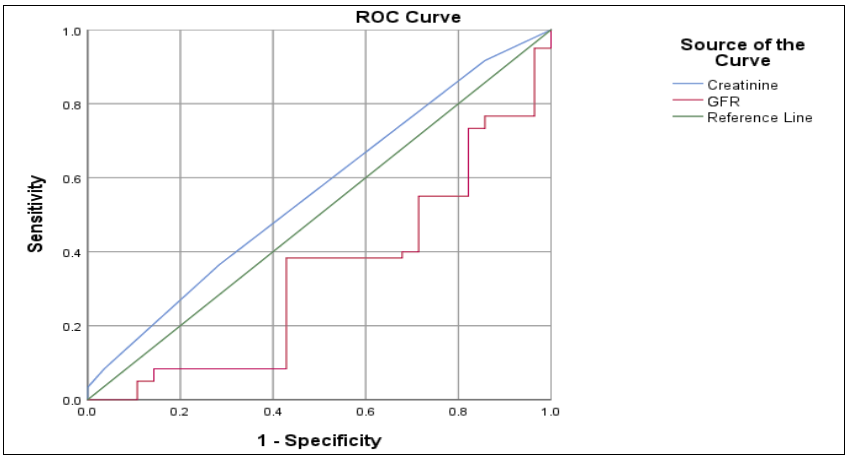


Figure 1: Receiver operating characteristic curve for renal function test.

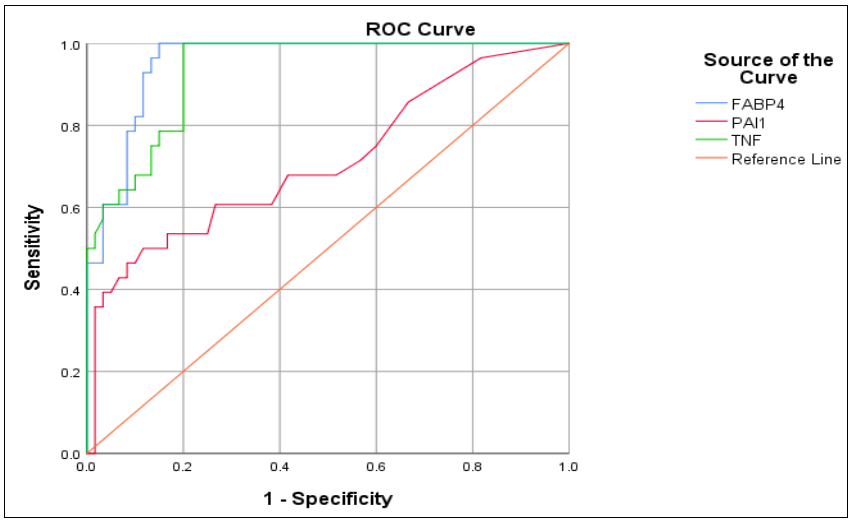


Figure 2: Receiver operating characteristic curve for immune and biochemical parameters.

4 DISCUSSION

Several studies in kidney disease have suggested that lower levels of PAI-1 protect against kidney fibrosis, while deletion of PAI-1 levels has been shown into promotes cardiac fibrosis, in vitro and vivo studies have suggested that the levels of PAI-1 may be have a pro-fibrotic and anti-fibrotic impacts. Studies in isolated and cultured astrocytes have indicate that anti-fibrotic impacts of the PAI-1 levels may be due to inhibition of interstitial collagenase during the onset of fibrosis [14].

Other study did agree with the result of current study, several years ago, the relationship between the levels of PAI-1 and (MS) with obesity was proven. It was found that patients with NAFLD have high levels of the PAI-1. This confirms the associated between thrombogenesis and MS [15].

The levels of TNF decreased significantly in the patients had disease less than one year than patients had disease duration more than one year at p-value < 0.05.

Also there is meta-analysis that does not agree with the result of our study, which included 27 studies including 698 healthy individuals and 1545 patients with NAFLD, circulating adiponectin levels were found to be decrease in patients with NAFLD compared to controls, they were also decreased more in patients with NASH than in patients with NAFLD, following the opposite direction to that of TNF-α. Interestingly, circulating adiponectin appears to be nonlinearly distributed in the NAFLD, being low in the NASH but increasing as the disease progresses from NASH to cirrhosis [16].

There is another study dis agreement with the result of current study, which some previous studies, it was noted that hypo-fibrinolysis seems to be associated with obesity, as the same changes were found in the obese control group. Some previous studies on the PAI-1 levels also indicated that altered fibrinolysis is more evident in the advance stages of disease [17].

In other study conducted, a possible relationship was discovered between levels of FABP4 and markers of liver injury in the serum, although there were no significant associations between them. Therefore, it is worth noting that these molecules are not reliable markers of disease, because about 75% of individuals with NAFLD don’t shows elevated levels of transaminase enzymes. However, the levels of FABP4 were positively correlated with GGT and CRP in the serum, indicating that FAPP in the serum is associated with liver injury and inflammation, which are features of advanced stages of the NAFLD [18].

Other studies that do not agree with the results obtained in current study, as it indicated that individuals with NAFLD have high levels of PAI-1, fibrinogen, von Willebrand factor, factor VII and C-reactive protein, which are known to be associated with an increased risk of strokes. This greatly supports in the vivo data on hypercoagulability in NAFLD. It also indicated a direct and highly significant relationship between PAI-1, fibrinogen, von Willebrand factor and VII factor with morphological criteria such as BMI and waist circumference (WC), but on the other hand, it also noted that there is a significant decrease in the levels of coagulating factors and a decrease in the levels of PAI-1 after losing weight induced by the exercise and dietary modification [15], [19].

5 CONCLUSIONS

This study provides compelling evidence that non-alcoholic fatty liver disease (NAFLD) is closely associated with changes in renal function and immunological biomarkers that may contribute to the early development of chronic kidney disease (CKD). Despite a non-significant elevation in serum creatinine levels among NAFLD patients, a statistically significant reduction in glomerular filtration rate (GFR) was observed, indicating early signs of renal impairment.

According to the ROC test, it was shown that all patients had negative results compared with the control group, where it was recorded that less than 1% of patients had their FABP-4 mean within the range of the control group. while API-1, the study noted 28% of patients had API-1 means more than or within the mean of the control group. Therefore, the FABP-4 and API-1 are considered excellent markers for predicting chronic kidney disease in the first place, followed by TNF.

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Assessment of the Effectiveness of Prolidase and Study of the Impact of Certain Antioxidants in Individuals Suffering from Beta Thalassemia in Anbar Province

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Keywords: Thalassemia, Prolidase, Antioxidants,

Abstract: Thalassemia is a common hereditary disease that is transmitted from parents to their children. As a result, many individuals do not follow the recommended guidelines, particularly before marriage. Early screening is considered an important measure to raise awareness among individuals for the early detection of thalassemia. The current study included 60 individuals (males and females) diagnosed with thalassemia, aged between 5 and 20 years, identified by specialists in hematology at Al Ramadi Hospital in Al Anbar Governorate, and 30 individuals (males and females) aged 5 to 20 years who are not affected by thalassemia, serving as a control group. Samples were collected at Al Ramadi Hospital for Women and Children / Thalassemia Unit.We discussed the effect of the disease on the activity of the Prolidase, which was found to significantly increase when exposed to this pathological condition (thalassemia). Therefore, we can rely on the estimation of elevated prolidase activity as a biomarker for the onset of health deterioration in individuals affected by thalassemia, thus preventing progression to advanced stages of the disease. The study aimed to estimate the effectiveness of the Prolidase in patients with thalassemia and compare it with a control group to understand the reason for the elevated enzyme activity in the patients. The measurement of levels of certain antioxidants, including (CAT, D3, MAD, GSH). The results showed a significant decrease in levels of (D3, GSH) at a significance level of ($P \leq 0.05$) in patients compared to the control group. At the same time, there was a significant increase in levels of (MAD, CAT) at a significance level of ($P \leq 0.05$) in the serum of patients compared to healthy individuals.

1 INTRODUCTION

Thalassemia is a hereditary blood disorder that leads to the production of abnormal hemoglobin due to a defect in one or more of the (alpha and beta) globin chains found in hemoglobin. This results in an imbalance in the production of these chains, causing a disturbance in the normal structure of hemoglobin, especially when one parent is a carrier of the disease, enabling transmission to the next generation [1]. When there is a disruption in the synthesis of one of the globin chains (alpha or beta), this leads to a reduced level of hemoglobin in red blood cells, affecting the oxygen transport process from the lungs to all body tissues, which causes the development of anemia in childhood and its persistence throughout life. Hemoglobin, which is composed of two proteins (alpha globin and beta globin), plays a crucial role in red blood cells for transporting oxygen to all body tissues. When the body is unable to produce one of these proteins, red blood cells cannot produce normal hemoglobin, rendering them unable to carry oxygen from the lungs to the tissues [2].

Prolidase is an enzyme classified under the number (EC 3.4.13.9) that catalyzes the hydrolysis of the substrate, leading to the breakdown of dipeptides that contain proline or hydroxyproline derivatives at the terminal end of the alpha carbon, resulting in free proline. Prolidase plays a significant role in the final stages of the degradation of proteins rich in amino acids, such as the breakdown of collagen. It contributes to cell growth by regulating growth factors and transcription, and it has important roles in various physiological aspects, pathological processes, and cell growth and differentiation [3]. The substrate that prolidase acts upon is imidodipeptides resulting from the degradation of collagen, which contain proline or

hydroxyproline at their ends. It exhibits activity in plasma and various organs such as the brain, heart, uterus, and red blood cells, but shows abnormal activity under conditions like liver disorders and osteoporosis [4]. Prolidase depends on manganese as a cofactor, associated with the vicinity of the active site, to carry out the activation process in the cleavage of dipeptides, breaking the bond in the (glycine–proline) compound to produce two compounds: glycine and L-proline. Therefore, Prolidase requires specific conditions with a temperature range of 35-55 °C and a pH range of 6-8, along with a narrow specificity regarding the substrate to hydrolyze only a few terminal peptide bonds, thereby regulating vital processes and promoting the breakdown of proteins into smaller peptides and amino acids. Its importance lies in the release and recycling of proline in the protein systems of bacterial enzymes [5].

These are a group of elements, complex compounds, enzymes, and vitamins that possess an important biological property for the bodies of many living organisms. They have the ability to inhibit the activity and formation of free radicals, which helps prevent or slow down the oxidation process by continuously removing active forms of oxygen and nitrogen from the body, rendering them unable to damage cells [6].

Glutathione (GSH) is considered a non-enzymatic, water-soluble endogenous antioxidant, existing in the form of a tripeptide composed of three amino acids (Glutamic Acid, Glycine, Cysteine). It plays a role in building and repairing tissues, combating free radicals, preventing and delaying cell damage, and detoxifying chemical substances within the liver. It has a low molecular weight and exists in both oxidized form (GSSG) and reduced form GSH [7]. GSH is regarded as a reducing agent capable of donating a hydrogen atom found in high oxygen-consuming and energy-producing organs such as the brain, kidneys, and liver, helping to maintain cell membranes from oxidation and oxidative damage. Therefore, there is an inverse relationship between GSH concentration in tissues and oxidative stress levels [8].

Malondialdehyde (MDA) is considered an antioxidant that results from the oxidative degradation of lipids that occurs spontaneously in the body's cells. MDA is one of the most important indicators used to investigate oxidative stress in body tissues, serving as a clear marker for lipid peroxidation. It is significant for monitoring oxidative damage caused by reactive oxygen species and estimating the end products of lipid oxidative degradation. MDA is produced through the oxidation of unsaturated fats, particularly those containing two or three double bonds, being a secondary product of the oxidation of polyunsaturated fatty acids after degradation by reactive oxygen species. Fatty acids are more susceptible to oxidative stress when they interact with free radicals in the process of lipid peroxidation, which primarily leads to MDA production [9], [10].

Catalase (CAT) is one of the oxidoreductase enzymes (1.11.1.6 E.C) that plays a crucial role in protecting cells from the toxic effects of hydrogen peroxide, as it catalyzes the decomposition of peroxide into O2 and H2O. Therefore, the levels of catalase enzyme in red blood cells of patients with mild thalassemia respond to oxidative threats, while in patients with severe thalassemia, the levels of antioxidant enzymes return to their normal levels due to the presence of normal red blood cells resulting from multiple blood transfusions [11].

Vitamin D is considered an essential nutrient for maintaining bone health and calcium balance, and for the alignment of the skeletal structure during periods of rapid growth from early childhood until puberty. It is obtained from external sources through the diet (such as fatty fish), and then it is transported to the liver, where it is converted by parathyroid hormone to 25-hydroxyvitamin D3. It then transforms in the kidneys into dihydroxyvitamin D3 (1,25-), with 25OHD being the circulating form of vitamin D in the serum. This vitamin plays a crucial role in regulating calcium absorption in the intestines, helping to maintain serum calcium concentration. Therefore, when vitamin D levels drop, conditions such as rickets and osteoporosis can occur [12], [13].

Research objectives:

1) To estimate the effectiveness of prolidase in patients with thalassemia and compare it with a control group.
2) To assess the levels of antioxidants by examining (VitD, GSH, MDA, CAT) in patients with thalassemia and comparing them with a control group.

2 MATERIALS AND METHODS

A case study was designed for thalassemia patients and a control group, totaling 90 samples from both patients and healthy individuals. Blood samples were collected from clinically diagnosed patients, comprising 60 samples: 30 from males and 30 from

females, aged between 5 and 20 years, at the Thalassemia Unit of Al-Ramadi Teaching Hospital. The control group consisted of 30 healthy samples, with 15 from males and 15 from females. A total of 5 mL of venous blood was drawn from thalassemia patients and healthy individuals using a single-use plastic syringe and was divided based on the type of examination. Then, 2 mL of blood was placed in test tubes containing an anticoagulant (EDTA) for measuring hematological variables, while the remaining 3 mL was placed in plastic test tubes without anticoagulant and left at room temperature until clotting occurred. The blood samples were centrifuged for 10 minutes at a speed of 3000 revolutions per minute to separate the serum, which was then aspirated using a micropipette and distributed into three numbered tubes to avoid repeated freezing and thawing that could affect enzyme activity. The samples were stored at a temperature of -20 °C until analysis, and the required biochemical tests were conducted later.

2.1 Estimation of Prolidase in Serum

The activity of prolidase was estimated by its catalytic action on its substrate, the dipeptide Glycine-Proline (Gly-L-Pro), to hydrolyze it, releasing the free amino acids glycine and proline. The amount of released proline was measured colorimetrically after it reacted with ninhydrin at a wavelength of 515 nm, using proline as a standard substance [14].

Serum was diluted by adding 250 µL of a dilution solution to every 50 µL of serum. The mixture was then incubated for 24 hours at 37 °C to prepare the diluted serum for addition. Following this, 100 µL was added to the control samples.

The activity of the prolidase enzyme in the blood samples under study was calculated based on the following (1) [15].

$$\textit{Prolidase Activity} = (\textit{Abs of test} - \textit{Abs control}) / (\textit{Abs of standard}) \times 2.4 \times [S]. \quad (1)$$

2.2 Determination of Glutathione Level

The modified method was used to determine the level of reduced GSH in the serum of thalassemia patients, which relies on the use of Ellman's reagent containing DTNB. This reagent reacts strongly with glutathione, reducing the thiol (SH) group to produce a yellow-colored complex that has a maximum absorbance at a wavelength of 412 nm. The concentration of the resulting product depends on the concentration of glutathione present in the serum [16].

A solution of sulfosalicylic acid was prepared by dissolving 2 g of the acid in 50 mL of distilled water and stored in the refrigerator until use. The phosphate buffer solution was prepared by mixing 30 mL of a sodium phosphate dibasic solution (Na_2HPO_4) at a concentration of 0.08 M, which was prepared by dissolving 0.284 g of Na_2HPO_4 in 50 mL of distilled water, with 15 mL of a KH_2PO_4 solution at a concentration of 0.6 M, which was prepared by dissolving 4.08 g of KH_2PO_4 in 25 mL of distilled water and then completing the volume to 50 mL. The Ellman's reagent solution was prepared at a concentration of 0.01 mmol by dissolving 0.004 g of DTNB in 100 mL of the phosphate buffer solution (pH=7.4) and stored in the refrigerator until use. Then, 150 µL of serum was added to a test tube containing 150 µL of a 4% sulfosalicylic acid solution and left for 5 minutes. The solution was then separated by centrifugation at 2000 rpm for 5 minutes. Following that, 4.5 mL of Ellman's reagent was added, and after 5 minutes, the absorbance was measured at 412 nm using a spectrophotometer.

The concentration of reduced GSH in the blood serum of patients and healthy individuals was calculated based on the following (2) [17]:

$$\textit{Conc. of GSH } (\mu mol/L) = (A \text{ at } 412 \text{ nm}) / (E \times L) \times 10^6 \quad (2)$$

2.3 Determination of VitD3 Concentration

Vitamin D labeled as biotin (OH-25) was estimated in the laboratory using an enzyme-linked immunosorbent assay (ELISA) kit. The color intensity, measured by a spectrophotometer at 450 nm, is proportional to the VitD3 concentration in the sample. The sample result is calculated directly from the standard curve by comparing the optical density (OD) of the samples and standard solutions [18].

The modified TBA-thiobarbituric acid reaction method was used to estimate the concentration of MDA, which represents one of the main products of lipid oxidation. The measurement depends on the reaction between malondialdehyde and TBA with lipid peroxides in an acidic medium to form a pink (TBA-MDA) complex, and its absorbance is measured at a wavelength of 532 nm [19].

Thiobarbituric acid (TBA) solution was prepared by dissolving 0.67 g of TBA with 20 g of trichloroacetic acid and 2 mL of glacial acetic acid, then completing the volume to 100 mL with distilled water. The solution was prepared immediately

before use. Trichloroacetic acid (TCA) solution was prepared in two concentrations: the first concentration of 17.5% was prepared by dissolving 17.5 g of TCA in 100 mL of distilled water, and the second concentration of 70% was prepared by dissolving 70 g of TCA in 100 mL of distilled water. Both solutions were kept in the refrigerator until use. Then, 150 µL of blood serum was added to a test tube, and 1 mL of 17.5% TCA solution was added to it, followed by 1 mL of TBA solution. The mixture was mixed well and then incubated in a boiling water bath at 100 °C for 30 minutes. The tubes were then cooled, and 1 mL of 70% TCA solution was added to them. The mixture was left at 37 °C for 20 minutes, and the supernatant was separated by centrifugation at 2000 rpm for 10 minutes. After that, the absorbance was measured at a wavelength of 532 nm using a spectrophotometer after the appearance of the pink color due to the formation of the colored complex.

The concentration of MDA in the blood serum of patients and healthy individuals was calculated based on the following (3) [20]:

$$Conc.\ of\ MDA\ (\mu mol / L) = (A_test - A_blank) / (E^{\circ} \times L) \times D \times 10^{6} . \quad (3)$$

2.4 Determination of Catalase Concentration

The activity of the catalase enzyme was estimated by measuring the decrease in absorbance resulting from the consumption of the substrate (hydrogen peroxide). Hydrogen peroxide reacts with ammonium metavanadate under acidic conditions to reduce vanadium to vanadium (III). Hydrogen peroxide is a strong oxidizing agent that forms a red-orange peroxovanadium complex that absorbs at 452 nm [21].

A buffered phosphate solution with a concentration of 50 mM and a pH of 7 was prepared. A hydrogen peroxide solution with a concentration of 10 mM was prepared using the buffered phosphate solution, and a vanadium reagent solution was prepared at a concentration of 0.01 M of ammonium metavanadate in 0.5 M sulfuric acid.

The activity of the catalase enzyme is calculated based on the following equation (4):

$$Catalase\ Activity\ of\ test\ (kU) = 2{,}303/t * \log(A1/A2) . \quad (4)$$

3 STATISTICS

Statistical analysis was conducted using the SPSS statistical package version (21). Data were evaluated using the arithmetic mean, and the means were compared using Duncan's multiple range test at a significance level of ($P \leq 0.05$). Analysis of Variance (ANOVA) was employed to find significant differences between the patient group and the healthy group, while Excel version (2010) was used to create graphical representations.

4 RESULTS AND DISCUSSION

The Prolidase activity is measured in serum blood.The activity of the prolidase enzyme was evaluated in the blood of patients with thalassemia and the control group. The results showed an increase in the activity of the enzyme in the serum of thalassemia patients. When a statistical comparison was made between the activity of the enzyme in the serum of thalassemia patients and the activity of the enzyme in the serum of apparently healthy individuals (the control group), it was found that there was a significant increase in the activity of the enzyme in individuals with thalassemia, as shown in Table 1 and Figure 1.

The results of the current study align with the findings of researchers (Cakmak) and (Belli) and their group .The increase in the activity of the prolidase is attributed to a dysfunction in collagen turnover, which leads to various disease conditions and disease progression. The prolidase may play a role in metabolic disorders in individuals with thalassemia, as collagen is a major extracellular component present in various tissues, including red blood cells. prolidase (Iminodipeptidase) is a homogenous enzyme that releases carboxyproline and hydroxyproline from prolidase , facilitating collagen turnover, cell reorganization, and growth in the body. The activity of the prolidase is a determining factor in the bioregulation of collagen, and the breakdown of tissue barriers is stimulated by proteolytic enzymes. Therefore, inhibiting the enzyme's activity may be considered a therapeutic target for patients with thalassemia. Although collagenases initiate the degradation of the peptide bonds in collagen, the final step of the breakdown is stimulated by the prolidase , where a significant decrease in collagen quantity has been observed in patients, which may be associated with an increase in prolidase activity in the tissues [22], [23].

Table 1: The activity of the prolidase in the blood serum of patients and the control group.

Parameters	Mean ± SD Groups					
	Male Patients	Female Patients	Total Patients	Male Control	Female Control	Total Control
Prolidase (IU/L)l	800.71 ± 5.78	796.06 ± 9.88	798.385 ±7.83	239.05 ± 25.11	229.07 ±3.42	234.06 ±14.26

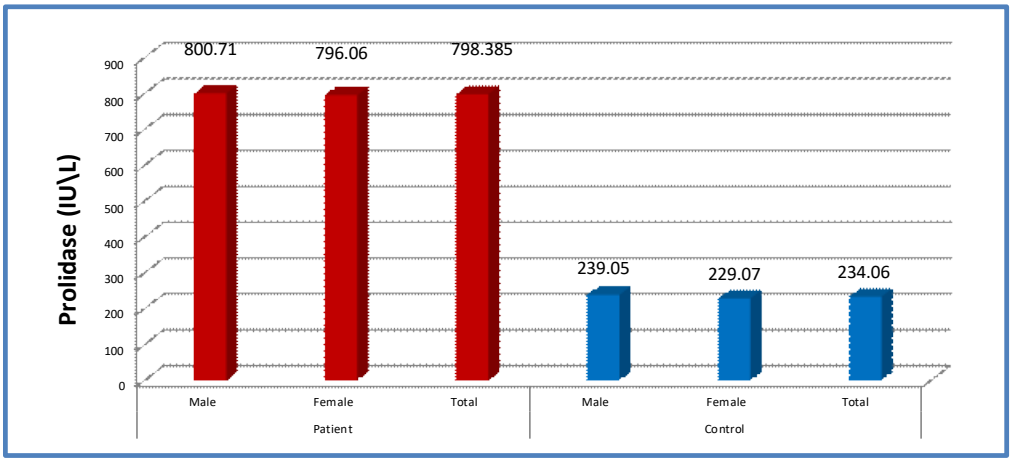


Figure 1: Comparison between different groups in the level of the prolidase.

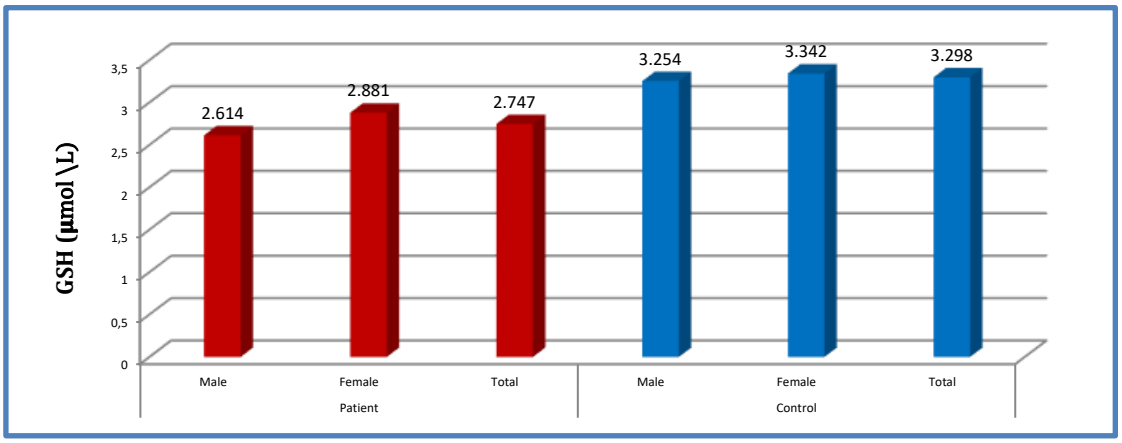


Figure 2: Comparison between different groups in GSH level.

Table 2: The effectiveness of antioxidants in the blood serum of the sick and healthy groups.

Parameter (Units)	Male Patients	Female Patients	Total Patients	Male Control	Female Control	Total Control
GSH (µmol/L)	2.614 ±1.11	2.88 ±1.01	2.747 ±1.01	3.254 ±1.72	3.342 ±1.50	3.298 ±1.35
MDA (µmol/L)	3.678 ±3.78	3.627 ±2.05	3.774 ±2.05	2.591 ±1.47	2.166 ±1.16	2.378 ±1.37
Vit D3 (ng/mL)	7.664 ±4.472	6.943 ±5.19	7.330 ±5.19	9.166 ±7.139	7.833 ±4.16	8.498 ±2.07
CAT (K/mL)	0.832 ±0.21	0.688 ±0.21	0.760 ±0.19	1.023 ±0.21	0.129 ±0.24	1.086 ±0.52

3.1 Estimation of Antioxidant Levels

The levels of antioxidants (CAT, D3, MAD, GSH) were estimated in Table 2 for thalassemia patients and control groups according to gender. Comparisons between patient groups and control groups showed a significant increase in the level of (MAD, CAT) and a significant decrease in the level of (GSH, D3) at the probability level ($P \leq 0.05$) in the serum of thalassemia patients compared to (control groups).

3.2 Estimation of GSH Concentration Levels

The level of GSH was estimated in the blood of patients with thalassemia and the control group. The results showed a decrease in the level of GSH in the serum of thalassemia patients. When a statistical comparison was made between the activity of GSH in the serum of thalassemia patients and the activity of GSH in the serum of apparently healthy individuals (the control group), it was found that there was a significant decrease in the activity of GSH in people with thalassemia compared to the healthy group, as shown in Table 2 and Figure 2.

The results of the current study, as shown in Table 2, indicated that the level of GSH in the serum of thalassemia patients is lower than that of healthy individuals (control group). These findings are consistent with those of the researcher Hadeer Hayder [24]. Furthermore, these results align with the findings of researchers Salih and Khalid and their group [25], who reported a significant decrease in the concentration of glutathione GSH in the serum of thalassemia patients compared to the glutathione levels in the serum of healthy individuals (control group). The results of this study are also in agreement with the study conducted by Attia and his team [26], where they found a reduction in the concentration of glutathione GSH due to excessive production of hydrogen peroxide. Glutathione GSH serves as a key reductant within cells, making it highly sensitive to oxidative stress, and it has several important functions, such as protecting against oxidative stress, regulating gene expression, and reducing the activation of programmed cell death. Glutathione GSH is one of the essential antioxidants for recycling vitamins E and C, and it is very effective in helping the body combat free radicals. Thus, glutathione GSH participates in the cellular defense system against oxidative stress by detoxifying free radicals and reactive oxygen species. Therefore, the decreased level of glutathione GSH in thalassemia patients leads to increased cellular sensitivity to oxidative stress .

3.3 MDA Concentration Levels

MDA level was estimated in the blood of patients with thalassemia and the control group. The results showed an increase in the level of MDA in the serum of thalassemia patients. When a statistical comparison was made between the activity of MDA in the serum of thalassemia patients and the activity of MDA in the serum of apparently healthy individuals (the control group), it was found that there was a significant increase in the activity of MDA in people with thalassemia compared to the healthy group, as shown in Table 2 and Figure 3.

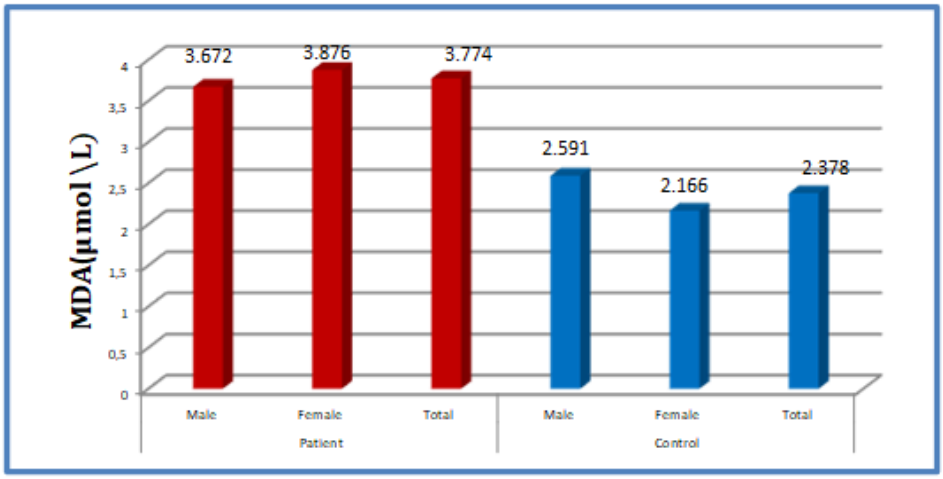


Figure 3: Comparison between different groups in MDA level.

The results of the current study, as shown in Table 2, indicated that the level of MDA in the serum of thalassemia patients is higher than the level of MDA in healthy individuals (control group). These findings are consistent with those reached by the researcher Lubis and his group [27], They investigated the levels of MDA in thalassemia patients dependent on blood transfusions, revealing that the MDA levels in these patients are significantly higher than those in healthy individuals due to the increased amount of iron in the patients, which enhances oxidative stress and leads to elevated MDA levels. Therefore, iron overload is a serious issue in transfusion-dependent thalassemia. Additionally, the current study results align with those of researcher ALAYUNT and his team [28], who found that MDA values are higher in thalassemia patients compared to the control group. It is believed that the reduced oxygen utilization capacity in thalassemia patients may play a role in the elevated levels of MDA. The oxidant and antioxidant properties were investigated in childhood thalassemia patients receiving regular blood transfusions and chelation therapy, allowing the researcher to find higher levels of MDA in thalassemia patients compared to the control group. The elevated levels of malondialdehyde MDA are considered a good indicator of oxidative damage in the blood of patients with thalassemia, as it is the end product of the oxidation of polyunsaturated fatty acids, which is commonly used as a marker for lipid peroxide levels. Therefore, the iron overload in thalassemia patients leads to tissue oxidative injury and an increase in MDA concentration. Thalassemia patients are primarily exposed to oxidative stress due to the accumulation of iron in their bodies. Thus, maintaining antioxidant systems could be beneficial in protecting thalassemia patients from more severe complications of the disease. Anemia in patients with thalassemia leads to hemolysis in the peripheral circulation and ineffective erythropoiesis, causing accelerated red blood cell death. The increase in iron resulting from continuous blood transfusions or oxidative stress in thalassemia patients leads to a significant consumption of antioxidants. Hence, the main goal of blood transfusion is to address anemia, suppress ineffective erythropoiesis, and inhibit the increased absorption of iron from the gastrointestinal tract, as iron accumulation can lead to toxicity and damage to the tissues where iron is deposited [29].

3.4 Estimating Vitamin D3 Levels

Vitamin D3 levels were estimated in the blood of patients with thalassemia and the control group. The results showed a decrease in the level of Vitamin D3 in the serum of thalassemia patients. When a statistical comparison was made between the effectiveness of Vitamin D3 in the serum of thalassemia patients and the effectiveness of Vitamin D3 in the serum of healthy individuals (the control group), it was found that there was a significant decrease in the effectiveness of Vitamin D3 in people with thalassemia compared to the healthy group, as shown in Table 2 and Figure 4.

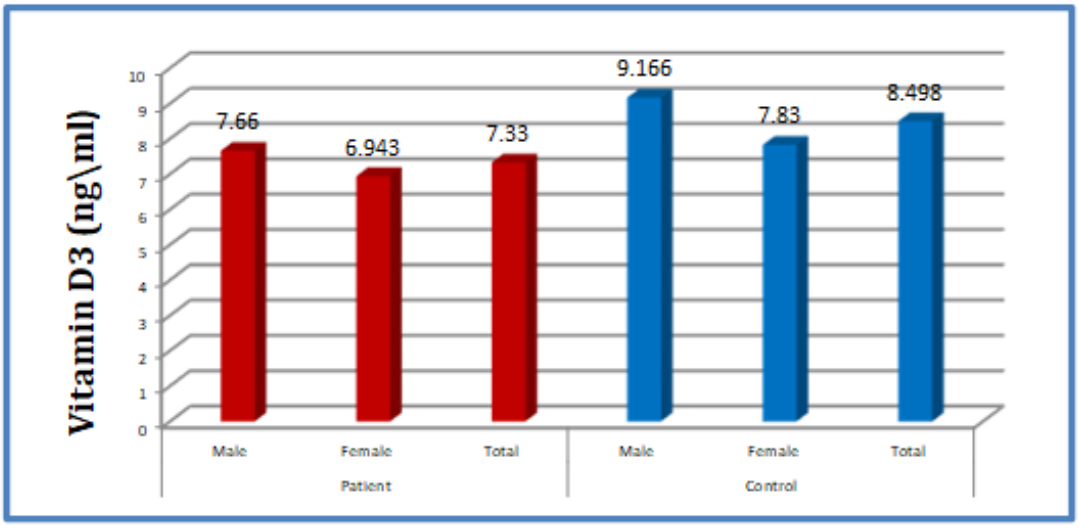


Figure 4: Comparison between different groups in terms of Vitamin D3 level.

The results of the current study, presented in Table 2, indicated that the level of Vitamin D3 in the serum of thalassemia patients is lower than the level of Vitamin D3 in healthy individuals (control group). These findings are consistent with those reported by researcher Alzubaidi and his team , who found a decrease in Vitamin D3 levels in thalassemia patients compared to the control group due to iron deposition in the liver and skin, which disrupts the process of converting Vitamin D from one form to another, leading to an increased risk of fractures and osteoporosis due to the regulatory effect of Vitamin D on bone cells. The results of the current study also align with those of researcher Al-Rubae and his team , indicating that thalassemia patients are at greater risk of Vitamin D deficiency due to limited sun exposure, low bone mass, impaired calcium metabolism, and increased iron absorption, which reduces calcium absorption since high ferritin levels interfere with the production of 25-hydroxy Vitamin D in the liver due to increased iron in the liver, negatively affecting bone metabolism who found that children with thalassemia receive regular blood transfusions at specified time intervals, which may lead to iron deposition in the liver, resulting in a decrease in the synthesis of Vitamin D-25OH [30], [31]. Anemia resulting from iron deficiency significantly impacts the intestinal absorption of fat-soluble vitamins such as Vitamin D. Consequently, patients with thalassemia show a notable deficiency in Vitamin D3 levels due to poor vitamin absorption and inadequate dietary intake necessary to maintain normal Vitamin D levels. This leads to liver disorders that hinder the liver's ability to convert Vitamin D into its active form, due to iron overload in the liver or improper functioning of endocrine tissues, resulting in the progression of clinical manifestations of Vitamin D deficiency. Vitamin D3 is considered an antioxidant that protects biological membranes and other cellular components from oxidative damage, which contributes to its depletion and reduced effectiveness in thalassemia patients due to iron deposition in the liver and their inability to convert Vitamin D3 into its active form. Moreover, low levels of Vitamin D3 in patients can significantly contribute to decreased bone mass and the onset of osteoporosis due to increased pressure on the outer membrane containing pain fibers, leading to generalized bone pain. It has been observed that elevated ferritin levels in patients correlate with reduced Vitamin D levels, which affects heart function in these patients [32].

3.5 Catalase Enzyme Concentration Levels

The level of CAT was estimated in the blood of patients with thalassemia and the control group. The results showed an increase in the level of CAT in the serum of thalassemia patients. When a statistical comparison was made between the activity of CAT in the serum of thalassemia patients and the activity of CAT in the serum of apparently healthy individuals (the control group), it was found that there was a significant increase in the activity of CAT in people with thalassemia compared to the healthy group, as shown in Table 2 and Figure 5.

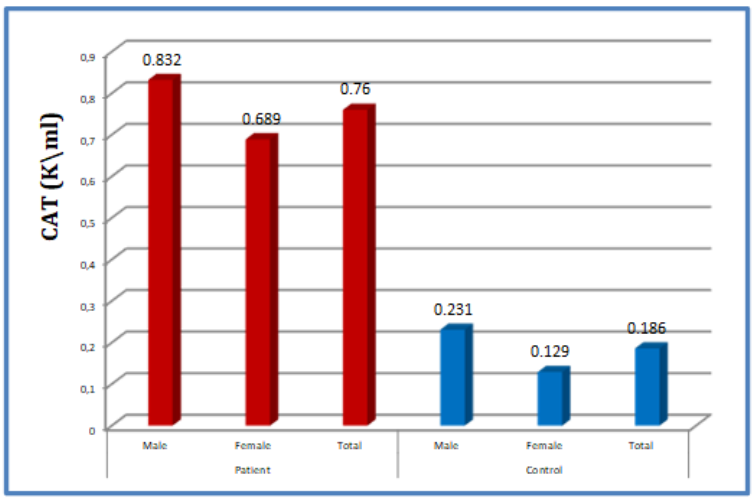


Figure 5: Comparison between different groups at the CAT level.

The results of the current study in Table 2 show that the level of CAT in thalassemia patients is higher than the level of CAT in healthy individuals (control group). These results are consistent with the findings of researcher Melo and his team [33], who observed an increase in catalase levels in patients compared to healthy individuals (control group) due to an increase in oxidative factors such as H2O2, causing the oxidation of components in red blood cells. The enzyme catalase is responsible for detoxifying cells and protects hemoglobin from oxidative damage. The elevated activity of catalase in red blood cells leads to increased levels of oxidative factors like H2O2, thus causing oxidation of cell components. An increase in levels of antioxidant enzymes such as CAT and glutathione peroxidase (GPX) in red blood cells of patients with mild thalassemia responds to the rising threat of oxidation, whereas in patients with severe beta thalassemia, the levels of antioxidant enzymes return to their normal levels due to the presence of normal red blood cells from multiple blood transfusions. It has been found that catalase occupies a place among the relevant biocatalysts due to its exceptional catalytic rate and thermal stability in destroying harmful hydrogen peroxide and producing water and oxygen in living systems by reducing free radical damage, making it an important biomarker. It has the highest turnover rate among enzymes, enhancing antioxidant defense systems in cells [34].

5 CONCLUSIONS

The findings of this study confirm that the flower bud extracts of *Rosa damascena*, particularly the ethanolic and aqueous forms, exhibit significant antibacterial activity against a range of clinically important Gram-positive and Gram-negative bacterial strains. Among the two, the ethanolic extract demonstrated superior inhibitory effects, notably against *Staphylococcus aureus*, *Serratia*, and *Pseudomonas aeruginosa*, suggesting that ethanol may be more efficient in extracting key antimicrobial phytochemicals.

The measurement of prolidase levels can be used as an initial indicator to identify individuals with thalassemia, and the elevated levels of prolidase in the serum of thalassemia patients contribute to iron balance. There is oxidative stress in the serum of thalassemia patients compared to healthy individuals, indicated by higher levels of MDA and lower levels of GSH, while CAT levels increased in thalassemia patients compared to the control group. Additionally, the levels of vitamin D3 decreased in thalassemia patients compared to healthy individuals. We can conclude that the significant increase in the levels of the studied biochemical indicators may be due to certain diseases, such as kidney, heart, and liver diseases, which are also characteristic features of beta thalassemia patients.

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Molecular Study of Multidrug-Resistant Integrons Associated with Acinetobacter baumannii Isolated from Clinical Samples

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Keywords: Acinetobacter baumannii, Antibiotic Resistance, Multidrug Resistance, Integrons, Clinical Isolates.

Abstract: The study investigated the prevalence of class one, two, and three integrons in A. baumannii isolates obtained from patients admitted to government hospitals in Diwaniyah (General Diwaniyah, Women and Children, al Hussein, and Burns Hospital) in Al-Qadisiyah Governorate, Iraq. From September 2024 to January 2025, 150 non-duplicate samples were collected, including 21 (14%) wound swabs, 36 (24%) burn swabs, 32 (21.33%) blood samples, 34 (22.66%) urine samples, and 27 (18%) tracheal swabs. After initial identification on MacConkey agar, blood agar, and chromium agar, the samples were chemically tested and Gram-stained. The VITEK2 compact system was used to confirm the diagnosis. This study showed that 117 (78%) of the samples showed bacterial growth, of which 40 (34.18%) were confirmed as A. baumannii, distributed as follows: 3 isolates (14.28%) from wounds, 6 isolates (16.66%) from burn swabs, 13 isolates (40.62%) from blood, 6 isolates (17.64%) from urine samples, and 12 isolates (44.44%) from tracheal swabs. The data indicated that tracheal swabs had the highest percentage of A. baumannii isolation, followed by blood, urine, burns, and wounds (44.44%, 40.62%, 17.64%, 16.66%, and 14.28%, respectively). Antibiotic susceptibility testing was performed using the Vitek device for 20 antibiotics. The purity and concentration of genomic DNA were measured using a Nanodrop instrument, and a conventional PCR reaction was set up to detect integrons using specific primers provided by Promega. The products were analyzed by agarose gel electrophoresis. The highest prevalence was found for class I integrons (23 samples, 57.5%), followed by class III (9 samples, 22.5%), and class II (6 samples, 15%) out of the 40 A. baumannii-positive samples.

1 INTRODUCTION

Acinetobacter baumannii is one of the most significant challenges facing healthcare facilities and hospitals worldwide, as very few effective antibiotics are available to treat the conditions it causes. It has attained therapeutic significance due to its exceptional capacity to acquire or control resistance factors, establishing it as one of the most notable multidrug-resistant (MDR) organisms. Discontinue the existing antibiotic treatment owing to the increasing incidence of antimicrobial resistance [1].

Acinetobacter baumannii surfaced as one of the most aggressive and dangerous organisms in ESKAPE, an acronym for the group of microorganisms that includes(Enterococcus faecium, Staphylococcusaureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonasaerugnosa, and Enterobacter species) [2]. Due to its introductory or easy accession of several mechanisms of resistance to multiple antibiotics and its veritably long survival in the terrain, survival indeed in dry environmental elements is linked to its capability to form biofilms.This point, together overuse of antibiotics and rwiththe magpie, has allowed A. baumannii to live and acclimatize impeccably to healthcare surroundings, therefore representing an important source of spread of this opportunistic bacterium [3] Acinetobacter baumannii, classified as an ESKAPE pathogen, accounts for 2-10% of clinical Gram-negative infections. It induces complications in long-term hospitalized persons and those who are immunocompromised. It is often established as colonies in the esophagus and respiratory tract. It can beget sanitarium- acquired pneumonia and generally targets wettish apkins similar as mucus, bloodstream infections, and meningitis [4]. To date,thir were multi classes of integrons have been described in colorful Gram-negative bacteria, and these play a crucial part in the rearrangement and spread of resistance genes. All integrons consist of two primary components: a conserved 5' segment, which includes the Int.ase gene, and the major integration site (attI), which has a unique 3' conserved segment. The most common

introns are first class, and integrons have an important part in the emergence of multidrug resistance patterns through the development of antimicrobial resistance in Gram-negative bacteria [5]. Class I integrons comprise three open reading frames in the 3' conserved sequence(3' CS) region. Class II integrons, whose conserved 3' member contains five tns genes, are responsible for the movement of transposable rudiments, generally associated with the Tn7 transposable element family [6], Class 3 introns have also been reported, but their conserved 3' member has not been characterized.[7] This work aims to investigate the frequency of class one, two, and three integrons obtained from A. baumannii isolates [8].

2 MATERIAL AND METHODS

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2.1 Patient

A total of one hundred fifty different clinical samples were collected from visitors and hospitalized patients in governmental hospitals in Al-Qadisiyah province. (Al- Diwanyiah Teaching Hospital, Maternary and Children Teaching Hospital and Al-Diwanyiah Burns Center), during the period from septmber/ 2024 to jenuary /2025. The clinical specimens were randomly collected from patients, and checked to recognize Acinetobacter baumannii isolates in this cross-sectional study. which included 21 (14%) wound swabs, and 36 (24%) burn swabs, 32 (21.33%) blood samples, 34 (22.66%) urine samples and 27 (18%) tracheal swabs were collected for analysis. These patients were males and females of different age groups, ranging from 1 month to 80 years including 26 males and 14 females.

2.2 Isolation and Identification of Isolates

Traditional methods have been used to identify bacteria, as well as by the phenotype of the causative organism, using bacteriological methods, including culture on selective media,Gramstaining, microscopic,characteristics,colony morphology,and biochemical identification tests. A. baumannii can be difficult to identify using traditional culture media, especially In this study, Acinetobacter chromium agar was used, which is a selective medium used for the accurate and rapid detection of A. baumannii, as it grows in the form of red colonies after an incubation period of 24 hours.[9].

2.3 Primers DNA Extraction and Polymerase Chain Reaction

Using the NCBI Genbank database, PCR primers were designed in this study, as shown in Table 2.

By using (Presto™ Mini gDNA Bacteria Kit) Bacterial genomic DNA was extracted from bacterial isolates as and done according to company instructions gene was calculated by using Optimase Protocol Writer™ online application and done by PCR thermocycler. See table 1.

2.4 Antibacterial Agents Susceptibility of A. baumannii Isolates

The isolates of Acinetobacter baumannii were subordinated to antibiotics vulnerability test by using Vitek2 compact system. All isolates were examined against 20 antibiotics agents related to 8 antibiotic classes. According to Clinical and Laboratory Norms Institute (CLSI 2024).

2.5 Ethics in Study Management

The present study has been managed according to recommendations companion gained from the College of biology, University of Al- Qadisiyah. The task of collecting samples from rehabilitated cases was eased according to an sanctioned executive order (numbered as 30/4161 in 1/9/2024) issued by the College of biology, University of Al- Qadisiyah, and it was approved by the directors of visited hospitals. The study did n't include interdicted natural accoutrements or genetically modified organisms.

Table 1: Thermocycler PCR reaction condition.

NO	Integron name	PCR Amplicon (bp)	Initial denaturation Temp./time	Denaturation Temp./time	Annealing Temp./time	Extension Temp./time	Cycles	Final extension Temp./time
1	*IntI-I*	243	95°C /5 min.	95°C/30sec	52.7°C/30sec	72°C/1min.	35	72°C/5min.
2	*IntI-II*	788	95°C /5 min.	95°C/30sec	55.2°C/30sec	72°C/1min.	35	72°C/5min.
3	*IntI-III*	600	95°C /5 min.	95°C/30sec	56.7°C/30sec	72°C/1min.	35	72°C/5min.

Table 2: The Primers Sequences used in this study.

Primer	Sequence (5'-3')	Product Size	Reference orGenbank
IntI-I	F- TCTCGGGTAACATCAAGG	243	10
	R- AGGAGATCCGAAGACCTC		
IntI-II	F- CACGGATATGCGACAAAAAGG	788	11
	R TGTAGCAAACGAGTGACGAAATG		
IntI-III	F- AGTGGGTGGCGAATGAGTG	600	11
	R- TGTTCTTGTATCGGCAGGTG		

3 RESULTS

3.1 Isolation of Bacterial Growth in Clinical Specimens

An aggregate of one hundred fifty different clinical samples were collected from callers and rehabilitated cases in governmental hospitals in Al- Qadisiyah fiefdom.(Al- Diwanyiah Teaching Hospital, Maternary and Children Teaching Hospital and Al-Diwanyiah Burns Center), during the period from septmber/ 2024 to jenuary/ 2025. The clinical samples were aimlessly collected from cases, and checked to fete Acinetobacter baumannii isolates in thiscross-sectional study.

The results in the current study revealed that 147 (81.6) samples had been given positive growth while 33(18.3) samples showed no growth asappeare (Figure 1).

3.2 Identification of A. baumannii Isolates

The confirmatory diagnosis of the suspected isolates was made by using the GN ID Card of the VITEK 2 compact system, (about 99% accuracy).The results confirm that all 40 (34.18%) of the collected isolates were identified as Acinetobacter baumannii isolates . According to the definite diagnosis of A. baumannii isolates , they distributed as showed in Figure 2. The number and percentage of A. baumannii isolates recorded in Table was 40 isolates distributed as 3 isolates (14.28%) from wound ,6 isolates (16.66%) from burn swabs , 13 isolates(40.62%) from blood, 6 isolates (17.64%) from urine samples and 12 isolates (44.44%) from trachea swabs, however, the data showed the rise percentage of A. baumannii were isolated are blood samples , trachea swabs followed by urin, bourn and followed by wound (44.44%),(40.62%),(17,64%),(16.66%)and (14.28%) respectively.

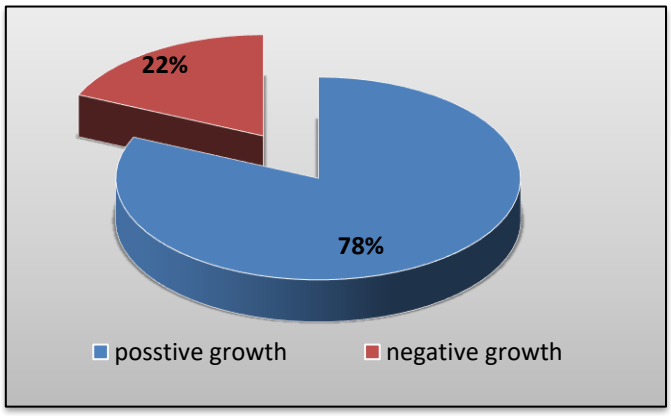


Figure 1: The percentages of clinical specimens.

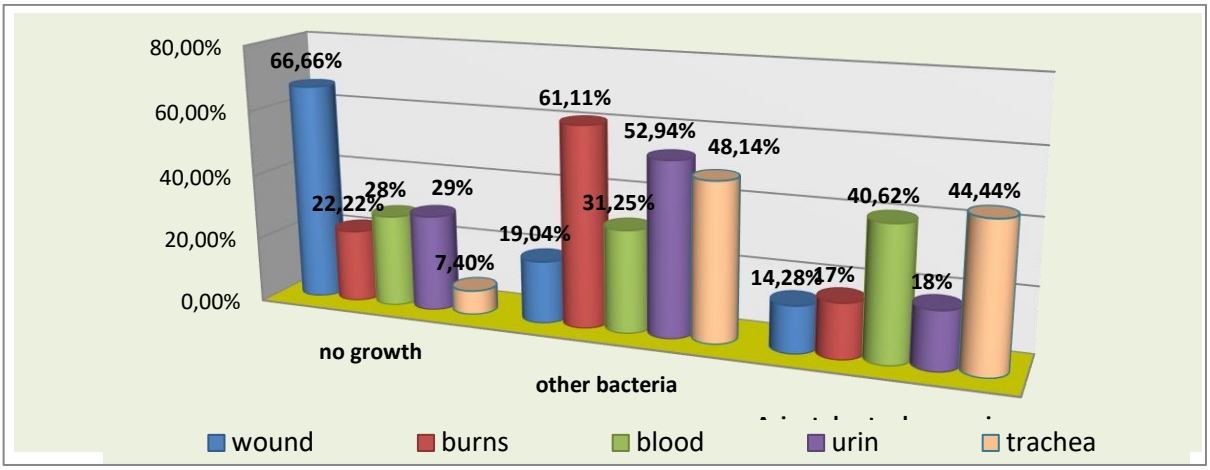


Figure 2: Distribution of bacterial growth according to source of samples.

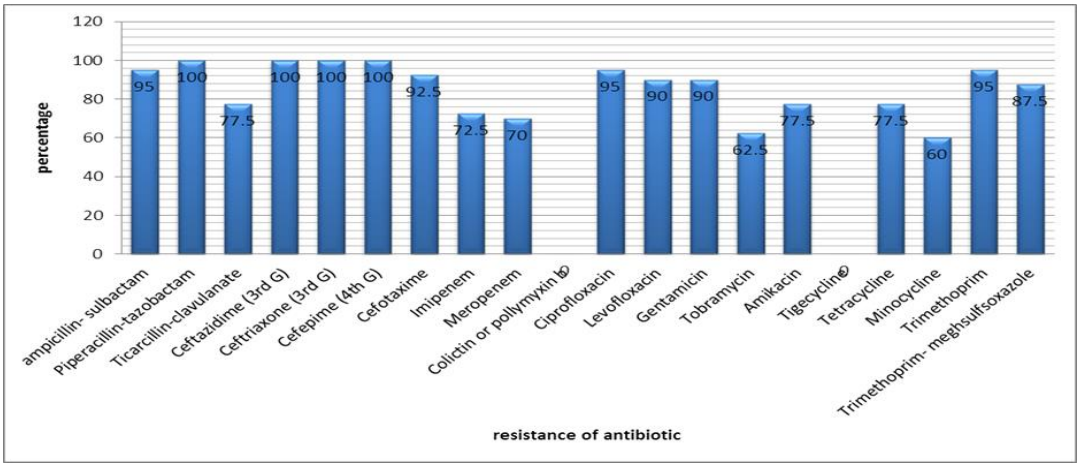


Figure 3: Antibiotics susceptibility pattern of 40 A. baumannii isolate.

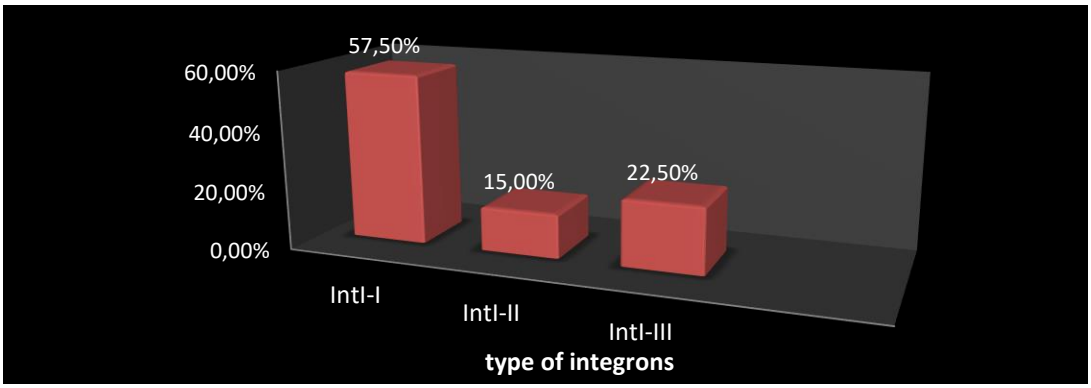


Figure 4: Distribution of integrons of 40 A. baumannii isolates.

3.3 Antibacterial Agents Susceptibility for Isolates of A. baumannii

Forty Acinetobacter baumannii isolates underwent antibiotic susceptibility testing with the Vitek2 compact system. recommendations all results were interpreted and all isolates were classification as very susceptible, intermediate, or resistant to each tested antibiotics agent show as Figure 3 . The antibiotic susceptibility test revealed the maximum resistance (100%) was recorded for Cephalosporins (3th and 4th G) and for penicillin (Piperacillin) . There was growing resistance to carbapenems, Imipenem (82.5%), and Meropenem (70%). According to present results the isolates showed a considerable resistance to Levofloxacin and Ciprofloxacin (95%). (90%). For Ampicillin/Sulbactam (95%), Piperacillin/Tazobactam (100%) and for Ticarcilline/Clavulanic Acid the resistance rate were (77.5%). The lowest resistance rates were found to found minocycline (60%) and tobramycin (62.5%). While antibiotics that are very sensitive are tigecycline (0%) and colistin or pollymyxin(0%).

4 DISCUSSION

The current study suggest that the percentage of A. baumannii isolated from the trachea and blood samples were (44.44%), (40.62) respectively. it differs from where's a study that obtained highest percentage (72.2%) of A. baumannii isolates from burn patients ,otherwise in Egypt, a study documented the prevalence of A. baumannii in patients with burns and wound infections were (3.8% and 4.8%) respectively,in this study the percentage of A. baumannii isolated from burn and wound swabs were (16.66%) and (14.28%) respectively [12]. this result in concordance with local study done in Al-Diwanyiah city who documented that A. baumannii isolated from burn and wound swabs were (7.36%) and (3.63%) respectively [13]. Moreover Previous local study had documented that A. baumannii rates isolated from burn and wound swabs were (36.5%) and (34.1%).While, A. baumannii isolation rates from burns and wound were recorded as (12.5% , 8.5%) in Baghdad Hospitalsand (2.8% and 0.6%) in Babylon [14].

A. baumannii can cause a wide variety of opportunistic infections due to its ability to infect virtually anywhere in the human body when the host's immune system is compromised, making it dangerous especially for the elderly and patient in ICUs [15]. additionally, patients with immunocompromised Due to weak immunity in some patients, they are more susceptible to the risk of developing urinary tract infections, which may be asymptomatic, as urinary tract infections can develop into symptomatic infections and eventually into bacteremia and then sepsis, which may lead to death [16].

PCR was used to detect several classes of integrons. produced by the genes encoding produced by the genes encoding Class 1 Integrons were found in 23 (57.5%) out of 40 samples, class 2 Integrons in 6 (15%) out of 40 samples, class 3 Integrons in 9(22.5%) out of 40 samples (Fig. 4). Although the emergence of multidrug resistance in A. baumannii isolates is highly complex and requires further study to understand the mechanism, it can be linked to the fact that one of the ways bacteria acquire antibiotic resistance is through resistance genes located on integrons. Transposable elements among bacteria facilitate the dissemination of antibiotic resistance,

especially in Gram-negative bacteria. Integrons were identified by the amplification of an internal segment of the intronase gene in 77.5% (31 of 40) of A. baumannii isolates, suggesting that these elements are prevalent among multiresistant isolates of this species. Among the 40 clinical isolates, Class 1 introns, recognized as the predominant category of mobile introns in multidrug-resistant Acinetobacter baumannii clinical strains, have been validated globally.

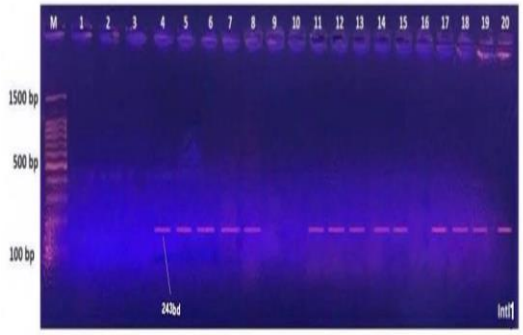


Figure 5: Distribution of class 1 integron (243 bp) among 40 A. baumannii isolates.

Figure 5 shows the frequency of the first class integrons among the isolates. The result of the polymerase chain reaction (PCR) was 243 base pairs. 23 samples appeared to carry the first integron out of 40 samples.

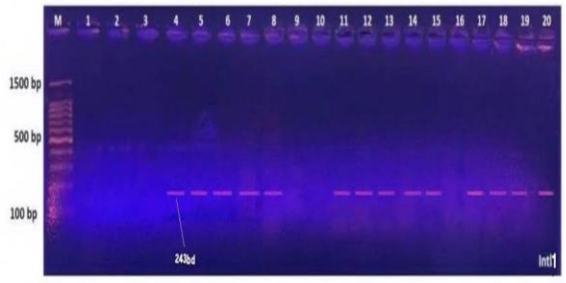


Figure 6: Distribution of class 1 integron (243 bp) among 40 A. baumannii isolates.

Figure 6 shows the frequency of the first class integrons among the isolates. The result of the polymerase chain reaction (PCR) was 243 base pairs. 23 samples appeared to carry the first integron out of 40 samples.

The current analysis revealed the presence of class 1 integrons in clinical isolates of A. baumannii, aligning with prevalence rates documented in other locations, such as Poland, where the occurrence of class 1 integrons was 63.5%, and Taiwan. The present investigation indicates a prevalence of class 1 integrons at 57.5%. Nevertheless, it significantly exceeds the percentages seen in Turkey (6.4%) and Iran (7.5%). 22 Substantial and notable percentages have also been documented in Korea (89.3%) and Egypt (85%) (18). This data indicates that class 1 integrons are the most common and extensively dispersed among multidrug-resistant Acinetobacter baumannii in the critical care units of Diwaniyah hospitals. Iraq. 2(5) samples contained both class I and class II integrins. While 5(12.5) samples contained both class I and class III integrins. The spread of integrins and their appearance in further than one class may be related to the increase in antibiotic resistance of Acinetobacter baumannii and the expansion of the range of resistance, as the maturity of samples came XDR.

A research on MDRAB in Brazil identified class 2 integrons in 23 samples, aligning with the present work that discovered 6 class 2 integrons in A. baumannii strains. Taheri-Kalani's research examined the prevalence of class 1, 2, and 3 integrons in A. baumannii isolates in Tehran, revealing that class 2 integrons were present in 14 samples.

Despite contradicting the findings of the present research, which revealed the existence of class 2 integrons in a restricted number of multidrug-resistant A. baumannii strains, this strain was recognized as the predominant type in investigations by Mirnejad (82) and Kamalbeik (67.5) (20). It also contradicts studies conducted in Korea, China, Poland, Iran, and Thailand could not identify any class 3 integrons. In the present investigation, we detected class 3 integrons with a prevalence of 22.5%. A. baumannii isolates in our present investigation conducted in Iraq [21].

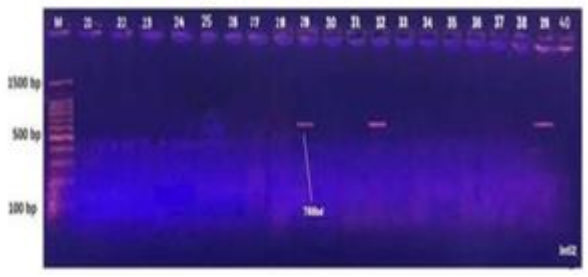


Figure 7: Distribution of class 2 integron (788 bp) among 40 A. baumannii isolates.

Figure 7 shows the frequency of the first class integrons among the isolates. The result of the polymerase chain reaction (PCR) was 788 base pairs. 6 samples appeared to carry the second integron out of 40 samples.

5 CONCLUSIONS

The studies demonstrate the presence of integrons, namely class II and I integrons harboring antibiotic resistance genes, which are instrumental in the virulence characteristics of multidrug resistance and extended drug resistance (XDR) in Acinetobacter baumannii.

A. baumannii was isolated from burns, wounds, and urine samples using conventional methods and the Vitek2 compact system. Antibiotic susceptibility and multidrug resistance tests were performed on the isolates. Most isolates were found to be extensively resistant (XDR) and resistant to most available antibiotics, with the exception of tigecycline and colistin, which are susceptible. This resistance may result from the acquisition of integrons containing antibiotic resistance genes. The majority of positive isolates were obtained from patients in intensive care units and premature infants. This is attributable to the bacteria's adaptation and capacity to endure prolonged periods on desiccated surfaces, including instruments, materials, and devices, as well as the utilization of ventilators, catheters, and incubators for premature infants. In addition, the infection may be transmitted from healthcare workers.

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Molecular Detection of the Relationship between Biofilm Production and Antibiotic Resistance in Staphylococcus Haemolyticus

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Keywords: Antibiotic Resistance, Biofilm, Staphylococcus Haemolyticus, Virulence Factors.

Abstract: To evaluate antibiotic resistance, biofilm formation, and the detection of virulence-associated genes this study was conduct. Clinical specimens samples were obtained from patients in Mosul city. susceptibility of Antibiotic was assessed using the Kirby-Bauer method with nineteen antibiotics. Biofilm production was tested using the tube adherence and microtiter plate methods. PCR was used to identifying virulence and resistance-related genes. Results showed a 94.7% resistance to ampicillin/cloxacillin and ceftriaxone, and 100% sensitivity to vancomycin and nitrofurantoin, with variability in resistance to other antibiotics. Biofilm formation was observed in all isolates. The PCR results showed that the SH gene was present in all isolates. The mecA gene, linked to methicillin resistance, was found in 88.88% of isolates, the tetK gene (tetracycline resistance) in 83.33%, and the ermC gene (erythromycin resistance) in 26.31%. Virulence factor genes, hla and fnbB, were detected in 100% and 73.68% of isolates, respectively. The findings highlight the significant antibiotic resistance, biofilm-forming ability, and horizontal gene transfer in Staphylococcus haemolyticus, emphasizing the global concern of multidrug-resistant bacteria.

1 INTRODUCTION

Bacterial strains that are resistant to antibiotics pose a major risk to human health. Fewer antibiotics are still effective against infectious diseases due to rising antibiotic resistance in common human infections [1]. In ordinary clinical care, coagulase-negative staphylococci (*CoNS*) are among the most commonly recovered microorganisms. Over the past few decades, their prevalence has risen gradually in tandem with medical advancements, particularly with regard to the use of foreign body devices [2]. *CoNS* have become important pathogens in nosocomial settings due to their function in preserving homeostasis. Numerous research have looked into the molecular causes of this development and have found several potential virulence factors related to the pathogenicity of *S. aureus* [3].

A vital component of the Staphylococcus genus, *S. haemolyticus* is important in Healthcare-associated illnesses due of its great resistance to drugs like methicillin and its ability to produce biofilms [4]. Is a nosocomial infection-causing acquired opportunistic pathogen (5). Compared to other coagulase-negative staphylococci, *S. haemolyticus* strains, especially those causing hospital-acquired infections, are more resistant to antibiotics. There is compelling evidence that it can transfer resistance genes to other types of staphylococci. Meningitis, endocarditis, prosthetic joint infections, bacteremia, sepsis, peritonitis, and otitis media are among the severe infections associated with this bacterium, especially in individuals with weakened immune systems [6]. Because a strain that is resistant to various drugs is becoming a serious hazard in healthcare facilities around the world [7]. It also plays a significant function in the propagation of resistance genes, which can lead to the production of more virulent and an epidemic clones [8], [9].

The exceptional ability to develop antibiotic resistance, especially against oxacillin, limits the available treatment options for catheter-associated infections that do not respond to methicillin. Additionally, this may increase the risk of sepsis and raise the morbidity and mortality rates of patients [10]. The existence of insertion sequences *(Iss)* in this organism facilitates gene addition and deletion, promoting frequent genomic rearrangements that enable the acquisition of drug resistance genes, hence enhancing adaption and survival in hospital environments [11]. Is well known for its capacity to form biofilms, which are crucial for the transmission of disease [12]. The formation of biofilms, which contribute to hospital-acquired infections such as

catheter-associated diseases, is the main characteristic of pathogenic isolates. Additionally, they secrete substances called hemolysins, enterotoxins, and fibronectin-binding proteins that enhance bacterial adhesion and invasion [6]. In the synthesis stage of biofilm formation, the fibronectin binding proteins (*fnbB*) are crucial. By homophilic contacts or protein binding to receptors on the surface of nearby cells, *fnbB* facilitates the formation of biofilms [13].

The abundance of insertion sequences and resistance to many drugs are two characteristics of its genome that cause hospital infections, this strain has been linked to an increase in infections and cases of multidrug resistance, which makes it a serious concern to public health [14], [15]. Given its remarkable adaptability and capacity for survival in hospital settings, particularly on medical equipment, *S. haemolyticus* emerges as a key contributor to nosocomial infections brought on by multiresistan [16]. Although being, the second most normally discovered *CoNS*, little is known about its virulence factors and antibiotic resistance.

Clarifying antibiotic patterns and molecular classification of resistance and virulence factor genes in *S. haemolyticus* isolates from different clinical samples is the goal of this investigation.

2 MATERIAL AND METHODS

2.1 Study Design

Isolation and Identification: 450 samples were collected from various medical sources, including urine, blood, sputum, and eye swabs, and different ages of both females and males from Ibn Al-Athir Hospital for Children and Ibn Sina General Hospital for the period from July 2024 to November 2024.

2.2 Methods

Traditional method: Using the streaking technique from the clinical sample, the organism is isolated or cultured on a solid medium containing agar and blood. Staphylococci can be isolated from samples that may contain other microbes using mannitol salt agar with 7.5% sodium chloride, which facilitates the growth of salt-resistant staphylococci [17]. After incubating them aerobically for 24 hours at 37 degrees Celsius, the isolates are stained with Gram stain and subjected to standard biochemical tests, including catalase, oxidase, DNase, coagulase, VP, nitrate, PYR, urease, novobiocin, and polymyxin B sensitivity [18].

Confirmed method: Commercial biochemical test systems like VITEK2 species-level identification. Established biochemical techniques and recently created substrates serve as the foundation for the GP identity card. Forty-three biochemical assays are used to measure resistance, enzymatic activity, and carbon source utilization.

Antibiotic susceptibility test: Nineteen tablets of antibiotics were used (Bioanalyses) : Penicillin G (10 µg), Tetracycline (30 µg), Clindamycin (2 µg), Vancomycin (30 µg), Meropenem (10 µg), Ceftriaxone (30 µg), Cefotaxime (10 µg), Ceftazidime/clavulanic acid (30/10 µg), Cefoxitin (30 µg), Trimethoprim (10 µg), Azithromycin (15 µg), Levofloxacin (5 µg), Oxacillin (1 µg), Amikacin (10 µg), Ampicillin/Cloxacillin (25/5 µg), Amoxicillin (25 µg)., Gentamicin (10 µg), Nitrofurantoin (100 µg), and Fusidic acid (10 µg).

Methicillin resistance was identified through preliminary phenotypic viewing for diminished sensitivity of the isolates to cefoxitin (30 µg) and oxacillin (1 µg) disks (Mast, UK) utilizing the disk diffusion method, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (2023) [19] standards.

Biofilm formation assay was performed in accordance with the: a) Tube Adherence Test (TM). Brain Heart Infusion (BHI) broth was inoculated with tested strains and incubate at 35°C for 2 days. Test tubes were dyed with crystal violet, and experiments were conducted three times, categorized as nonexistent, mild, moderate, and powerful [20].

Biofilm formation test using Microtiter Plate: Bacterial isolates are added to brain heart infusion medium, incubated for 24 hours at 37°C. fill the first well of the first row with 200 microliters of the negative control, or non-bacterial brain-heart infusion medium. Then, 200 microliters of the bacterial suspension are added to the first three wells of the third row. The wells are cleaned with saline solution, stained with 0.1% crystal violet, and then diluted with 99% ethanol. The optic-density (OD) is v using ELISA equipment at a wavelength of 630 nano-meters [21].

Molecular identification of genes linked to antibiotic resistance and pathogenicity: The genetic DNA of all *S. haemolyticus* strains under investigation was extracted using the Gene aid kit (Taiwan) the recommended steps from the manufacturer were followed. After that, Measurements were made of the genetic DNA's content and purity [22]. To identify the relevant genes in the genetic DNA of *S. haemolyticus*, six sets of primers were used to confirm the bacteria at the

genetic level using the *SH* gene and to identify the virulence factors *fnbB*, *hla* and the antibiotic resistance genes *mecA*, *tetK*, *ermC*, as shown in Table 2.

The (PCR) was conducted utilization the *GoTaq* G2 Green Master Mix from Promega (USA) in a 20-microlitre reaction volume for molecular identification. We added 200 nanograms of DNA template, with a primer concentration of 2 micromoles each, next the producer guidance. The PCR produce were divided on a 2% agarose gel and staining with red safe dye. A 100 base pair DNA ladder from New England Bio Labs, UK.

2.3 PCR Conditions

The PCR program including an primary denaturation at 95°C for 3 min., following by denaturing at 94°C for 45 seconds, annealing at the temperatures specified in Table 1, and expansion at 72°C for 45 seconds. A final extension step was performed at 72°C for 3 minutes.

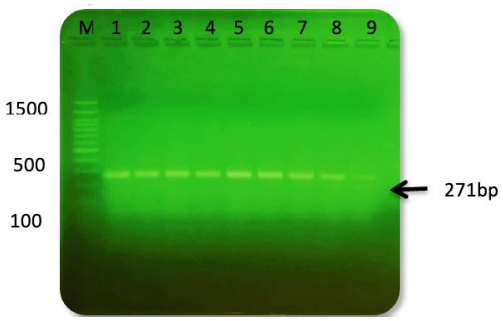


Figure 1: Agarose Gel electrophoresis for SH gene in S.haemolyticus .Lane (M): p DNA Lanes (1-9)PCR product of SHgene (271 bp).

Table 1: Biofilm production method.

Step	Description
1. Bacterial Culture Preparation	The isolates were grown in TSB medium at 37°C for 24 hours.
2. Inoculation	A standardized bacterial suspension (0.5 McFarland) was prepared and inoculated into 96-well polystyrene plates.
3. Incubation	Plates were incubated at 37°C for 24-48 hours.
4. Washing	Wells washed for 3 times by PBS
5. Staining	Biofilms were stained with 0.1% crystal violet for 15 minutes.
6. Washing & Drying	Excess stain was removed, and plates were air-dried.
7. Quantification	Absorbance was measured at 570 nm to determine biofilm formation levels.

Table 2: Primer DNA sequences used in this study.

Gene	Sequences (5'-3')	Product Size (bp)	Annealing Temperature (°C)	Source of Primer
SH	F: GGTCGCTTAGTCGGAACAAT R: CACGAGCAATCTCATCACCT	271	52	(Pereira et al., 2010)
mecA	F: TAGAAATGACTGAACGTCCG R: TTGCGATCAATGTTACCGTAG	154	50	(Pereira et al., 2010)
tetK	F: GTAGCGACAATAGGTAATAGT R: GTAGTGACAATAAACCTCCTA	360	46	(Manoharan et al., 2021)
ermC	F: GCTAATATTGTTTAAATCGTCAATTCC R: GGATCAGGAAAAGGACATTTTAC	572	47	(Manoharan et al., 2021)
hla	F: TGGGCCATAAACTTCAATCGC R: ACGCCACCTACATGCAGATTT	72	53	(Pinheiro et al., 2015)
fnbB	F: TAAATCAGAGCCGCCAGTGGAG R: GTCCTTGCGCTTGACCATGTTC	416	57	(Eltwisy et al., 2021)

3 RESULTS

Number of 450 clinical samples yielded nineteen isolates (4.4%) were positive for S. haemolyticus. The growth of the bacteria on blood agar is characterized by opaque white colonies surrounded by a zone of complete hemolysis.

All isolates were negative for oxidase, DNase, and coagulase tests, variable in mannitol fermentation, VP, nitrate, and PYR tests, positive for the catalase test, sensitive to polymyxin B, and susceptible to novobiocin. The results were confirmed using the VITEK2 technique.

All isolates identified by morphological and biochemical methods were confirmed by molecular methods using the SH diagnostic gene for S. haemolyticus bacteria, as shown in Figure 1.

3.1 Antibiotic Susceptibility

The findings demonstrated a distinct variation in the isolates' resistance to the employed antibiotics (see Fig. 2). The isolates showed resistance to ampicillin/cloxacillin and ceftriaxone at a rate of (94.7%), fusidic acid (89.4%), azithromycin (84.2%), gentamicin (78.9%), cefotaxime (73.6%), penicillin G and tetracycline (63.1%), levofloxacin, ceftazidime/clavulanic acid, and amikacin (57.8%), amoxicillin (52.6%), clindamycin (15.7%), and meropenem (10.5%). While the isolates were all susceptible to the medicines nitrofurantoin and vancomycin, some isolates exhibited multidrug resistance (MDR), especially in those that showed resistance to methicillin.

Eighteen strains (94.73%) produced biofilms in the tube out of 19 isolates, according to the results of the biofilm formation test conducted using the tube adhesion method. The isolates' capacities to form biofilms differed; twelve isolates formed the biofilm in a moderate manner, while six isolates were classified as weak or strong. Additionally, the microtiter Plate method was used to examine S. haemolyticus capacity to produce biofilms, and 17 out of 19 isolates (89.47%) had favourable results. When compared to the control sample, the results showed varying degrees of biofilm formation.

3.2 Molecular Studies

As for the virulence genes used in the study, found that 19 out of 19 isolates tested positive for the hla gene, which is 100% (see Fig. 3). This is consistent with the phenotypic characterization of S. haemolyticus that produces complete hemolysis on blood agar, The results of their study were (94.7%) and (100%) respectively for the appearance of the hla gene.

As for fnbB gene it was found 14 out of 19 isolates test positive with a gene appearance rate of 73,68% out of a total of 19 isolates. Detailed results for fnbB gene detection are shown in Fig. 4. Eight positive isolates of the mecA gene were detected out of nine resistance isolates, with the gene appearing at a rate of 88.88%. (see Fig. 5)

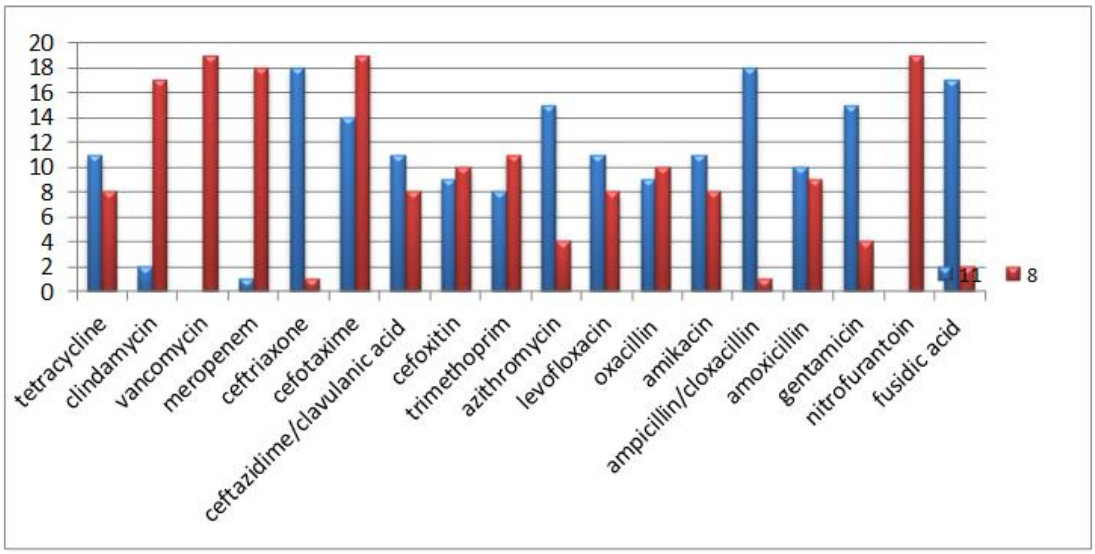


Figure 2: Antibiotic resistance profiles of Staphylococcus haemolyticus Isolate.

About 10 of the 12 resistance isolates tested positive for the tet-K gene, representing 83.33% (see Fig. 6).

Nineteen isolates the *ermC* gene was detected in around 5 of them, with a frequency of 26.31% (see Fig. 7).

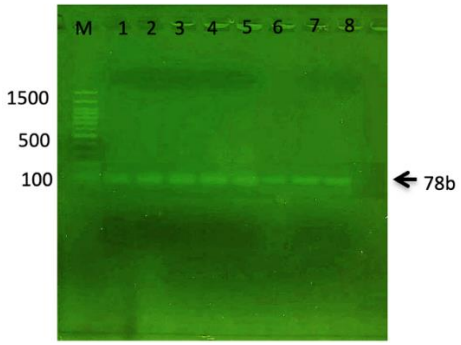


Figure 3: Agarose Gel electrophoresis for hla gene in S. haemolyticus. Lane(M):100 bp DNA ladder Lanes (1-8) PCR product of hla gene (78 bp).

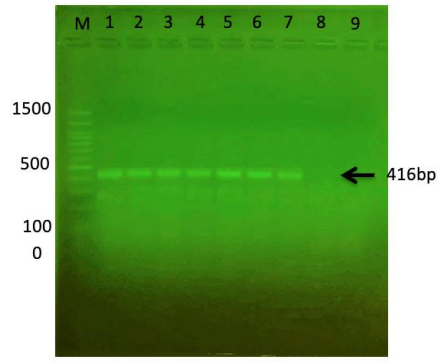


Figure 4: Agarose Gel electrophoresis for fnbB gene in S.haemolyticus. Lane(M):100 bp DNA ladder Lanes (1-9) PCR product of fnbB gene (416 bp).

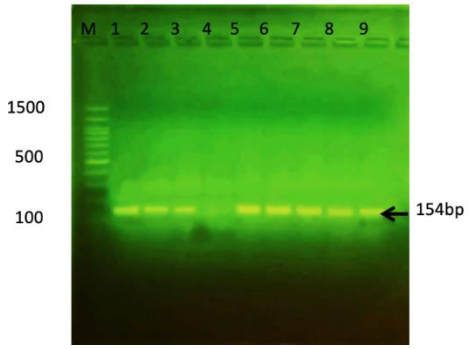


Figure 5: Agarose Gel electrophoresis for mecA gene in S.haemolyticus. Lane(M):100 bp DNA ladder ,Lanes (1-9) PCR product of mecA gene (154 bp).

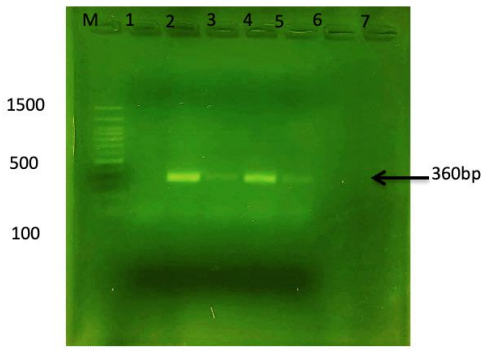


Figure 6: Agarose Gel electrophoresis for tet k gene in S.haemolyticus. .Lane(M):100 bp DNA ladder (BioLabs), Lanes (1-7)PCR product of tet k gene(360 bp).

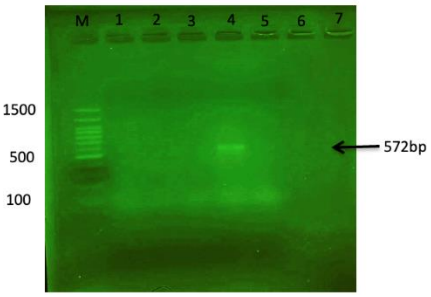


Figure 7: Agarose Gel electrophoresis for ermC gene in S.haemolyticus.Lane(M): 100 bp DNA ladder (BioLabs),Lanes (1-7)PCR product of ermC gene (572bp).

3 DISCUSSION

The research shows a link between the development of biofilms and resistance to antibiotics in *S. haemolyticus*, reinforcing its identity as a bacteria that's hard to treat with multiple drugs. The elevated levels of resistance seen against ampicillin/cloxacillin and ceftriaxone point to a hurdle in therapy treatmen = [23] .Potential therapeutic options are available, due, to the effectiveness of vancomycin and nitrofurantoin [24]. Most of the isolates having mecA suggest a methicillin resistance mechanism widely, which complicates treatment approaches even more [25]. The discovery of tetK and ermC genes displays how S.haemolyticus can resist tetracyclines and macrolides effectively [26]. The impressive capability of forming biofilms, in each isolate boosts the survival and resilience of bacteria by facilitating the transfer of resistance and virulence genes, like *hla* and *fnbB* [27], [28].

These results highlight the importance of enhancing infection prevention practices and exploring treatment approaches, for addressing

multidrug *S.haemolyticus* infections. The significant increase, in resistance levels seen in *S. haemolyticus* strains to drugs like β lactams and aminoglycosides underscores the escalating problem of drug resistance, in environments [29,30]. The fact that many people respond well to vancomycin and nitrofurantoin indicates that these antibiotics continue to be treatment choices [31], [32]. The significant link, between methicillin resistance and multi drug resistance (MDR) underscores the importance of *mecA* mediated resistance. The findings, on biofilm formation show that the majority of strains are capable of sticking and creating biofilms. This process boosts the lasting power of bacteria and their resistance, to antibiotics [33], [34].

The differences, in how biofilms are formed were observed in both the tube adhesion and microplate methods. This indicates that certain strains could be more concerning in infections because they show stronger adherence capabilities [35], [36]. These discoveries highlight the importance of exploring treatment approaches and implementing infection control measures, against infections caused by *S. haemolyticus* due the protective function of biofilms for bacteria, against the bodys immune system and antibiotics. The molecular study shows that *S. haemolyticus* isolates contain a number of genes related to virulence and resistance [37], [38]. The widespread identification of the *hla* gene corresponds with the behavior noted on blood agar plates. This highlights its significance, in causing disease [39,40]. The occupancy of the *fnbB* gene, in 73.68 % of the isolation indicates an ability, for adhesion and biofilm creation boosting bacterial survival and potential for causing infections. The significant occurrence of the *mecA* gene, at 88% in methicillin strains provides more evidence for the connection, between creating biofilms and resistance to multiple drugs [41], [42]. The identification of *tetA* (83%) and *ermB* (26%) genes illustrates how *S. haemolyticus* can withstand tetracyclines and macrolides respectively, in settings. These discoveries highlight how crucial it is, for surveillance practices observing resistance mechanisms and applying infection control strategies, against *S. haemolyticus* related infections [43], [44].

5 CONCLUSIONS

This research emphasizes the levels of resistance pattern, towards antibiotics and the ability of S. haemolyticus strains from samples in Mosul to form biofilms efficiently. These strains exhibited resistance against β lactams and aminoglycosides well as macrolides but displayed consistent susceptibility towards vancomycin and nitrofurantoin. The presence of mecA,tetK and ermC genes authenticates their resistance against drugs; meanwhile virulence factors such as genes likehlaand fnbbcontribute towards their nature. These discoveries highlight the importance of implementing infection control procedures and exploring alternative therapeutic approaches, for tackling infections associated with S.haemolyticus.

Results showed a 94.7% resistance to ampicillin/cloxacillin and ceftriaxone, and 100% sensitivity to vancomycin and nitrofurantoin, with variability in resistance to other antibiotics. Biofilm formation was observed in all isolates. The PCR results showed that the SH gene was present in all isolates. The mecA gene, linked to methicillin resistance, was found in 88.88% of isolates, the tetK gene (tetracycline resistance) in 83.33%, and the ermC gene (erythromycin resistance) in 26.31%.

ACKNOWLEDGMENT

All thanks for University of Tikrit for their supporting of this work.

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Investigating Myo-Inositol Oxygenase and Renal Biomarkers in Acute Kidney Injury

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Keywords: Acute Kidney Injury, Myo-Inositol Oxygenase, Kidney Functions, Kidney Diseases.

Abstract: Acute kidney injury (AKI) refers to an abrupt renal damage episode or failure, which can occur within a few hours or days. Myo-inositol oxygenase (MIOX) is an enzyme involved in the breakdown of myo-inositol. MIOX catalyzes the oxidation of myo-inositol to form D-glucuronate, an important step in the metabolism of myo-inositol. Research has suggested potential links between MIOX and kidney function. The objective of this study to show the role of MIOX on kidney diseases. Methods: Case-control study was conducted in Anbar Dialysis Center. 50 participants suffering from AKI and the other group of 50 participants of healthy participants. Demographic information for both groups was obtained (age, Body Mass Index "BMI", duration of disease), in addition to laboratory tests (urea, creatinine, uric acid, calcium, and MIOX). Groups were compared using the t-test and descriptive analysis. Pearson's correlation coefficient was used to find the relationship between different variables. Results: In this case-control study Urea, creatinine, and uric acid have higher concentrations and are statistically significant in acute kidney injury cases than in healthy controls. In addition to a decrease in the concentration of calcium and MIOX with high significant differences between the two groups. Conclusion: Acute kidney injury is associated with an elevation in urea, creatinine, and uric acid and a decrease in calcium and MIOX. Studies have shown that MIOX expression levels are up-regulated in response to renal injury, suggesting a role for this enzyme in the pathogenesis of AKI.

1 INTRODUCTION

Acute kidney injury (AKI) is when kidneys suddenly stop working properly. It can range from minor loss of kidney function to complete kidney failure. AKI normally happens as a complication of another serious illness [1]. MIOX is a renal-specific, proximal tubule protein that is increased in the plasma of animals and critically ill patients with AKI. MIOX preceded the elevation in Serum creatinine by approximately two days in human patients [2]. Myo-inositol oxygenase (MIOX) is an enzyme that plays a critical role in inositol metabolism by catalyzing the oxidative cleavage of myo-inositol to glucuronic acid. While the understanding of inositol and its metabolic pathways has advanced in recent years, the specific involvement of inositol oxygenase in Acute Kidney Injury (AKI) is an emerging area of research [3]. Recent studies have indicated that alterations in inositol metabolism, particularly changes in the activity of inositol oxygenase, may contribute to the pathogenesis of AKI. Dysregulation of inositol metabolism has been implicated in oxidative stress and inflammatory responses, both of which are key contributors to the development and progression of AKI [4].

In this study, we investigated the role of MIOX enzyme in the diagnosis of AKI as an early biomarker as compared with diagnostic parameters such as urea, creatinine, uric acid, and calcium.

2 METHODS

2.1 Study Design

This study was prepared during the period from November 2023 to February 2024. 100 people participated in it. They are split into two groups. The first group consisted of 50 patients with Acute Kidney Injury, (male and female) to evaluate levels of Urea, Creatinine, Calcium, Uric acid, and Myo-Inositol Oxygenase. Inclusion criteria included patients with abdominal pain or typical symptoms suggestive of Acute Kidney Injury who presented to the Dialysis Center, Anbar Health Department Depending on the diagnosis, the diagnosis was based on clinical presentation and history of AKI, which was confirmed by Ultrasound, CT-Scan, and Lab tests. The exclusion criteria were patients with liver diseases, renal transplant, and pregnant women. The second group included 50 controls (male, female), Healthy people without any disease, and was parameters were measured like the previous group (cases). The age period for the case study was 30-70 years and for the control group was 26-65 years. This study received ethical approval from the University of Anbar, Ethical Approval Committee, (Project No. 06) on 06/07/2024.

2.2 Procedure

A venous blood sample was collected in position from the anticubital vein of each case and control subject. Four milliliters of blood samples from patients (cases) and control. The blood samples were collected in a gel tube and left for 20 minutes at room temperature. After coagulation, sera were separated by centrifugation at 2000 xg for 10 min and divided into small aliquots. Immediate measurements of Creatinine, Uric acid, Calcium, and urea, were done using appropriate enzymatic and colorimetric methods. The rest of the sera was stored at -20 ˚C until assayed for serum Myo-Inositol Oxygenase it was measured using enzyme-linked immunosorbent assay (ELISA) kits by ELK Biotechnology kit (Lot No. 46661314).

2.3 Statistical Analysis

The statistical analysis was done by Statistical Package for the Social Sciences (SPSS) and used a descriptive model to show the results of demographic information. In addition, a paired t-test was conducted to find significant differences between the variables of the study groups. Also, person correlation to find the relation between study parameters. The receiver operating characteristic (ROC) curve and the area under the curve (AUC) were used to assess the specificity and sensitivity of the study parameters.

3 RESULTS

A total sample size of 100 was collected, out of which 50 were cases and 50 were controls, and the data obtained was subjected to statistical analysis. The results are presented under the headings of various parameters considered for this study.

Table 1 and Figures 1 and 2 show demographic information for the sample study for case and control groups. To find the comparison between Age and BMI, a paired t-test and found Mean± SD with a range of values. The significant differences between groups were determined with confidence interval (CI) (99.9) and probability (less than 0.01). The results showed the mean ± SD of age for cases was 51.9 ± 11.8 years with a range (30-70) years while the mean ± SD for the control group was 44.1±11.8 years and the range was (26-65) years with high significant differences between the groups of the study and a p-value (0.002). Also, the results of BMI showed that the control group had a high mean± SD value of 25.5± 3.96 with a range (17.7-35.1) while the case group had a BMI mean± SD 24.1± 3.64 and the range was (18.6-34.5) with p= (0.049).

The distribution of sex and duration of disease for the study samples (cases and controls) groups was analyzed by using frequency analysis. The result of sex showed the male participant was 24 samples with a percentage (48%) for patients and 29 samples with percent (52%) for the control group. In addition, the female participants for patients was 26 samples with percent (52%), and for the control 21 samples with percent (42%).

Duration of disease is very important to be noted and studied because it determines whether the disease is in the acute stage or chronic stage. The study was designed to take the duration of the disease by weeks. Table 1 showed that 17 samples of the study samples had a duration of disease with a range from 1 to 4 weeks, also 8 participants had a duration of disease from 5 to 8 weeks. 18 samples had a duration of disease from 9 to 12 weeks and at last 7 participants had more than 12 weeks of disease duration.

Table 1: The sociodemographic information (age, sex, BMI and duration of disease for the sample study.

Characteristics		Cases	Controls	P-value
Age (Years)	Mean ± SD	44.1 ± 11.8	51.9 ± 11.8	0.002
	Range	30 - 70	26 - 65	
Sex [n(%)]	Male	24 (48%)	29 (58%)	0.390 NS
	Female	26 (52%)	21 (42%)	0.390 NS
	Total sample size	50 (100%)	50 (100%)	
BMI (Kg/m2)	Mean ± SD	24.1 ± 3.64	25.5 ± 3.96	0.049
	Range	18.6 – 34.5	17.7 – 35.1	
Duration of disease (weeks) [n(%)]	1-4	17 (34%)	0	
	5-8	8 (8%)	0	
	9-12	18 (36%)	0	
	More than 12	7 (14%)	0	
Statistically significant if P<0.01; *P values from unpaired t-test (Age, BMI). *P value from Z test for two proportions (sex) Duration of disease (AKI) for cases without a control group.				

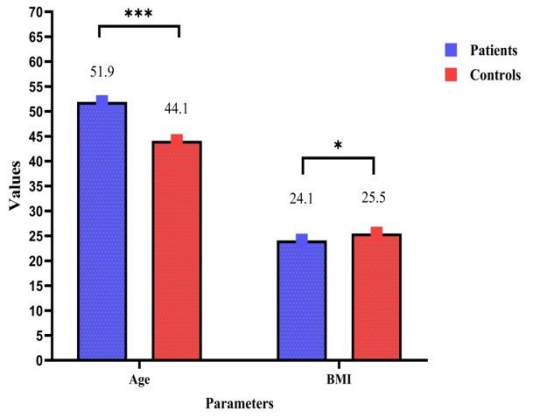


Figure 1: The mean and p-value of age and BMI for study samples.

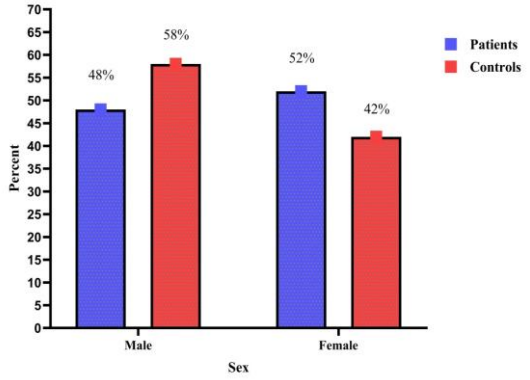


Figure 2: The distribution of Sex for study samples.

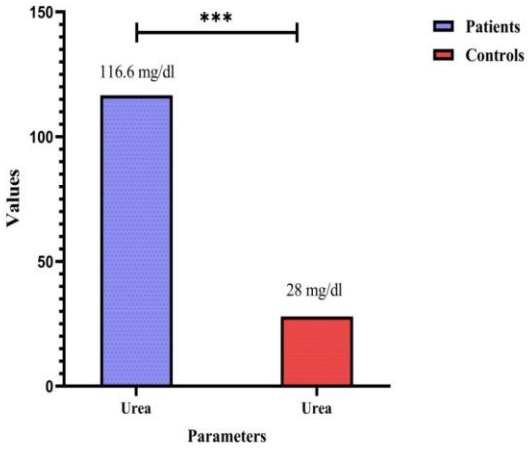


Figure 3: The mean and p-value of Blood Urea for study samples.

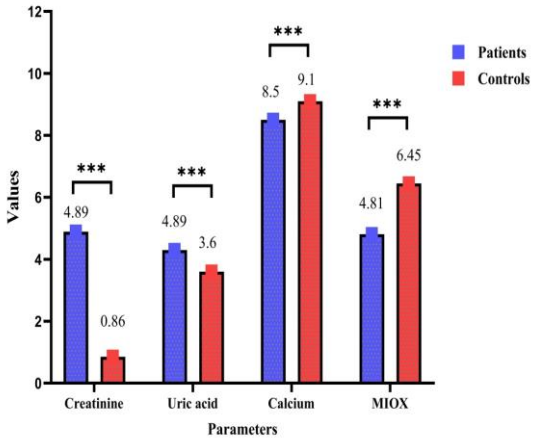


Figure 4: The mean and p-value of serum Creatinine, Uric acid, Calcium, and MIOX for study samples.

Table 2: The Mean ± SD, range, and differences of study parameters among study groups.

Characteristics		Case	Control	P-value
Urea (mg/dl)	Mean ± SD	116.6 ± 45.9	28.0 ± 5.3	0.0001
	Range	62.00 - 241.00	19.00 - 37.00	
Creatinine (mg/dl)	Mean ± SD	4.89 ± 2.2	0.85 ± 0.21	0.0001
	Range	2.0 – 10.6	0.50 – 1.20	
Uric acid (mg/dl)	Mean ± SD	4.3 ± 1.2	3.6 ± 0.43	0.001
	Range	2.9 – 7.8	3.0 – 4.7	
Calcium (mg/dl)	Mean ± SD	8.5 ± 0.53	9.1 ± 0.71	0.0001
	Range	7.9 – 10.0	8.0 – 11.0	
MIOX (ng/ml)	Mean ± SD	4.81 ± 1.7	6.45 ± 1.3	0.0001
	Range	2.0 – 7.5	4.1 - 9.2	
Statistically significant if P<0.05; *P values from unpaired t-test (Urea, creatinine, uric acid, calcium, MIOX).				

Table 2 in addition to Figures 3 and 4 shows the mean, standard deviation (SD), and range with Asymptotic significant differences for Urea, Creatinine, Uric acid, Calcium, and Myo-Inositol oxygenase (MIOX) for cases and control group.

The result appears that urea concentration was higher in cases than controls with mean±SD (116.6±45.9) and the range of value was (62-241 mg/dl) while the control group had mean±SD (28±5.3) and range (19.0-37.0 mg/dl) with high significant differences and p = (0.0001). Serum creatinine is considered an indicator of kidney diseases and is used as a diagnosis parameter for acute and chronic kidney injury [5]. The result in Table 2 showed the mean±SD for cases was (4.89±2.2) and range (2.0-10.6 mg/dl) while the mean±SD for the control group was (0.85±0.21) and the range was (0.5-1.2 mg/dl) with significant differences and p = (0.0001)

The metabolism and excretion of uric acid were done inside the kidney. Uric acid is a waste product that's from purine breakdown, so when the kidney has an injury it leads to decreased excretion of uric acid and concentration elevated in blood circulation [6]. The result of a recent study showed that the mean±SD concentration of uric acid for patients was (4.3±1.2) with range (2.9-7.8 mg/dl), while the mean±SD concentration for the control group was (3.6±0.43) and the range was (3.0-4.7 mg/dl) with significant differences and p = (0.001).

Most percent of total calcium in the human body is reabsorbed by kidney tubules. Kidney injury or any disease that affects on kidney leads to decreased reabsorption of calcium to blood and decreased concentration in the body [7]. The study result showed that the mean±SD concentration of calcium decreased in cases (8.5±0.53) and the range was (7.9-10.0 mg/dl) while the mean±SD concentration in the control group was (9.1±0.71) and the range was (8.0-11.0 mg/dl) with high significant differences and p = (0.0001).

Myo-inositol oxygenase (MIOX) is mainly expressed in the kidneys. There is little information available on the function of the MIOX enzyme in the diagnosis of AKI, even though it is one of the rare organ-specific enzymes, specifically a renal-specific enzyme [2]. The study results showed that the mean±SD of MIOX for patients was less than the cases group (4.81±1.7) with range (2.0-7.5 ng/ml) while the mean±SD concentration for control groups was (6.45±1.3) and the range was (4.1-9.2 ng/ml) with high significant differences between the study groups and the p = (0.0001).

The study results showed a high correlation between blood urea and serum creatinine with r = 0.466 and p = (0.0001). In addition, the uric acid concentration relation has an inverse correlation with age which means the concentration decreases with age increase with r= -0.421 and p = (0.001). The explanation for this result is that kidney function decreases when age increases same thing with all body organs. The study found also a high correlation between uric acid and urea for AKI patients with r = 0.492 and a p = (0.0001). On the other side, the result showed a high correlation between uric acid and creatinine with r=0.618 and p = (0.0001). Myo-inositol oxygenase (MIOX) concentration showed a correlation with creatinine and uric acid with r = 0.256, 0.302, and p = (0.037 and 0.017) respectively. The results of the correlation are clarified in Table 3.

Table 3: Spearman's ρ correlation coefficient between AKI clinical parameters, biochemical parameters, and MIOX.

Parameters Patients	Pearson r	P-Value
Urea - Creatinine	0.466**	0.0001
Uric acid - Age	- 0.421**	0.001
Uric acid - Urea	0.492**	0.0001
Uric acid - Creatinine	0.618**	0.0001
MIOX - Creatinine	0.256*	0.037
MIOX – Uric acid	0.302*	0.017
**Correlation is significant at the 0.01 level (1-tailed). * Correlation is significant at the 0.05 level (1-tailed). NS: Non-significant		

Table 4: Receive Operator Curve analysis for study parameters to diagnose AKI.

Parameter	Area-under curve	Specificity	Sensitivity	Cut-off value	Sig.
MIOX	0.738	78%	58%	6.41	0.0001
Urea	1.0	100%	100%	49.5	0.0001
Creatinine	1.0	100%	100%	1.6	0.0001
Uric acid	0.666	98%	38%	4.25	0.0001
Calcium	0.188	100%	0 %	12.0	0.004

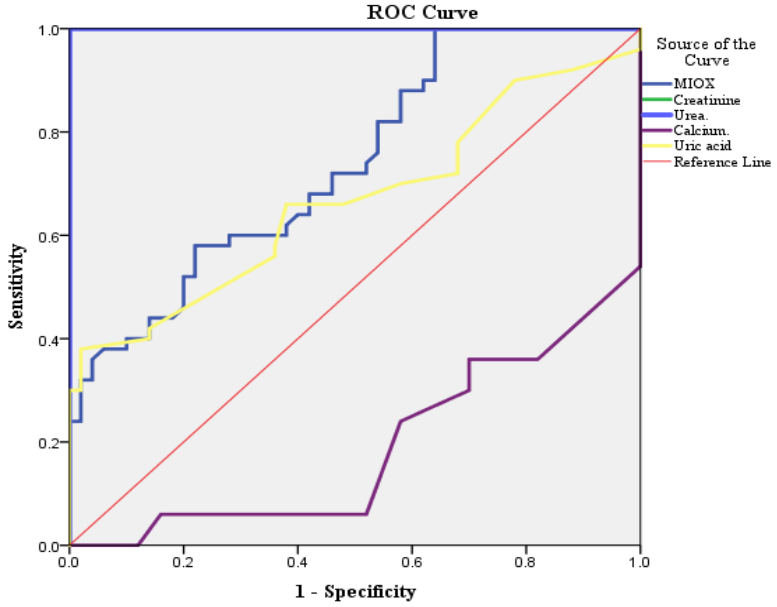


Figure 5: The ROC curve, specificity and sensitivity for Urea, Creatinine, uric acid, MIOX, and calcium among study groups.

The receiver operating characteristic (ROC) curves were generated for MIOX and other parameters to give an initial assessment of critical values for a diagnosis of AKI. As shown in Table 4 and Figure 5, the area under the curve (AUC) was calculated as 0.738 for MIOX with a cut-off value (6.41) and significant differences with p = (0.0001). Other parameters showed highly significant differences for AUC with different Specificity and sensitivity. The cut-off value indicates that the value is considered as critical value for acute kidney injury disease.

4 DISCUSSION

Our study showed that progress in age leads to an increased probability of acute kidney injury. Acute

kidney injury (AKI) is becoming more common in people of all ages, but older patients (those over 65) are more vulnerable to developing AKI because of the age-related kidney damage that affects their structure and function, their decreased renal reserve, the presence of comorbidities, and their diminished capacity to recover [8]. This study corresponds with our study.

There are multiple possible processes explaining the correlation between a high body mass index and the development of AKI. Initially, obesity leads to certain hemodynamic alterations in the glomerulus, including glomerular hyper perfusion and hyper filtration because of compromised natriuretic-related renin and angiotensin system activation [9]. Second, obesity can lead to a decrease in the number of functional nephrons in obese patients by increasing the hemodynamic and metabolic load on each glomerulus [10]. Third, in obese patients, adipocytes may serve as a location of production for oxidative stress and activated inflammatory cytokines [11].

Paller et al showed that the duration of ischemia for 60 minutes was sufficient for ischemia-reperfusion injury to occur. Serum BUN and creatinine levels indicated adequate renal damage because they were higher in all ischemia-reperfusion groups as compared to the control group [12]. This study agrees with our study results.

The concept that uric acid, an ancient biological component, may be causing inflammatory pathways that exacerbate acute kidney damage has led to the resuscitation of uric acid as a possible mediator of acute kidney injury (AKI) [13]. Mild hyperuricemia has since been shown to have pro-inflammatory and anti-angiogenic properties [14]. Renin-angiotensin system activation, increased reactive oxygen radicals, inflammatory mediators (MCP-1, ICAM), vascular responsiveness, and vascular smooth muscle proliferation and migration are all brought on by uric acid [15]. There is a study that proves mounting evidence that uric acid is a potential causative agent in AKI. Uric acid may increase the risk for AKI via both systemic effects of hyperuricemia and local effects due to crystalline and non-crystalline effects of urinary uric acid on tubules [16]. A linear correlation has been observed recently between the development of dialysis-dependent AKI during hospitalization and blood uric acid levels [17]. All previous studies correspond with our study that was conducted on AKI patients and appears that uric acid as a waste product increases in AKI patients.

The findings of a study conducted by Thongprayoon, C. et al. showed a significant correlation between the risk of AKI in the hospital and the blood calcium level of cases admitted into the hospital. Additionally, it has been suggested that abnormality in calcium levels has an impact on vascular tone, especially renal arteries [18]. This study agrees with the results obtained in our study. Prior research has indicated the risk of AKI in patients with different serum calcium levels [19], [20]. Low serum ionized calcium might be related to the severity of the illness or sepsis [21], [22]. The illness-related release of inflammatory cytokines reduces the parathyroid gland's ability to secrete parathyroid hormone (PTH) and increases end-organ resistance to PTH [23]. Furthermore, calcitriol production is also suppressed during this severe illness [24].

Myo-inositol oxygenase is a novel biomarker that may be used as an indicator for AKI. Our study found that MIOX is decreased in patients and this study agrees with the previous study [25], [26]. The study done by Cuma Mertoglu, et al. found MIOX values were significantly higher in the AKI group compared to the control group with a P = (0.0016) [25]. This study was conducted with our result. Gaut et al. demonstrated that MIOX is a renal-specific proximal tubule protein using the Western blot approach. In the present investigation, we also found that the MIOX levels in the AKI group were considerably higher. Furthermore, for AKI, MIOX's diagnostic sensitivity and specificity were 53.8% and 81.5%, respectively. Naturally, this outcome is crucial for using MIOX in AKI diagnosis. Nonetheless, as MIOX is an enzyme found in the proximal kidney tubules, MIOX elevations may result from any type of tubular injury [2]. Previous studies agree with this study's results. Another recent study by Tominaga, T. et al. has shown that overexpression of MIOX is responsible for the overproduction of reactive oxygen species (ROS), which contributes to tubulointerstitial injury in obesity [27]. Moreover, in a new study, MIOX overexpression increased renal injury in diabetic nephropathy due to oxidative stress, increased ROS, and led to the disruption of antioxidant systems [28]. Moreover, MIOX expression has been reported to be low in extra-renal organs with diabetic complications such as neuropathy, retinopathy, and cataracts [29]. The study of (Tom Jose Kakkanattu, et.al,) found that serum MIOX levels and Urine Protein Creatinine Ratio (UPCR) upon hospital discharge may be utilized alone or in combination with other variables, such as serum creatinine, as a predictive biomarker for renal recovery from community-acquired AKI [26].

4 CONCLUSIONS

This study highlights the clinical significance of Myo-Inositol Oxygenase (MIOX) as a potential biomarker for acute kidney injury (AKI), alongside traditional renal function indicators such as urea, creatinine, uric acid, and calcium. The findings clearly demonstrate that patients with AKI exhibit significantly elevated levels of urea, creatinine, and uric acid, and significantly decreased levels of calcium and MIOX compared to healthy individuals. These patterns affirm the role of oxidative stress and impaired renal filtration in AKI pathogenesis. The decline in MIOX concentration among AKI patients is particularly noteworthy, considering MIOX is a kidney-specific enzyme expressed in proximal tubules. Although earlier studies indicated MIOX overexpression in renal stress models, our findings align with clinical observations where severe or sustained AKI may lead to tubular injury and enzyme depletion or impaired release. The ROC analysis confirms the diagnostic utility of MIOX, showing moderate sensitivity and good specificity, indicating its value in supporting early detection strategies for AKI when combined with traditional markers.

ACKNOWLEDGMENTS

The authors acknowledge all participants in the study, in addition to the University of AlMaarif to provide the necessary assistance in providing all the needs to complete this study.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study received ethical approval from the University of Anbar, Ethical Approval Committee, (Project No. 06) on 06/07/2024.

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Cytokines and Kidney Function Biomarkers in Entamoeba Histolytica-Infected Patients

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Keywords: Entamoeba Histolytica, IL-1β, IL-8, IFN-γ, Renal Biomarkers, Amebiasis.

Abstract: This study investigates the immune response and kidney function in individuals infected with Entamoeba histolytica in Kirkuk, Iraq. Samples were obtained from hospitals and private laboratories between October and December 2024. Among 220 stool samples analyzed for E. histolytica cysts and trophozoites, 130 were excluded due to chronic or concurrent infections. The remaining participants were categorized into two groups: 70 infected individuals and 20 healthy controls. Blood samples were assessed for cytokine levels (IL-1β, IL-8, IFN-γ) and kidney function biomarkers (Creatinine, Urea, BUN). Statistical analysis using SPSS revealed significant elevations ($p \leq 0.05$) in IL-1β, IL-8, Creatinine, Urea, and BUN levels in infected individuals, suggesting immune activation and potential kidney function impairment. However, no significant differences were noted in IFN-γ levels or the BUN/creatinine ratio. These findings highlight the impact of parasitic infections on immune regulation and kidney health, underscoring the need for improved diagnostic and therapeutic strategies in endemic regions.

1 INTRODUCTION

Entamoeba histolytica, a tissue-invasive protozoan, is considered one of the anaerobic intestinal parasites responsible for approximately 50 million infections worldwide, with a mortality rate exceeding 1,00,000 annually [1]. The parasite invades the intestinal mucosa, causing amoebic colitis and potentially liver abscesses [2]. Amoebic infection is the third leading cause of parasitic-related deaths globally and in Iraq after malaria and schistosomiasis [3]. The infection is widespread in Kirkuk [4] and in developing countries such as Bangladesh, India, tropical African nations, and certain regions of Mexico and Brazil [5]. It is increasingly observed among the populations of both developing and developed countries, including the United States and European nations, due to international travel and migration from endemic areas [6]. Most parasitic infections tend to be asymptomatic. However, common symptoms associated with them include abdominal pain, discomfort, vomiting, and bloody diarrhea [7]. In severe cases, the infection can lead to complications such as gastroenteritis, malnutrition, and malabsorption. Children are more susceptible to intestinal protozoan infections, primarily due to the poor social and economic conditions of individuals, which is considered the main factor contributing to the spread of intestinal parasites [8]. When Entamoeba histolytica infects the host, the parasite binds to the epithelial layer of the colon and the mucosal layer through the secretion of Gal/GalNAc lectin by the parasite's trophozoite cells. After attachment, the parasite begins to degrade the epithelial cells using hydrolytic enzymes and surface proteins, particularly cysteine proteases[9]. This leads to the infection of intestinal cells and the destruction of extracellular matrix components. The parasite enters the initial defence against parasitic conditions, particularly the mucosal gut layer [10]. Interleukin-1 beta (IL-1β) is a basic inflammatory cytokine that contributes to the regulation of inflammation and immune system responses [11]. A group of stimuli, including lipopolysaccharides (LPS), TNF-α, and other signaling molecules, stimulates activated macrophage cells and various immune cells to produce it [11], [12]. E. histolytica leads to amoebiasis diseases, leading the colon epithelial cells to produce IL-8. Amoebic elements and substances released post-cell lysis may facilitate this process by improving the ejection of IL-8 mRNA [12].

The inflammatory reaction and tissue damage in gastrointestinal amoebiasis are mainly started by IL-8, which is vital in drawing neutrophils to the site of disease [13], [14]. Consequently, IL-8 acts an essential part in the pathophysiology of amoebiasis by connecting the virulence of E. histolytica with the immune response [15]. The aim of this study was to assess the effect of amino dysentery infection on some immune variables, such as Beta-1 Beta (IL-1β), Interleukin-8 (IL-8), and Interferon Gamma (IFN-γ). In addition, the study aims to assess the kidney function in individuals with Amoebic infection by measuring the levels of urea, creatinine, urea nitrogen in blood (BUN), and BUN/Creatinine.

2 MATERIALS AND METHODS

2.1 Study Population and Sample Collection

Initial study samples (stool) collected from 220 individuals during the period from October 2024 to December 2024 of both genders (aged 18-65 years) who visited Kirkuk hospitals and private laboratories in Kirkuk.

2.1.1 Inclusion Criteria

The study included 90 participants after excluding 130 cases based on predefined criteria. Inclusion required: (1) signed informed consent, (2) varying gastrointestinal symptoms (diarrhea, abdominal pain, dysentery), and (3) microscopic confirmation of Entamoeba histolytica cysts or trophozoites. All demographic and clinical data were meticulously documented before analysis.

2.1.2 Exclusion Criteria

Participants were excluded from the study if any of the following criteria matched:

1) Diagnosis of chronic metabolic disorders (diabetes, chronic renal insufficiency).
2) The presence of synchronous infection (bacterial, viral, or parasitic confirmed by laboratory tests).
3) Using antibiotics or immunosuppressants within 4 weeks before collecting samples.

These criteria aimed to reduce confusing variables in analysis of cytokines and kidney function. The final number of samples suitable for analysis was stabilized to 90 valid samples. Samples are kept in sterile containers with tightly closed covers to maintain the safety of the sample and maintain moisture. A medical syringe was used to take 90 samples of blood, which were then placed in the test tubes with gel. After that, Samples were centrifuged at 3000 ×g for 15 min at 4°C (Eppendorf 5804 R, Germany) with a balanced rotor configuration. Serum was aliquoted and stored at -20 °C (Thermo Scientific) for ≤2 weeks, avoiding freeze-thaw cycles..

2.2 Microscopic Examination

To determine the cystic and trophozoite stages in samples of stool, the samples were examined both directly and through a light microscope. The samples were divided into two groups: 70 samples from parasite-infected patients and 20 samples from healthy individuals. To make sure participant diversity and more accurately reflect the disease's local prevalence in the area, samples were taken from both public hospitals and private laboratories. A wooden stick was used to take a faeces sample, that was then put on a glass slide with a drop of regular saline. After that, a cover slip was set at an angle to keep air bubbles from developing. The microscope was used to examine the slide, first at a small magnification (10X) and then at high magnification. The exact same steps were employed for making a second slide, and one droplet of Lugol iodine stain—which was previously prepared using Luna's method (1968)—was added. This was accomplished through the addition of 10 grammes of iodine crystals after five grammes of potassium iodide had been dissolved in 1000 millilitres of distilled water. After slowly stirring the mixture until it was dissolved, it was filtered and put in sterile, tightly-sealed containers. The Entamoeba histolytica (tissue-invasive protozoan) parasite's cyst stage was coloured by the pigment. The samples were taken from several parts of the sample in order to improve the possibility of locating it.

2.3 Laboratory Analyses

2.3.1 Cytokine Measurement (ELISA)

The Enzyme-Linked Immunosorbent Assay (ELISA) method was employed using an ELISA reader and specific kits provided by the Chinese company Sunlong for the serum samples in the study. In this test, specific antibodies targeting key Cytokines (Interleukin-1 beta, Interleukin-8, and Interferon gamma) were used. These antibodies coated the

surface of 96 wells in a microtiter plate, as per the manufacturer's instructions (Sunlong,China). After completing the steps according to the guidelines, the color change was measured using an ELISA reader at a wavelength of 450 nm.

2.3.2 Renal Function Tests

Serum creatinine levels in individuals with infection were measured using the Jaffe Kinetic test (22, 2002). In this method, creatinine in the serum sample reacts with picric acid in an alkaline solution (alkaline picrate) from the reagent, resulting in the formation of an orange-colored complex. The amount of creatinine in the test samples was calculated based on the intensity of the color developed over a fixed period. The color intensity was measured using the fully automated Cobas C311 analyzer to detect creatinine levels in serum [16], [17]. The urea levels in the serum were analyzed using urease to produce ammonium and carbonate. Subsequently, L-glutamate was generated through reactions between ammonium and 2-oxoglutarate in the presence of glutamate dehydrogenase and the coenzyme NADH. During this reaction, 2 moles of NADH were oxidized to NAD+ for each mole of urea hydrolyzed. The rate of decrease in NADH concentration was directly proportional to the urea concentration in the serum sample, which was determined photometrically using the fully automated Cobas C311 analyzer [16], [17]. The blood urea nitrogen (BUN) was calculated according to the formula provided by [16], as follows:

$$BUN = Urea/2.14.$$

2.4 Statistical Analysis

The results were analyzed statistically by SPSS to determine significant differences between groups using the independent t-test. Subsequently, the magnitude and type of correlation between variables were assessed using the Pearson correlation coefficient [18].

3 RESULTS AND DISCUSSION

3.1 Microscopic Examination

The microscopic examination results in the current study, as shown in Table 1, revealed an infection rate of 31.818% (70 samples) from a total of 220 stool samples examined microscopically. These samples were collected from patients visiting the govermental Hospital in Kirkuk and private clinics between October 2024 and December 2024.

Table 1: Infection rate with E.histolytica by microscopic examination in the study groups.

Microscopic Examination	Positive Samples	Negative Samples
220	70 (31.818%)	150 (68.18%)

The cyst stage is characterized by a round or oval transparent body containing three to four nuclei and a chromatoid body, as shown in Figure 1. The trophozoite stage is characterized by an irregular protoplasmic mass with protrusions extending in all directions. It contains a single nucleus with a central nucleolus that regulates and controls the cell's functions, as shown in Figure 2.

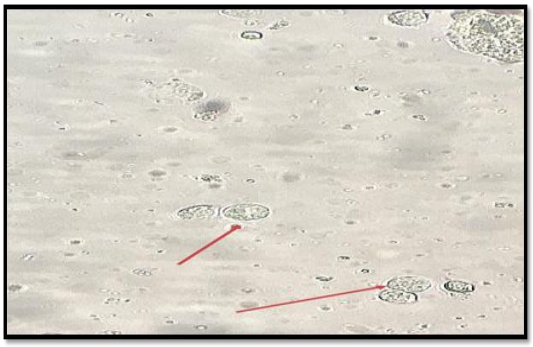


Figure 1: Cyst stage of Entamoeba histolytica stained with Lugol's iodine under 40X magnification.

The infection rate of this study was recorded at 30.22%. The findings also agreed with [19] in Dhi Qar, [20] in Kalar, who reported infection rates of 29.9%, 31.6%, respectively. These results are higher than those recorded by [21] in Kirkuk,, who reported an infection rate of 27.7%.

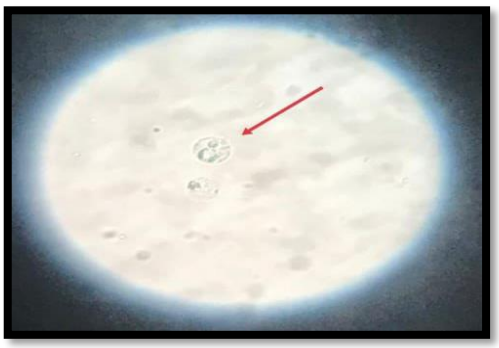


Figure 2: Trophozoite stage of Entamoeba histolytica stained with Lugol's iodine under 40X magnification.

3.2. Cytokine Profile

3.2.1 IL-1β Level

The results presented in Table 2 showed a significant increase ($P \leq 0.05$) in the level of Interleukin 1-beta (IL-1β) in individuals infected with Entamoeba histolytica (18.42 ± 3.98 ng/ml) when compared to healthy or uninfected individuals (14.81 ± 3.61 ng/ml)

Table 2: IL-1β level in the study groups.

Parameters Groups	Interleukin IL-1β ((ng/ml	Sig.
Control (Healthy) N =20	14.81 ± 3.61 B	0.012
Patients (Infected) N = 70	18.42 ± 3.98 A	

The results are presented as mean ± standard deviation, where (N) indicates the sample size. Different letters denote significant differences ($p \leq 0.05$), while similar letters indicate no significant differences.

These results are in agreement with the study by [22], which indicated a significant increase in this variable in infected individuals compared to healthy controls. These results can be explained by the inflammatory response triggered by the invasion of the parasite into body tissues. Inflammatory reactions and tissue damage result from the protozoa's stimulation of the host epithelial cells in order to release pro-inflammatory Cytokines, like interleukin-1 beta (IL-1β). Additionally, amoebic proteins possess a capacity to induce caspase-1 activity and activate proIL-1β, the precursor form of IL-1β, each of which increase inflammation [23]. Numerous vital processes are involved in the creation of amoebic proteins related with the pro-IL-1β precursor of interleukin-1 beta, To become its active state, pro-IL-1β must be cleaved by caspases, specifically caspase-1, after being created as a precursor that remains inactive [8]. Multiple triggers, for example pathogen-associated molecular patterns (PAMPs), lack in host cells yet present in specific pathogens, can cause this reaction [24]. In E. histolytica sickness, interaction between the amoeba and host immune cells may produce pro-IL-1β by inflammatory signal pathways [8].

3.2.2 Interferon Gamma Level

There are not any significant variations ($P < 0.05$) in the concentrations of gamma interferon between people infected (123.16 ± 6.32 ng/ml) with the Entamoeba histolytica (tissue-invasive protozoan) E. histolytica, according to the results shown in Table 3 when compared to healthy or uninfected individuals (128.24 ± 12.26 ng/ml).

Table 3: Interferon Gamma level in the study groups.

Parameters Groups	IFN-ϒ (ng/ml)	Sig.
Control (Healthy) N =20	128.24 ± 12.26 A	0.58
Patients (Infected) N = 70	123.16 ± 6.32 A	

The results obtained line up with a number of earlier investigations that showed no discernible difference in gamma interferon levels between infected and healthy controls. Moreover, amoebic infections appear to possess no impact on gamma interferon levels. This result is consistent with studies by [11] and [12], in which a group of individuals received a vaccine which contained the parasite's infectious stage. Studies have showed that although individuals produced more gamma interferon in response to the immunisation, there were no significant differences in gamma interferon levels between infected and non-infected individuals. Due of the particular immune system reaction mechanisms involved, amoebic infection may not have a major impact on gamma interferon (IFN-γ) levels. To study, when amoebic proteins occur, phagocytic cells infected with the parasite Entamoeba histolytica do not consistently produce more IFN-γ or produce more tumour necrosis factor (TNF-α) than when they are stimulated with lipopolysaccharide (LPS) [25]. There was no statistically significant relation between gamma interferon and interleukin-1 beta ($r = 0.17$, Sig = 0.27), or between gamma interferon and interleukin-8 ($r = 0.14$, Sig = 0.37), . There are many kinds of causes of this, including:

1) Temporal Variability: There is a time disparity in the secretion of these Cytokines. Gamma interferon appears early in the course of infection, whereas the interleukins mentioned are typically secreted in the later stages of infection [26]. This temporal difference may prevent a statistical correlation between their levels.

2) Sample Size: The small sample size could also reduce the statistical power to detect meaningful correlations, even if they exist.

3.2.3 Interleukin 8 Level

Patients showed significantly higher IL-8 levels (29.65 ± 5.50 ng/ml) compared to healthy controls (23.47 ± 6.12 ng/ml; p = 0.003) (see Table 4).

Table 4 Interleukin 8level in the study groups.

Parameters Groups	Interleukin 8 (ng/ml)	Sig.
Control (Healthy) N =20	23.47 ± 6.12 B	0.003
Patients (Infected) N = 70	29.65 ± 5.50 A	

These results are consistent with the study by [27]. which indicated that interleukin 8 levels are significantly higher in patients compared to healthy individuals. The elevated levels of interleukin 8 (IL-8) in amoebic infections, particularly those caused by Entamoeba histolytica, are primarily attributed to the direct stimulation of colonic epithelial cells by the parasite. This stimulation, either by the parasite itself or its components, leads to increased expression of the IL-8 gene in these cells via post-transcriptional mechanisms. Over time, this results in enhanced secretion of IL-8 [28]. Furthermore, the inflammatory response is amplified by Cytokines such as IL-1β, which activate NF-κB pathways, promoting IL-8 production and attracting immune cells to the site of infection [29]. The Pearson correlation coefficient indicates a strong positive correlation between interleukin 8 and interleukin 1 beta (r = 0.998, sig = 0.00) on one hand, while no statistically significant correlation was observed between interleukin 8 and gamma interferon (r = 0.14, sig = 0.38) on the other hand. This finding was explained earlier (in the interpretation of the results for interleukin 1 beta and interferon gamma).

3.3 Renal Function Test

The results presented in Table 5 [25] show a significant increase (P ≤ 0.05) in the level of urea in individuals infected with the Entamoeba histolytica (tissue-invasive protozoan) E. histolytica (41.99 ± 3.96) when compared to healthy or uninfected individuals (37.40 ± 4.35). These results are consistent with the study by [25], which indicated a significant increase in urea levels in infected individuals compared to healthy controls.

Table 5: Kidney Function biomarkers levels in study groups.

Group Parameters	Control N =20	Patients N = 70
Urea (mg/dL)	37.40 ± 4.35 B	41.99 ± 3.96 a
Creatinine (mg/dL)	0.18 B 0 . 8 8	1.01± 0.16 a
BUN (mg/dl)	80.04 ± 9.30 B	89.86 ± 8.47 a
BUN/Creatinie ratio	20.73 ± 4.57 A	19.70 ± 2.62 a

The elevated urea levels in the blood may be attributed to the breakdown and degradation of proteins due to kidney involvement in the disease, leading to an increase in urea concentration. One explanation for this is that the last product of protein metabolism is urea [9]. These findings are in line with a study by [30], which found that people infected with E. histolytica had lower urea levels. The results presented in Table 5 show a significant increase (P ≤ 0.05) in the creatinine level in individuals infected with the Entamoeba histolytica (tissue-invasive protozoan) E. histolytica (1.01 ± 0.16) when compared to healthy or uninfected individuals (0.88 ± 0.18).

These results are consistent with the study by (Mohammed et al. 2022). However, this study differs from a study conducted by [31], which reported a slight decrease in creatinine levels in infected individuals [32].The results presented in Table 5 show a significant increase (P ≤ 0.05) in the BUN level in individuals infected with the Entamoeba histolytica (tissue-invasive protozoan) E. histolytica (89.86 ± 8.47) compared to healthy or uninfected individuals (80.04 ± 9.30).

These results are consistent with the study by [32], which indicated an elevation in BUN levels during amoebic dysentery infection. The statistical results analyzed using the SPSS program showed a significant increase (p ≤ 0.05) in the levels of (IL-1β), (IL-8), creatinine, urea, and BUN. In contrast, no significant difference was observed in the levels of interferon gamma (IFN-γ) and the BUN/Creatinine ratio between the two groups. Urea levels may also be accompanied by rising IL-1β levels.

This suggests how the inflammatory response impacts renal function. The kidneys' decreased ability to remove metabolic waste as a result of an increase in inflammatory Cytokines (such IL-1β) might cause urea to build up in the blood. The correlation results also showed a strong positive correlation (r = 0.00)

between the levels of Interleukin-1β and Interleukin-8 (r = 0.998, sig = 0.00).

This can be interpreted by the fact that Entamoeba histolytica stimulates the production of Interleukin-8 in colon cells as part of the inflammatory response during infection. At the same time, the presence of Interleukin-1β enhances the secretion of Interleukin-8, contributing to the inflammatory environment associated with amoebic dysentery [23], [29].

The statistical analysis of the BUN/Creatinine ratio values showed no significant differences between the patient group (20.73 ± 4.57) when compared to the control group (19.70 ± 2.62). The BUN/Creatinine ratio is an important indicator for assessing kidney function, with normal values typically ranging from 10:1 to 20:1. However, in the case of amoebic infection, no specific or well-documented ratios exist. According to prior studies, modifications to this ratio could be a symptom of underlying medical disorders such as dehydration or cut kidney function, each of which can be greatly impacted by severe amoebic infections [33].

IL-1β and creatinine indicate a slight positive correlation of roughly 0.45. Putting it another way, there may be a relationship between high IL-1β and creatinine levels. The inflammatory impact of the amoeba parasite on renal function may account for this. The infection's immunological and inflammatory effects can make it harder for the kidneys to remove waste. IL-1β and urea share an a little positive link, with a correlation value of 0.38. While urea and IL-1β have a fairly beneficial relationship, rising blood

A Pearson correlation value of 0.51 shows a substantial connection between IL-1β and BUN. Given the strong positive relationship between blood urea nitrogen (BUN) and IL-1β, it is possible that elevated BUN levels are linked to raised IL-1β levels. This result indicates that a decrease in kidney function may be associated to the inflammatory response triggered by the amoeba parasite. BUN levels can rise as a result of acute inflammation brought on by high IL-1β levels that impair the kidneys' ability to clear out waste.

The inflammatory response carried on by the amoeba infection may affect renal function, as indicated by the moderate major positive correlations observed between IL-1β, creatinine, urea, and BUN. High levels of IL-1β are probably causing inflammation in the kidneys, leading to decreases in their capacity to filter waste and raising blood levels of creatinine, urea, and BUN.

4 CONCLUSIONS

Statistical analysis using SPSS revealed significant elevations ($p \leq 0.05$) in IL-1β, IL-8, Creatinine, Urea, and BUN levels in infected individuals, suggesting immune activation and potential kidney function impairment. However, no significant differences were noted in IFN-γ levels or the BUN/creatinine ratio. Given there were notable increases in interleukin 1 beta and interleukin 8 levels, which indicate the activation of the inflammatory response, we reach our conclusion that individuals infected with the Entamoeba histolytica (tissue-invasive protozoan) have an exaggerated immune response. Significant rises in creatinine and urea levels additionally show the infection's detrimental effects on renal function. However, there were no appreciable variations in the BUN/Creatinine ratio or gamma interferon levels between infected and healthy people. These findings emphasise how crucial it is to assess organ function indicators and immunological markers when trying to create efficient diagnosis and treatment plans.

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Effect of Antioxidant Activity of Naringin and CoQ10 Against Acetaminophen-Induced Nephrotoxicity in Male Rats

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Keywords: Antioxidant, CoQ10, Kidney Function, Naringin, Acetaminophen, Nephrotoxicity.

Abstract: Acetaminophen overdose is a known cause of nephrotoxicity, primarily through oxidative stress mechanisms. This study evaluated the potential renoprotective effects of the antioxidants naringin and coenzyme Q10 (CoQ10) against acetaminophen-induced kidney damage. Forty-two male rats were divided into six groups (n=7): control, acetaminophen (1g/kg), naringin (100mg/kg), CoQ10 (100mg/kg), acetaminophen + naringin, and acetaminophen + CoQ10. Treatments were administered orally for 60 days. Serum levels of urea, creatinine, calcium, phosphate, and the activities of antioxidant enzymes (SOD, GPx) and malondialdehyde (MDA) were assessed. Acetaminophen administration caused a significant ($p<0.05$) decrease in SOD, GPx, calcium, and phosphate levels, alongside an increase in MDA, urea, and creatinine levels compared to the control. Co-treatment with both naringin and CoQ10 significantly ameliorated these changes. Notably, naringin exhibited a more potent protective effect, normalizing kidney function parameters and oxidative stress markers to levels comparable with the control group. The findings demonstrate that naringin and CoQ10 confer protection against acetaminophen-induced nephrotoxicity by attenuating oxidative stress. Naringin proved to be a more effective therapeutic agent in this model, suggesting its potential for clinical application in preventing drug-induced kidney injury.

1 INTRODUCTION

Free radicals are uneasy molecules characterized by having an unpaired electron in their outer shell, which makes them highly reactive to cells, proteins, and DNA within the body. These molecules are generated during normal metabolic functions or through external factors like pollution, radiation, and smoking. The body's antioxidant defense system is crucial in counteracting their damaging effects [1].

An increase in free radicals because of a weakened antioxidant system can result in cellular damage and contribute to the onset of various chronic diseases, including cancer, heart disease, and arthritis. Unregulated oxidation also accelerates aging and can lead to nervous system disorders [2].

Naringin exhibits protective properties against chemotherapy-induced kidney toxicity by mitigating oxidative stress, cell apoptosis, autophagy, and oxidative DNA damage [3]. Naringin has a protective effect against nephrotoxicity in male rats by modulating lipid peroxidation, enhancing antioxidant defenses, and suppressing pro-inflammatory cytokines, while also lowering urea and creatinine concentrations. The findings demonstrated an improvement in mitochondrial activity and a decline in lipid peroxidation, confirming naringin's potential to safeguard the kidneys from drug-induced toxicity [4]. Studies have also demonstrated that naringin boosts the action of antioxidant enzymes, including glutathione peroxidase (GPx) and catalase (CAT), thereby enhancing the kidneys' defense against oxidative stress and preventing cellular damage [5].

CoQ10 is essential for electron transfer in mitochondrial oxidative phosphorylation. It functions as a powerful antioxidant, stabilizes cellular membranes, and serves as a crucial cofactor in ATP synthesis through oxidative phosphorylation, protecting proteins and DNA from oxidative damage [6]. CoQ10 is used to prevent oxidative stress caused by cisplatin, which causes inflammation, necrosis, and apoptosis in kidney tissues through its antioxidant actions. Similarly, CoQ10 is used to mitigate the poisoning of anthracyclines, tamoxifen, and doxorubicin [7]. levels of Coenzyme CoQ10 in the Plasma are significantly depressed in patients with chronic kidney disease (with or without

dialysis), in contrast to normal levels, and there is evidence that the supplementation of CoQ10 may ameliorate kidney function and lower the need for dialysis in chronic kidney disease patients [8].

Acetaminophen is one of the most widely consumed over-the-counter antipyretic and painkiller drugs [9]. Although the drug's mechanism of action remains unclear, it has historically been classified with "non-steroidal anti-inflammatory drugs" due to its suppression of the pathway of the cyclooxygenase (COX) enzyme [10]. Acetaminophen-induced kidney injury involves activation of the cytochrome P450 enzyme pathway, specifically in the renal cortex, binding of the drug to prostaglandin endo-peroxidase, formation of a toxic metabolite (NAPQI) in the renal medulla, deacetylation of the drug by N-deacetylase enzymes, and generation of harmful free radicals that cause renal necrosis [11]. This review aims to determine the role of naringin and the enzyme CoQ10 as antioxidants and their ability to reduce the negative effects of oxidative stress on the kidneys and improve their function.

2 MATERIALS AND METHODS

2.1 Preparation of Solution

2.1.1 Acetaminophen

The dosage of 1 g /kg [12], was obtained from the pharmacy. A stock solution was then prepared and the corresponding dose was administered to each male animal based on its weight.

2.1.2 The Naringin

The extract, at a concentration of 0.1 g/kg [13], was obtained from the pharmacy and dissolved in 10 ml of 0.9% normal saline to prepare the stock solution. Based on the body weight of the animals, the appropriate dose was administered orally.

2.1.3 The Coenzyme CoQ10

In relation to CoQ10, the concentration was 0.1g/kg [14], purchased from the Al-Razi Laboratory Supplies Store." It was dissolved in 10 ml of normal saline 0.9% to get the stock, then adjusted according to their individual's body.

2.2 Experimental Design

Following a one-week acclimatization period, a total of forty-two (42) male rats were randomly allocated into six experimental groups (n=7 per group). The groups were designed as follows:

- The control group: They were orally administered normal slain 0.9 % for 60 day.
- Naringin group: Dosage with Naringin at 0.1g/ kg orally only for 60 day.
- CoQ10 group: received orally CoQ10 (0.1g/ kg) only for 60 day.
- Acetaminophen group: received orally acetaminophen at dose of 1 g/kg 60 day.
- Acetaminophen + Naringin: received acetaminophen 1 g/kg, then after 4 h given Naringin at a concentration 0.1g/ kg orally for 60 day.
- Acetaminophen + CoQ10 group: gave orally acetaminophen 1g/kg after 4h given CoQ10 0.1g/ kg for 60 day.
- * seven rats in each group.

2.3 Indicators and Antioxidants. Estimation of Action Glutathione Peroxidase (GPx) in the Serum

The level of selenium-dependent glutathione peroxidase was estimated according to the method [15].

2.3.1 Determination of Malondialdehyde in the Serum

Malondialdehyde levels in serum were estimated using a modified method developed by researchers [16]. Absorbance intensity is measured at 532 nm.

2.3.2 Estimation of Superoxide Dismutase (SOD) Activity in the Serum

SOD enzyme activity was estimated using a spectrophotometer [17].

2.4 Kidney Function Indicators

2.4.1 Assessment of Urea Level in the Blood Serum

Urea levels were estimated using a spectrophotometer according to [18].

2.4.2 Estimation Level of Creatinine in the Blood Serum

Serum creatinine levels were estimated based on [19]. using the colorimetric method (Jaffe's reaction), where creatinine reacts with basic buffers to produce a colored compound. The absorbance was measured using a spectrophotometer.2

2.5 Estimation Levels of Ion in the Serum

2.5.1 Assessment Levels of Calcium Ion in Serum

The calcium ion level in serum was estimated using the Roche (COBAS C311) device. According [20].

2.5.2 Estimation of Phosphate Ion Level in the Serum

Ion level was estimated using the (COBAS C311) device from Roche/Hitachi. measured according to [21].

2.6 The Analysis

Concerning the analysis of study results, the " Statistical Program for Social Sciences (SPSS), version 22 and ANOVA test were used to extract the significant differences between all the groups. The LSD at P-values (<0.05) were dependent in the present study [22].

3 RESULTS

3.1 Effect of Treatment with Coenzyme CoQ10, Naringin, and Acetaminophen on the Action of SOD, MDA, and GPX

Through statistical analysis of study results, acetaminophen led to a decrease ($P \leq 0.05$) in contrast to the control group and the rest of the study groups, while the treatment with CoQ10 showed a slight rise in antioxidant levels contrast to the drug group. The naringin treatment was more effective, as an increase in levels of antioxidants was observed compared to the group that received both acetaminophen and CoQ10 Table 1.

3.2 Effect of the Treatment with Coenzyme CoQ10, Naringin, Anddrug of Acetaminophen on Levels of Creatinine, Urea, Calcium, and Phosphate

Table 2 shows an increase ($P \leq 0.05$) in creatinine and urea levels in the group treated with acetaminophen only, compared to the control and other groups. On the other hand, a decrease in creatinine and urea levels was observed in the groups treated with naringin or CoQ10, respectively, when compared to the drug-treated group.

Table 1: Effect of coenzyme CoQ10, naringin and acetaminophen on the action of SOD, MDA, and GPX.

Groups of study	Mean ± SD			
	Samples Number	SOD (IU/ L)	MDA (IU/ L)	GPX (IU/ L)
Control	7	43.28±1.14 b	49.11± 0.04 c	56.23± 0.07 b
Naringin (100mg/ kg)	7	48.26± 1.17 a	31.94± 1.02 d	62.13± 1.08 a
Q10 (100mg/ kg)	7	44.11± 0.45 b	51.35± 0.07 c	54.88±1.67 b
Acetaminophen (1g/ kg)	7	29.85± 0.08 d	65.46± 0.39 a	38.77± 1.06 d
Acetaminophen + Naringin	7	42.89± 0.05 b	50.69± 0.09 c	54.95± 1.02 b
Acetaminophen + Q10	7	38.45± 1.33 c	58.09±1.33 b	38.45± 1.33 c
LSD		3.91	6.57	5.18

Different letters show significant differences through of the study groups in P-value <0.05.

Table 2: Effect of coenzyme CoQ10, naringin and acetaminophen on levels of creatinine, urea, calcium, and phosphate.

Groups of study	Mean ± SD				
	Samples Number	Creatinine (mg/dl)	Urea (mg/dl)	Calcium (mmol/L)	phosphate (mmol/L)
Control	7	0.29±0.12 c	28.23± 1.11 c	9.68± 1.18 a	5.51± 0.17 a
Naringin (100mg/ kg)	7	0.27±0.03 c	23.56± 1.07 c	9.97± 1.23 a	5.59± 0.29 a
Q10 (100mg/ kg)	7	0.32±0.13 c	30.09± 1.09 c	9.35± 0.23 a	5.23± 1.01 a
Acetaminophen (1g/ kg)	7	0.65±0.09 a	41.15± 0.750 a	7.16± 0.06 c	3.21± 0.06 c
Acetaminophen + Naringin	7	0.30±0.06 c	28.69± 1.12 c	9.57± 0.65 a	5.45± 0.18 a
Acetaminophen + Q10	7	0.41±0.049 b	35.19± 0.06 b	8.56± 0.03 b	4.49± 0.67 b
LSD		0.08	4.95	0.78	0.69

Different letters show significant differences through of the study groups in P-value <0.05.

In contrast, showed a significant decrease in levels of calcium and phosphate ($P \leq 0.05$) in group that received the drug only, compared to the other experimental groups. An increase in calcium and phosphate levels was observed when the animals were treated with naringin and CoQ10 compared to the group that received acetaminophen. Table 2.

4 DISCATION

The results display a decrease in the antioxidants (GPX) and (SOD), accompanied by a significant increase in (MDA) level in the group treated with the drug acetaminophen only when compared with the control group. The results are similar to the study [23], which measured the level of GSH and GPx1, as well as the level of MDA. These results were consistent with the study [24], in which a reduced level of glutathione and an increased level of malondialdehyde were detected. This may be explained by the fact that the 1g/kg dose may have induced oxidative stress in kidney tissues by promoting lipid peroxidation, thereby increasing the formation of different free radical species that cause histological damage in the kidneys. The high production of the reactive compound NAPQI after a high dose leads to the depletion of cellular glutathione and damage to mitochondrial proteins, leading to the induction of oxidative stress, which can result in DNA damage, cell necrosis, and cell death. The result is that the dose causes damage to the kidney cells [25]. The increase in the levels of these parameters in the serum is an indicator of the drug-induced nephrotoxicity in the kidneys of animals, as acetaminophen depleted the antioxidant enzymes in kidney tissue and increased lipid peroxidation, which led to a disruption of the homeostasis process, cell death, tissue necrosis and, finally, functional impairment in the kidney, which leads to the accumulation of metabolic wastes in the serum, as shown by some studies [26].

In addition to the above, the results also showed that treating rats with acetaminophen + CoQ10 resulted in a significant increase in (SOD) and (GPX), while significantly decreasing the (MDA) compared to the drug group, because CoQ10 acts as an antioxidant, but it can become a pro-oxidant when oxidants are present, as occurs after high doses of acetaminophen. Acetaminophen affects the body's antioxidant system, damaging the liver and kidneys via toxic metabolites such as NAPQI, which interact with and deplete glutathione, leading to decreased levels of GPX and SOD, along with increased MDA. Alternatively, CoQ10 may exacerbate oxidative stress when the natural antioxidant system is impaired, leading to increased peroxides, notably MDA and changes in GPX [27].

In contrast to the above, the results also showed that treating rats with acetaminophen + naringin extract led to no significant change in the enzymes (SOD) and (GPX), and in the level of (MDA) compared to the control. The current research is somewhat in agreement with the study [28]. This is due to the effectiveness of the chemical compounds of naringin extract, which remove free radicals and prevent inflammatory responses by inhibiting the production of inflammatory cytokines, thus preventing cell damage by enhancing the natural

defenses of antioxidants such as glutathione (GSH), catalase, and SOD [29].

In light of the results of the current study, an elevation in the levels of creatinine and urea was observed in the group treated with acetaminophen. These results were consistent with those of some studies [30]. This increase may be explained by the toxic effect of acetaminophen, which causes a decline in the function of the renal glomeruli. As a result, filtration rates decline due to oxidative stress induced by the drug's toxicity. Several histopathological changes in renal tissue have been documented, triggered by acetaminophen consumption, such as hemorrhage in some convoluted tubules of renal tissue and necrosis of the lining epithelium, along with edema within the interstitial tissue of the renal cortex and shrinkage of some renal glomeruli, which may cause significant increases in creatinine and urea levels. Some studies have indicated shrinkage of renal glomerular cells, resulting in a reduction in the total filtration surface area and consequently a decrease in filtration rate and waste excretion [31].

Contrary to the above, a significant reduction in calcium ion and phosphate levels was observed in the aforementioned group when compared with the control. This is because of the fact that high doses of acetaminophen can lead to nephrotoxicity, limiting the kidneys' ability to reabsorb calcium and phosphate, leading to decreased levels in the blood. Damage to the renal tubules can also cause increased loss of calcium and phosphate. Alternatively, it may be due to acetaminophen-induced oxidative stress in the kidneys, which leads to increased consumption of vital minerals such as calcium and phosphate for cellular recovery and regeneration.

As for the group treated with acetaminophen + coenzyme CoQ10, a decrease in urea and creatinine was observed when compared with the drug group. This improvement can be attributed to the significant role that CoQ10 plays in protecting the interstitial tissue of the renal cortex from damage caused by oxidative stress by reducing the free radicals and their harmful effect on the kidney tissue, which results in an improvement in its functions, restoring its normal composition, and returning urea and creatinine to their normal levels [32]. CoQ10 also plays an important role in regenerating antioxidants, including (vitamins E and C). Vitamin C plays a role in reducing inflammation and oxidative stress on the kidneys, and improving the function of the renal tubules or nephrons [33].

Additionally, CoQ10 treatment resulted in an increase in calcium and phosphate levels compared with the control and drug groups. The results were somewhat consistent with a study [34]; CoQ10 also has a role as an antioxidant that includes scavenging ROS and thus reducing the oxidation of low-density lipoprotein and protecting the kidney tissue from renal damage [34].

In contrast, urea and creatinine levels did not show a significant change in the group that was treated with acetaminophen + naringin compared to the control. This lack of change could be attributed to the fact that naringin has a clear role in protecting the kidneys under multiple abnormal pathological conditions [35]. Although acetaminophen triggers oxidative stress by activating the generation of reactive oxygen species, studies have indicated the protective effects of naringin's chemical components with antioxidant activity, such as phenols, polyphenols, and flavonoid glycosides [36]. Thus, it may have played a substantial role in enhancing the physiological function of the kidneys, which was positively reflected in the absence of a significant difference in the creatinine and urea levels in rats of this group.

Calcium and phosphate levels in the blood did not demonstrate a notable change in this group compared to the control. This is because calcium and phosphate levels are linked to kidney function, and the unchanged levels of these in the blood indicate that naringin plays a role in protecting the kidneys from oxidative stress [37]. Studies have also indicated that naringin plays a substantial role in bone protection by affecting estrogen receptors and promoting mineral deposition. It also increases calcium absorption and promotes its deposition in bones [38].

5 CONCLUSIONS

This study demonstrates that acetaminophen at a dose of 1 g/kg induces significant nephrotoxicity in male rats, as evidenced by increased oxidative stress (elevated MDA, decreased SOD and GPx), impaired kidney function (elevated urea and creatinine), and disrupted electrolyte balance (decreased calcium and phosphate).

Both naringin and coenzyme Q10 showed a protective effect against acetaminophen-induced kidney damage due to their antioxidant properties. They significantly attenuated the oxidative stress and restored kidney function parameters and ion levels towards normal.

Notably, naringin exhibited a more potent renoprotective effect than coenzyme Q10. The group receiving naringin in combination with acetaminophen showed results that were not statistically different from the healthy control group across most parameters, indicating almost complete prevention of kidney damage. In contrast, the coenzyme Q10 group showed a significant improvement compared to the acetaminophen group, but not a full return to baseline.

In conclusion, naringin can be considered a highly effective natural agent for protection against drug-induced nephrotoxicity. Further clinical studies are warranted to explore its potential therapeutic application for humans.

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Study of Inhibitory Estimates of Probiotics and Extent of their Synergistic Effect with some Antibiotics Against Antibiotic-Resistant Bacteria that Cause Urinary Tract Infections in Children

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Keywords: Probiotics, Urinary Tract Infections, Antibiotics, Synergism, Children.

Abstract: The prevalence of urinary tract infections (UTIs) in children caused by antibiotic-resistant bacterial strains represents a serious public health problem. The aim of this in vitro study was to evaluate the direct antimicrobial activity of commercially available probiotic strains and to investigate their synergistic interaction with antibiotics against clinical isolates of uropathogens. From 160 urine samples, 65 antibiotic-resistant bacterial isolates (both Gram-positive and Gram-negative) were obtained. Their identification and resistance profiling were performed using the VITEK-2 system. The antimicrobial activity of probiotics and their synergism with antibiotics were assessed using the agar diffusion method. The results showed that certain probiotic strains, particularly L. rhamnosus and L. reuteri, exhibited significant inhibitory activity against uropathogens, whereas others (Bifidobacterium) were inactive. The strongest synergistic effect was observed with the combination of probiotics and tetracycline. The findings demonstrate that the antimicrobial effects and synergism with antibiotics are strictly strain-dependent and vary with the antibiotic's mechanism of action. This in vitro study highlights the potential utility of probiotics as adjunctive agents to overcome antibiotic resistance and emphasizes the need for further investigation of strain-specific interactions.

1 INTRODUCTION

One of the most prevalent forms of bacterial infections in children is urinary tract infections (UTIs). occur frequently in both females and boys. After starting in the urethra, the infection spreads to the kidney tissue. The infection's name reflects its place of origin [1]. The incidence rate is 1% for boys and 3% to 5% for girls. Studies have shown that the prevalence of urinary tract infections in children is greater than the prevalence of bacterial meningitis, pneumonia, and bacteremia. The most important risk factors for urinary tract infections (UTI) in children are gender, age, recurrence of urinary tract infections, uncircumcised children, fever, and bladder catheterization [2]. The most prevalent pathogen, *Escherichia coli*, is responsible for about 85% to 90% of UTIs. *Staphylococcus aureus*, *Klebsiella pneumonia*, *Proteus species*, and *Enterococcus species* are next in line. [3].

The use of antibiotics has significantly reduced the incidence of urinary tract infections in patients, but the overuse of these antibiotics has led to the emergence of resistant strains that have different strategies to resist many drugs and the spread of super-infections caused by these organisms [4]. Finding innovative and successful therapeutic approaches is so essential. Numerous probiotic strains have been used to cure or prevent urinary tract infections by reducing the quantity of dangerous bacteria and maintaining an acidic environment [5]. Scientific studies have confirmed the ability of probiotics to inhibit pathogens, reduce inflammation, and reduce lactose tolerance. Probiotics are defined as live microorganisms that, when ingested in appropriate amounts, boost the host's health. [6].

Nowadays, therapeutic biology is used extensively in food and medicine and is of significant interest worldwide [7]. Probiotics is the Greek word that consists of (pro and bios), which means life, and it was first recognized by scientists (Stillwell, Lilly) in 1965. It was possible for these microscopic

organisms to release substances that promoted the growth of other microscopic organisms. The scientist verified that in 1989 (Roy Fuller). The success of probiotics, the possibility of using it in treatment, and their ability to benefit the health of the host. [8], Characterized by bacteria *L. plantarum*, it has great medical importance and is a bacilli gram positive. Catalase test negative result increases the pH of the medium in which it is present, and also plays a role in *L. plantarum*. It plays an important role in colonizing urinary tract epithelial cells within 24 hours of incubation and thus has antibacterial properties. [9].

2 MATERIALS AND METHODS

2.1 Collection and Isolation of Pathogens

The current study was carried out between August 8, 2024, and November 11, 2024. 65 isolates of bacteria resistant to antibiotics were isolated from 160 samples of patients under the age of 15 who visited several hospitals in Mosul, including Al-Salam Teaching Hospital, Mosul General Hospital, and Al-Khansaa Teaching Hospital. Samples of children under one year were collected using the adhesive plastic bag method. The samples were incubated for 24 hours at 37º in aerobic conditions after being cultivated on MacConkey and Blood agar. The isolates were identified by colony morphology, biochemical testing, and microscopic examination [10]. The diagnosis was confirmed using a VITEK-2 compact system utilizing ID-GNB and ID-GPB cards (BioMerieux, France) [11].

2.2 Collection and Isolation of Probiotics

In this study, some types of probiotic bacteria found in pharmacies and from reliable sources were used, in addition to types obtained from the University of Mosul, College of Agriculture. Species isolated from vaginal swabs and Activia milk were activated by growing them in MRS broth. Then incubated under anaerobic conditions for 24 hours at.37ºc After the incubation period, take 0.1It was grown on medium MRS agar. The identity of the isolates was determined through cultural characteristics and biochemical tests as stated in [12] and used it in the study.

2.3 Determination Of AST and Minimal Inhibitory Concentration of Antibiotic Using the Vitek2 Compact System

Antibiotic susceptibility tests and minimum inhibitory concentrations (MICs) for all isolates under study were carried out to identify the prevalence of antibiotic resistance among isolates by using the VITEK-2 compact system [13]. In this study, we used two types of sensitivity cards: AST card for Gram-positive bacteria (AST-580) and the AST card for Gram-negative bacteria (AST-N222).

2.4 Testing the Inhibitory Activity of Isolates Probiotics Invitro

2.4.1 Estimation of Inhibitory Activity of Precipitate Organisms Probiotic

Carried out as described in [14-16]. After minor adjustments, 0.1 ml of bacterial suspension was added, with a concentration of $1.5x10^8$ bacterial cells/ml. Using a cotton swab, they were applied in three different directions to the Muller-Hinton agar medium while the plate was rotated at a 60° angle. The plates were then left to dry at room temperature for ten to fifteen minutes. A sterile corkborer was used to create wells of five millimeters in the middle of the Muller-Hinton agar on the pathogenic bacteria that had grown. For control and comparison, 0.1 ml of the bacterial isolate's suspension Probiotics was added to each well, and one well was filled with MRS broth free of bacterial growth and incubated for 24–48–72 hours at 37ºC surrounding the holes were measured following the incubation time, and the results were recorded and compared with the control coefficient that contained MRS broth without vaccination [17].

2.4.2 Estimation of Inhibitory Activity of Filtrate Probiotics

The Well diffusion experiment was utilized to determine the inhibitory activity of therapeutic filtrate probiotics, with a few modifications. The impact of liquid culture filtrate on the growth of isolates of therapeutic organisms Probiotics (0.5) MacFarlane Within test tubes filled with MRS broth after adjusting the pH to 5.7, the tubes were incubated at 37ºC for 24 hours under anaerobic conditions. Following incubation, the tubes were centrifuged at 4000 rpm for 10 minutes to extract the bacterial-free supernatant. The liquid was then

filtered through filters having a 0.45 micrometer diameter. In the process, the turbidity constant was compared with the suspension of harmful bacteria. The suspension was then applied to Mueller Hinton agar medium using a sterilized cotton swab. 50 µL of liquid culture filtrate of isolates probiotics was transferred to the solid medium after holes measuring 5 mm in diameter were created with a corkborer. and incubated for 24-48-72 hours at 37ºC. The inhibition zones surrounding the holes were measured following the incubation time, and the results were recorded and compared with the control coefficient that contained MRS broth without vaccination [17].

2.5 Combined Effects of Probiotics and Antibiotics Against Urinary Tract Infection Pathogens

The diffusion test was used with some modifications to measure the effectiveness of synergism in inhibiting pathogenic strains (*E. coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*). A bacterial suspension of pathogenic bacteria and turbidity comparison was prepared (0.5) with McFarland scale and brushed with a cotton swab on Mueller Hinton agar medium and 100 microliter of the probiotic that caused the highest inhibition was mixed with (100) µL (100µg/ml) of antibiotics (Tetracycline, Amikacin, Gentamycin, Amoxicillin, Ciprofloxacin, Cefuroxime). Then transfer (50) µL from the mixture to the holes and incubated for 24 hours at a temperature 37 ºC. After the incubation period, the inhibition zones around the holes were calculated, and the results were recorded and compared with the control coefficient containing MRS broth without vaccine [18].

3 RESULTS AND DISCUSSION

3.1 Isolation and Identification of Pathogens

Between August 8, 2024, and November 11, 2024, 160 urine samples were collected from children under 15 who had UTIs and visited hospitals in Mosul City. According to the findings, 65 samples were positive culture and 95 were negative culture, as indicated in Figure 1. The isolates were identified using the Gram stain, cell morphology, and culture characteristics in the blood agar and MacConkey agar. The diagnosis was confirmed by Vitek2 compact system. The rates of recurrent urinary tract infections are still high and lead to increased relapses and deaths around the world. The excessive and inappropriate use of antibiotics has led to a decrease in the effectiveness of antibiotics and an increase in bacterial resistance, not only at the individual level but also at the community level [19].

3.2 Percentage of Gram-Negative Bacterial Species

Figure 3 shows that Gram-negative bacterial isolates were represented by Escherichia coli 22(55%), Pseudomonas aeruginosa 6 (15%), Klebsiella pneumonia 5 (12.5%), Enterobacter cloacae 4(10%), Proteus mirabilis 2(5%) and Serratia marcescens 1(2.5%). The percentage of Gram-negative bacterial species is shown in Figure 3.

3.3 Percentage of Gram-Positive and Gram-Negative Bacterial Species

Distribution of bacterial isolates was as follows: 25/65 (38.5%) Gram-positive and 40/65 (61.5%) Gram-negative bacteria (Figure 1). Gram-positive isolates comprised: Staphylococcus haemolyticus 11 (44%), S. aureus 10 (40%), Staphylococcus hominis 2 (8%), and Enterococcus faecalis 2 (8%) (Figure 2).

3.4 Sensitivity of Isolates and Minimum Inhibitory Concentration to Antibiotics

All 65 isolates under study were subjected to antibiotic susceptibility testing and minimum inhibitory concentration (MIC) to determine the prevalence of antibiotic resistance. The MIC was determined by Vitek2 compact system which is based on a series of dilutions of antibiotics prepared in a card AST for Gram-positive and Gram-negative bacterial isolates, the antibiotic was considered sensitive if the value was MIC is at the lowest stopping point according to (CLSI-2024) and the results were MIC for isolation as follows:

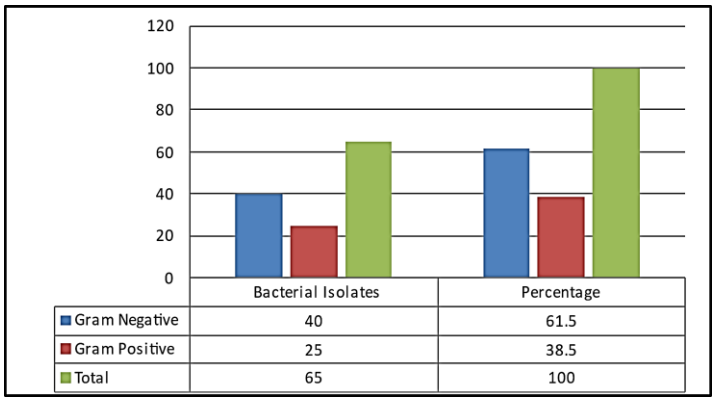


Figure 1: The demographic characteristics of the study.

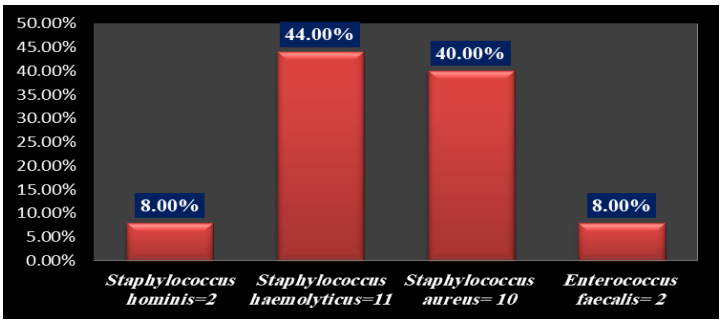


Figure 2: Percentage of Gram-positive bacterial species.

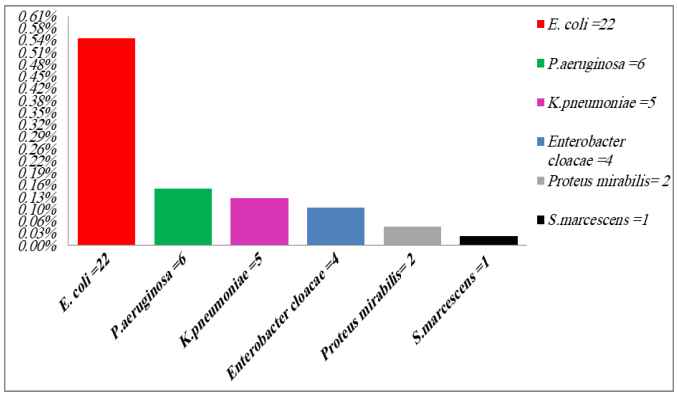


Figure 3**:** Percentage of Gram-negative bacterial species.

3.5 AST and MIC Profile for Gram Positive and Gram-Negative Bacteria

According to Table 1, all Staphylococcus aureus isolates were 100% sensitive to antibiotics (Moxifloxacin, Gentamycin, Teicoplanin, Linezolid, Vancomycin, Trimethoprim/Sulfamethoxazole) respectively. The minimum inhibition concentration was (⩾0.5µg) for each of (Vancomycin, Teicoplanin, Gentamycin) respectively, while the MIC was (⩽0.5µg, ⩽2µg, 10µg) for each of (Moxifloxacin, Linezolid, Trimethoprim/sulfamethoxazole) respectively. The highest resistance was recorded 70% by (Benzylpenicillin, Oxacillin) and the MIC was (⩾4µg, ⩾0.5µg) respectively, while the lowest resistance was recorded 10% by (Tetracycline, Rifampicin) and the MIC recorded was (2µg,1µg)

respectively. Staphylococcus haemolyticus isolates showed 100% resistance to (Oxacillin, Benzylpenicillin) and recorded MIC (⩾2µg, ⩾0.25µg, ⩾0.5µg) respectively and were 100% sensitive to antibiotics (Linezolid, Vancomycin, Tigecycline, Rifampicin) and the MIC was (⩾0.5µg,0,25µg) respectively and were 90% sensitive to antibiotics (Moxifloxacin, Clindamycin, Teicoplanin). Staphylococcus hominis showed 100% resistance to antibiotics (Oxacillin, Fusidic Acid, Erythromycin) and the MIC was (0.5µg, ⩾8µg, 4µg) respectively and were sensitive to many antibiotics 100% (Gentamycin, Clindamycin, Teicoplanin, Linezolid, Vancomycin, Tigecycline, Rifampicin) MIC was between (⩾0.25µg, 1µg). Enterococcus faecalis was 100% resistant to the antibiotic (Gentamycin) while 100% sensitive to (Teicoplanin, Linezolid) MIC (⩾0.5µg, 1µg) respectively. According to Table 2, the isolates of E. coli were 100% sensitive to each of the antibiotics (Imipenem, Meropenem, Amikacin, Colistin) and the MIC was (0.25µg⩾ µg2), while 100% resistant to the antibiotics (Ticarcillin) MIC is (⩾128µg). Klebsiella Pneumonia isolates were 100% resistant to each of the antibiotics (Aztreonam, Ticarcillin) and the MIC was (32µg, ⩾128µg) respectively, while the isolates recorded high sensitivity of 100% to the antibiotics (Imipenem, Meropenem, Amikacin, Colistin) recorded MIC (0.5µg, 2µg, ⩽0.25µg). Proteus mirabilis isolates were 100% resistant to each of the antibiotics (Piperacillin, Imipenem, Tobramycin, Colistin, Ticarcillin, Trimethoprim/ Sulfamethoxazole) and the MIC was (2µg, ⩾128µg), while the isolates were 100% sensitive to the antibiotics (Cefepime, Meropenem, Amikacin) and the MIC was (2µg, 16µg) respectively. The isolates of Pseudomonas aurogenosa were 83% resistant to each of the antibiotics (Ticarcillin/Clavulanic acid, Piperacillin, Imipenem, Meropenem) and recorded MIC (0.5µg, ⩽0.25µg, 32µg,) respectively while the highest sensitivity to antibiotics was recorded by Colistin was 66%, while the MIC was (⩽o.5µg). Enterobacter cloacae isolates were 100% resistant to the antibiotic Colistin and the recorded MIC (⩾0.5µg), while they were 100% sensitive to the antibiotics (Cefepime,

Meropenem, Amikacin, Imipenem) and the MIC was between (⩾0.25µg, 2µg). Serratia marcescen recorded 100% resistance to the antibiotic Colistin and the MIC (2µg). 100% sensitive to all antibiotics used in the study, MIC was between (⩾0.12µg, 4µg). Gram-positive bacterial isolates According to Table 1 showed high sensitivity to antibiotics (Vancomycin, Linezolid Teicoplanin, Tigecycline). The reason is attributed to their lack of mechanisms that help them resist drugs, such as the lack of sufficient defense mechanisms or the lack of resistance genes. Glycopeptides antibiotics (Teicoplanin, Vancomycin) act on the cell wall, which It represents the basic part of the bacterial cell, and (Tigecycline, Linezolid) work from the oxazolidinones, Tetracyclines class that inhibit protein synthesis, which leads to stopping protein synthesis. Staphylococcus aureus, Staphylococcus haemolyticus have a high level of resistance to(Oxacillin, Benzylpenicillin) This study agreed with a study conducted with Diaullah Mirza and a study in Rajshahi [20], [21] while it did not agree with a study in Egypt (Mahfouz et al., 2023) where the resistance rate was medium while it gave a resistance rate to each of Fusidic acid, Erythromycin, Clindamycin)) reached 20% and a study in Iraq and Jordan (AL Husain et al., 2020; [22] showed high resistance due to the presence of genes resistance. But gram-negative bacteria were between resistant and sensitive to the antibiotics used in the study, and the cause of resistance is attributed to the possession of ß-lactamase enzymes that break the ß-Lactams ring in antibiotics, and changes in the cell membrane in addition to the acquisition of mutated genes through plasmid transfer processes, but the cause of sensitivities The lack of these factors makes them more susceptible to drugs [23] in this study, according to Table 2 the most gram-negative bacterial isolates were highly sensitive to (Colistin, Imipenem, meropenem, Amikacin) and the results were consistent with studies in Mosul and Iran [24], [25] and the difference of the results with the study of performances in Erbil [26] showed the result Different proportions of 40% resistance against (Amikacin) When isolates showed moderate resistance to (Ciprofloxacin, Aztreonam), except for Klebsiella Pneumonia isolates, it was 100% resistant, but Pseudomonas aeruginosa isolates showed 66% high sensitivity to(Colistin) it was different from a previous study in Diyala (Mohammed, 2021) where isolates showed Multiple drug resistance. Antibiotic failure if the bacteria show moderate resistance and if not diagnosed in time. According to the study [27], with the increase of resistant bacteria, it led to the lack of therapeutic options in the treatment of bacterial infections, and we now face a big challenge in employing active treatment.

3.6 Probiotics Development and Isolation

Probiotic isolates were obtained as where it was activated on MRS broth. for 24 hours at 37°C under anaerobic conditions, then purified on MRS agar medium and incubated anaerobically for 48 hours at 37°C. The colonies were small to medium sized flat circular colonies. The colony color was white to creamy, shiny, sticky, usually smooth, with edges and slightly convex, while the cells appeared under the microscope as long or short rods, sometimes oval, in pairs or Single and Gram positive [28]. It is non-spore-forming and negative for catalase, oxidase, indole and urease tests and positive for carbohydrate fermentation.

3.7 Study of the Inhibitory Activity of Probiotic Isolates

The inhibition activity of the samples obtained was tested using MRS agar medium. against pathogenic bacterial isolates and selection of the most resistant to antibiotics. As shown in Figure 4 several methods were used to study the inhibitory activity of probiotics, as the rate of inhibition diameters for the isolate (L. rhamnosus, L. reuteri) against pathogenic bacteria ranged between 17-24mm, while the rate of inhibition diameters for the bacterial isolate (L. plantarium) ranged between 15-20mm. As for the bacteria isolated from the vagina, an inhibition rate was recorded between 6-10mm, while no inhibition was recorded by the milk isolate (Bifidobactrium).



Figure 4: The diameters of inhibition of probiotics against pathogens.

An inhibiion rate was recorded by the commercial isolate consisting of several types of probiotics, and it was between 14-20mm. The highest inhibition rate was recorded by the types used in this study against the pathogenic bacteria (Proteus mirabilis), as the inhibition rate ranged between 19-24mm, while the inhibition rate varied against other pathogenic types, as shown in Table 3. It was also found in this study that the ideal incubation period at which the diameters of inhibition reached their highest measurements was 24 hours, which is consistent with what was stated by [29] While no inhibition was recorded by the probiotic filtrate or it was of little effect for all pathogenic bacterial isolates.

3.8 The Combined Effect of Probiotics and Antibiotics

According to the results shown, the most efficient isolates were selected from probiotics were used in the study of the synergistic effect with the antibiotic. Three pathogenic isolates that were most resistant to antibiotics were selected. The results of the synergistic effect between the antibiotic and probiotics were as shown in Table 4, where the synergism between the antibiotic Tetracycline with the probiotic isolates used recorded the highest inhibition and was between (21-33mm). The highest inhibition was on *Staphylococcus aureus* bacteria. No inhibitory effect was recorded when the Probiotic isolates were synergized with the antibiotics (Amoxicillin, Cefuroxime) against pathogenic isolates (*E. coli, Klebsiella Pneumonia*), while it was an inhibitor on *Staphylococcus aureus* isolates. Synergism was not recorded between the antibiotic Gentamycin with *L. plantarium* and the product containing different types of probiotics against the *Klebsiella pneumonia* isolate. The use of commercially obtained combinations of Probiotics against bacterial isolates causing urinary tract infections and resistant to multiple antibiotics was suggested. The selection of antibiotics was based on studying the synergistic effects for their use in clinical prescriptions in the hospital to treat infections. Most antibiotics had a synergistic effect with Probiotics. Using the diffusion method in the holes, it was observed that the diameter of the inhibition zone against pathogens when mixing (Tetracycline + Probiotics) was higher than the inhibition zone when mixing (Gentamycin, Amikacin) with Probiotics [30]. In this study, it was recorded that the effect of inhibiting the growth of pathogens by antibiotics alone was higher than the synergistic effect with Probiotics, which is consistent

with what was stated in the study [31], where synergy had negative effects and reduced the zone of inhibition of pathogen growth, contrary to what was stated in a study conducted by [18]. The synergy between antibiotics and Probiotics had higher inhibitory effects on the growth of pathogens than when used alone, so it seems that the type of antibiotic and the type of probiotic are important in creating synergistic effects. This depends on the mechanism of action of the antibiotic. For example, the antibiotic Tetracycline acts on the 30S subunit and inhibits protein synthesis. Therefore, when used separately, the antibiotic has a better effect on pathogens than when mixed with probiotics. Through our evaluation of the ability Probiotics inhibit the growth of pathogens and found their effect on both Gram-positive and Gram-negative bacteria, this is consistent with a study conducted in Iraq by [32]. While it differed from a study in Turkey conducted by [33], in which probiotics were found to be effective on Gram-positive bacteria only. The variation in inhibition may be related to the type of pathogenic bacteria, the type and quantity of the inhibitor, and its ability to inhibit.

Table 1**:** Antibiotics susceptibility tests in AST card for Gram-Positive bacteria based on CLSI,2024.

No o	AB	Gram-Positive bacterial isolates											
		Staphylococcus aureus (10)			*Staphylococcus haemolyticus (11)*			*Staphylococcus Hominis* (2)			*Enterococcus faecalis* (2)		
		R%	S%	MIC	R%	S%	MIC	R%	S%	MIC		S%	MIC
1	P	7 (70)	3 (30)	⩾0.03µg	11 (100)	0 (0)	⩾0.5µg	1 (50)	1 (50)	⩾0.03µg	-	-	-
2	OXI	7 (70)	3 (30)	⩾0.25µg	11 (100)	0 (0)	⩾4µg	2 100	0(0)	0.5µg	-	-	-
3	FA	2 (20)	8 (80)	⩾0.5µg	9 (82)	2 (18)	⩾0.5µg	2 100	0(0)	4µg	-	-	-
4	GM	0 (0)	10 (100)	⩾0.5µg	6 (55)	5 (45)	⩾0.5µg	0(0)	2 100	⩾0.5µg	2 100	0(0)	SYN
5	E	2 (20)	8 (80)	⩾0.25µg	9 (82)	2 (18)	⩾0.25µg	2 (100)	0(0)	⩾8µg	-	-	-
6	MO	0 (0)	10 (100)	⩾0.25µg	2 (18)	9 (82)	⩾0.25µg	1 (50)	1 (50)	⩾0.25µg	-	-	-
7	CM	2 (20)	8 (80)	⩾0.25µg	2 (18)	9 (82)	⩾0.25µg	0(0)	2 100	⩾0.25µg	-	-	-
8	TEC	0 (0)	10 (100)	⩾0.5µg	1 (9)	10 (91)	8µg	0(0)	2 100	1µg	0(0)	2 100	⩾0.5µg
9	LNZ	0 (0)	10 (100)	2µg	0 (0)	11 (100)	2µg	0(0)	2 100	2µg	0(0)	2 100	1µg
10	VA	0 (0)	10 (100)	⩾0.5µg	0 (0)	11 (100)	⩾0.5µg	0(0)	2 100	⩾0.5µg	1 (50)	1 (50)	4µg
11	TE	1 (10)	9 (90)	⩾1µg	9 (82)	2 (18)	⩾1µg	1 (50)	1 (50)	⩾1µg	1 (50)	1 (50)	⩾1µg
12	TGC	0 (0)	10 (100)	⩾0.12µg	0 (0)	11 (100)	0.25µg	0(0)	2 100	⩾1µg	0(0)	2 100	⩾0.12µg
13	RA	1 (10)	9 (90)	⩾0.5µg	0 (0)	11 (100)	⩾0.5µg	0(0)	2 (100)	⩾0.5µg	-	-	-
14	SXT	0 (0)	10 (100)	⩾10µg	5 (45)	6 (55)	⩾10µg	1 (50)	1 (50)	⩾10µg	-	-	-

Table 2: Antibiotics susceptibility tests in AST card for Gram-Negative bacteria based on CLSI,2024.

No	AB*	Gram-Negative bacterial isolates																		
		Escherichia Coli (22)			*Klebsiella pneumonia (5)*			*Pseudomonas (6) aeruginosa*			*Proteus mirabilis* (2)			*Enterobacter cloacae (4)*			*Serratia marcescen* (1)		
		R%	S%	MIC	R%	S%	MIC	R%	S%	MIC	R%	S%	MIC	R%	S%	MIC	R%	S%	MIC
1	P	10 (42.8)	12 (57.2)	8 µg	3 60%	2 40 %	⩽8	5 (83)	1 (17)	32µ g	1 (5 0)	1 (5 0)	64µ g	1 (2 5)	3 (7 5)	⩽8µg	0 0	1 1 0 0	⩽8µg
2	PE	22 (100)	0 (0)	⩾128 µg	4 80%	1 20 %	⩾12 8 µg	5 (83)	1 (17)	32µ g	2 1 0 0	0(0)	16µ g	2 (5 0)	2 (5 0)	⩾4µg	0 0	1 1 0 0	⩽4µg
3	C AZ	9 (38)	13 (62)	⩽1 µg	4 80%	1 20 %	16 µg	4 (66)	2 (34)	⩽1µ g	1 (5 0)	1 (5 0)	⩽1µ g	2 (5 0)	2 (5 0)	⩾1µg	0 0	1 100	⩽1µ g
4	G M	9 (38)	13 (62)	⩽1 µg	2 40%	3 60 %	⩾16 µg	3 (50)	3 (50)	⩽1µ g	1 (5 0)	1 (5 0)	8µg	2 (5 0)	2 (5 0)	⩾1µg	0 0	1 1 0 0	⩽1µg
5	FE D	5 (2 4)	17 (76)	⩽1 µg	3 60%	2 40 %	⩽1 µg	4 (66)	2 (34)	⩽1µ g	0(0)	2 1 0 0	4 µg	0 (0)	4 (1 00)	⩾1µg	0 0	1 1 0 0	⩽1µg
6	IP M	0 (0)	22 (10 0)	⩽0.25 µg	0 0%	5 100 %	⩽0.2 5 µg	5 (83)	1 (17)	⩽0. 25µ g	2 1 0 0	0(0)	2µg	0 (0)	4 (1 00)	⩽0.5µ g	-	-	-
7	M E M	0 (0)	22 (10 0)	⩽0.25 µg	0 0%	5 100 %	⩽0.2 5 µg	5 (83)	1 (17)	0.5µ g	0(0)	2 1 0 0	⩽0. 25 µg	0 (0)	4 (1 00)	⩽0.25 µg	0 0	1 100	⩽0.2 5µg
8	A K	0 (0)	22 (100)	⩽2 µg	0 0%	5 100 %	⩽2 µg	3 (50)	3 (50)	32µ g	0(0)	2 1 0 0	⩽2 µg	0 (0)	4 (1 00)	⩽2µg	1 1 0 0	0 0	⩽2µ g
9	TO b	8 (38)	14 (62)	⩽1 µg	2 40%	3 60 %	⩽1 µg	4 (66)	2 (34)	⩽1µ g	2 1 0 0	0(0)	⩾16 µg	2 (5 0)	2 (5 0)	⩾1µg	1 1 0 0	0 0	⩽1µ g
10	CS	0 (0)	22 (100)	⩽0.5 µg	0 0%	5 100 %	⩽0.5 µg	2 (34)	4 (66)	⩽0. 5µg	2 1 0 0	0(0)	⩾16 µg	4 (1 00)	0 (0)	⩽0.5 µg	1 1 0 0	0 0	2µg
11	CI P	12 (5 7)	10 (43)	⩽0.2 5 µg	3 60%	2 40%	0.5⩽ µg	3 (50)	3 (50)	⩽0. 06µ g	1 (5 0)	1 (5 0)	⩽0. 25µ g	1 (2 5)	3 (7 5)	⩾0.25 µg	0 0	1 1 0 0	⩾0.25µg
12	A Z	12 (53)	10 (47)	⩽1 µg	5 100%	0 0%	32 µg	-	-	-	1 (5 0)	1 (5 0)	⩽1 µg	2 (5 0)	2 (5 0)	⩾1µg	0 0	1 1 0 0	⩽1µg
13	Ti c	22 (1 00)	0 (0)	⩾12 8 µg	5 100%	0 0%	⩾12 8 µg	-	-	-	2 1 0 0	0(0)	⩾64 µg	2 (5 0)	2 (5 0)	⩾16µg	0 0	1 100	⩽8µ g
14	S	17 (8 1)	5 (19)	⩽20 µg	3 60%	2 40%	⩽20 µg	-	-	32µ g	2 1 0 0	0(0)	⩾32 0µg	2 (5 0)	2 (5 0)	⩾320 µg	0 0	1 100	⩽20 µg

Table 3: The inhibitory effect of probiotic strains against bacterial pathogens.

Pathogen bacteria / Probiotics	*S. auras*	*E. coli*	*Pseudomonas. aurogenosa*	*Klebsiella pneumonia*	*Proteus mirabilis*
L.rhamnosus, L. reuteri	Inhibition zone in diameter				
	17 mm	18 mm	17 mm	17 mm	24 mm
L.plantarium	16 mm	15 mm	20 mm	14 mm	20 mm
L.acidophilus	15 mm	15 mm	14 mm	6 mm	20 mm
Bifidobacterium	-	-	-	-	-
L.casei, L. paracasei, L gasseri. L. Salivarius, L. bulgaricus . fermentum, L	20 mm	15 mm	14 mm	16 mm	19 mm

Table 4: The antibacterial activity of probiotics and antibiotics alone and in combination against pathogen isolates based on the inhibitory zone's diameter.

Antibiotics	Zone of inhibition in mm of pathogenic bacteria and antibiotics											
	E. coli				Klebsiella pneumonia				Staphylococcus aureus			
	antib	1+ Antibio	2+ Antibio	5+ Antibio	antib	1+ Antibio	2+ Antibio	5+ Antibio	antib	1+ Antibio	2+ Antibio	5+ Antibio
GM	17mm	13mm	12mm	18mm	19mm	11mm	0	0	22mm	17mm	18mm	18mm
AK	23mm	21mm	20mm	22mm	20mm	16mm	15mm	10mm	19mm	15mm	15mm	14mm
CIP	5mm	0	0	0	23mm	18mm	21mm	16mm	12mm	0	0	0
AM	0	0	0	0	0	0	0	0	16mm	14mm	12mm	14mm
TE	33mm	30mm	23mm	21mm	25mm	22mm	23mm	23mm	38mm	30mm	30mm	33mm
CX	0	0	0	0	0	0	0	0	22mm	20mm	20mm	20mm

Abbreviations: GM= Gentamycin, AK =Amikacin, CIP= ciprofloxacin, AM =Amoxicillin, TET= Tetracycline, CX= Cefuroxime, Antib= Antibiotics, 2=L.plantarium,5= MIX of probiotics. 1=Lactobacillus rhamnosus, L. reuteri

Table 5: Comparison of the percentage of survival of different probiotic strains at different pH values.

Probiotics	pH at 3 hours				pH at 6 hours			
	pH 2	pH 3.5	pH5	pH 6.5	pH 2	pH 3.5	pH5	pH 6.5
L. rhamnosus, L. reuteri	54.16%	60%	68.7%	93.3%	28%	41.17%	87.5%	97.2%
L.plantarium	40%	38%	48%	96.5%	33.3%	35.3%	45%	1oo.o8%
L.casei, L. paracasei L gasseri. L. Salivarius L. bulgaricus L . fermentum,	33.3%	33.3%	98%	99.3%	48%	37.5%	100%	100.5%

3.9 Acidity Tolerance pH of Probiotic Isolates

Acidity tolerance was carried out according to the method described [34] with some modifications, incubation of Probiotics isolates anaerobically overnight in 5 ml of MRS broth at a temperature of 37°C, after incubation, the culture was diluted with 5 ml of MRS broth to obtain a bacterial suspension (10^7 CFU) and then were washed twice with Phosphate buffer solution(pbs) with a pH of 7.2, and resuspended in 5 mL of sterile MRS broth which was adjusted to pH use of 2, 3.5, 5, and 6.5 which has been modified by using hydrochloric acid (HCL)and incubated for 3 and 6 hours in the aerobical at 37ºc. With a temperature of 37ºc and anaerobic, the percent of survivors of the acid challenge was calculated as the ratio of bacterial concentrations of colonies counted in MRS after acid challenge (N1) divided by to the initial bacterial number concentration at time zero (N0):

$$Survivors\ (\%) = cfu/ml(N0)/cfu/ml(N1) \times 100.$$

According to Table 5 The difference in the tolerance of probiotics isolates to different degrees of pH to simulate the environment of the human stomach showed that all the isolates used in the study showed a general level of tolerance at pH (5, 6.5). These results agreed with the results reported by [35]. According to the reported study [36], the survival rate of isolates with pH was low.

4 CONCLUSIONS

This in vitro study was designed to evaluate the antimicrobial activity of different probiotics and their ability to act synergistically with antibiotics against clinical isolates of bacteria causing UTIs in children. The main conclusions are as follows:

- The antimicrobial activity of probiotics was shown to be strain-dependent. Lactobacillus rhamnosus and Lactobacillus reuteri demonstrated the highest in vitro efficacy.
- The synergistic effect between probiotics and antibiotics is not universal. It varies according to the specific probiotic strain–antibiotic combination. The strongest synergistic effect in our experiment was observed with tetracycline.
- In certain cases, the combination of a probiotic and an antibiotic may result in an antagonistic effect or show no difference compared to the antibiotic alone, underscoring the complexity and selectivity of these interactions.
- The obtained in vitro data highlight the potential utility of specific probiotic strains as adjunctive agents for enhancing the effectiveness of antibiotics and overcoming uropathogen resistance.

Thus, the results of the study indicate that selected probiotic strains may exhibit antimicrobial activity and enhance the action of certain antibiotics under in vitro conditions. Further, more comprehensive research is required to determine the mechanisms underlying these interactions and their potential clinical significance.

ACKNOWLEDGMENTS

We thank the collage of science, Department of biology for the facility provided

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Study of Serum IgM, IgG, IL-37 Levels and IL-37 Gene Polymorphism in Women Infected with Trichomonas Vaginalis in Baqubah City

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Keywords: Trichomonas Vaginalis, IgM, IgG, IL-37.

Abstract Trichomoniasis is the most prevalent non-viral sexually transmitted infection globally, relying on both innate and adaptive immunity to combat the extracellular pathogen Trichomonas vaginalis. This study included 45 infected women and 45 healthy controls in Baqubah, Iraq, from November 2023 to July 2024. Serum levels of IgM, IgG, and interleukin-37 (IL-37) were measured using ELISA, while genotyping of IL-37 SNP rs3811047 was performed using PCR-SSP. Results revealed the highest infection rate (14%) among women aged ≤25 years. Infected women showed a significant increase in IgM (2.09) and IgG (14.32) compared to controls (IgM: 0.07; IgG: 0.23), with p=0.00. IL-37 levels were also significantly elevated in the infected group (14.29) versus controls (7.35), p=0.00. Sanger sequencing of rs3811047 identified AA, GG, and AG genotypes, suggesting possible associations between A and G alleles and susceptibility to infection. The findings highlight a strong immunological response to T. vaginalis and potential genetic factors influencing host defence.

1 INTRODUCTION

Trichomonas vaginalis is the causative agent of trichomoniasis, a common human urogenital infection. It is the most common, treatable, non-viral sexually transmitted disease (STI) worldwide, with an estimated 156 million cases among men and women aged 15 - 49 years [1].

Trichomonas vaginalis is endemic in Iraq, where several epidemiological studies over the past decade in several Iraqi cities, along with Baghdad, Kufa, Najaf, and Mosul, have confirmed effective diagnosis of infection amongst ladies attending governmental hospitals [2], [3]. Although contamination fees are almost equal in ladies and men, occurrence and signs and symptoms are drastically better in ladies (99 million instances in ladies compared to eleven. Five million instances in guys) [1].

The maximum usually used diagnostic methods encompass microscopic examination of vaginal moist swabs, that is cheaper however insensitive [4]. Genetic investigations can shed slight on drug resistance, pathobiology, aetiology, and different epidemiological capabilities of Trichomonas vaginalis and perceive crucial genes [5]. Various strategies were used to perceive and hit upon Trichomonas vaginalis, which includes monoclonal antibody binding antigenic profiling and real-time polymerase chain reaction (RT-PCR) [6]. The host-parasite interplay in trichomoniasis is complicated. It relies upon at the host immune reaction to the trichomoniasis and the virulence elements of the parasite. This results in pathological consequences via the break out of the parasite from the permanent physical barrier of the lady reproductive tract after powerful mobile adhesion of the parasite to epithelial cells and a success destruction and phagocytosis of target cells, ensuing in continual contamination. The energetic or passive immune reaction of T cells of the inflamed host play essential roles in controlling or exacerbating trichomoniasis [7]. Cytokines, along with interleukins, are a huge organization of proteins, which includes peptides and glycoproteins, secreted by specific cells within the immune gadget. They are produced throughout the frame with the aid of diverse immune cells [8].

IL-37 is a dual characteristic cytokine with both intracellular and extracellular capabilities that has large and complex anti inflammatory and immunomodulatory results, slows the unfold of inflammation, and forestalls tissue harm because of inflammation. These outcomes are caused by suppressing the development of some inflammatory cells, producing cytokines, and activating transcription factors and signalling kinases [9].

This case-control study aimed to explore some immunologic parameters (IgM, IgG, IL-37) and the analysis of the genetic sequence of the single nucleotide polymorphisms (SNP of IL-37).

2 MATERIALS AND METHODS

2.1 Sample Collection and Laboratory Methods

The current study was conducted on women with Trichomoniasis who visited Al-Batoul Teaching Hospital and private gynecology clinics in Baqubah, Diyala Governorate, during the period from November 2023 to July 2024. Pregnant women were excluded from this study.

In this study, 5 ml venous blood was taken from the participants and divided into two parts: 3 ml was placed in gel-containing tubes for immunologic investigations IgM, IgG, IL-37 by ELISA technique. The other 2 ml was put in EDTA-containing tubes and kept in the refrigerator for genetic testing. DNA was then extracted using a Promega kit to conduct genetic screening and polymorphism screening of IL-37 using the Sequence-specific primer-PCR (PCR-SSP) technique.

Primers of IL-37F genes were: GTAAAACGACGGCCAGTGCACAGACCCAGT TGTTT, and of IL-37R were: CAGGAAACAGCTATGACGCTCATCTTTCCCG AGTTATC. After PCR amplification, agarose gel electrophoresis became used to confirm the presence of amplification. PCR changed into absolutely dependent on the extracted DNA standards.

2.2 Statistical Analysis

In this study, the Statistical Package for Social Sciences (SPSS-25) become used for statistical evaluation. The statistics have been usually dispensed and expressed as (mean ± SD). An unbiased pattern t-check and one-way evaluation of variance (ANOVA) have been used to evaluate between corporations. The genetic assessments had been finished by Hardy-Weinberg equilibrium (HWE), Fisher's exact chance and Sanger sequencing.

3 RESULTS

The cutting-edge study included 90 women divided into two groups. The first group comprised 45 patients infected with *Trichomonas vaginalis*, with ages ranging from 19 to 43 years and an average age of 32 years. The second group included 45 uninfected controls (healthy women), with ages ranging between 34 and 43 years and an average age of 34 years. The results demonstrated that the highest percentage of *Trichomonas vaginalis* infections (14%) occurred among women in the age group ≤25 years, while the lowest percentage (7%) was observed in the 26-30 years age group, as validated in Table 1.

Table 1: Distribution of study groups according to age.

Cases	≤25 years	26-30 years	31-35 years	36-40 years	≥41 years
Infected	14%	7%	10%	10%	9%
Control	10%	6%	8%	11%	15%
Total	24%	13%	18%	21%	24%

The results in Table 2 showed that there was a highly significant increase in the mean and standard deviation of IgM antibody in women with Trichomoniasis (2.09±0.77) compared to the mean and standard deviation of this antibody in healthy women (0.07±0.12) (p=0.00). The results in Table 2 also showed a highly significant increase in the mean and standard deviation of IgG antibody in women with Trichomoniasis (14.32±6.67) compared to the mean and standard deviation of this antibody in the control group (0.23±0.13) (p=0.00).

Table 3 shows observed and expected numbers and percentage frequencies and Hardy-Weinberg equilibrium (HWE) of IL37 SNP rs3811047 genotypes and alleles in trichomoniasis patients and controls.

The statistical analysis of associations between IL37 SNP rs3811047 genotypes or alleles in trichomoniasis and controls is summarized in Table 4.

Sanger sequencing was used to analyze the IL37 gene's rs3811047 SNP as shown in Figure 1.

Table 2: Mean and SD of immunoglobulins (IgM and IgG) among the study groups.

Antibody type	Mean±SD Infected women	Mean±SD Controls	P value
IgM	2.09±0.77	0.07±0.12	0.00**
IgG	14.32±6.67	0.13±0.23	0.00**

**There are significant differences at a significance level of 0.01.

Table 3: Observed and expected numbers and percentage frequencies and Hardy-Weinberg equilibrium (HWE) of IL37 SNP rs3811047 genotypes and alleles in trichomoniasis patients and controls.

Genotyping of IL37 SNP rs3811047	Patients group No. (%)		Control group No. (%)	
	Observed	Expected	Observed	Expected
AA	3(3.2)	15(16%)	0 (0.8)	0(16)
AG	10 (9.6)	50 (48%)	4 (2.4)	80 (48)
GG	7 (7.2)	35 (36%)	1 (1.8)	20 (36)
Total	20 (100.0)	100 (100.0)	5(100.0)	100 (100.0)
A	16 (40%)	Not estimated	4 (40%)	Not estimated
G	24 (60%)	Not estimated	6 (60%)	Not estimated
P-HWE	0.8522		0.136	

Table 4: Statistical analysis of associations between IL37 SNP rs3811047 genotypes or alleles in trichomoniasis and controls.

Type of Comparison		Relatives risk (%)	OR	95% CI	Fisher's exact probability
Patients Versus Controls	AA	9.1	2.20	0.12-38.81	1.000
	AG	6.0	0.25	0.03-2.12	0.341
	GG	22.0	2.69	0.31-23.23	0.628
Patients Versus Controls	A	37.8	1.40	0.26-3.87	1.000
	G	10.0	1.00	0.26-3.87	1.000
OR - odds ratio, 95% CI - confidence intervals					

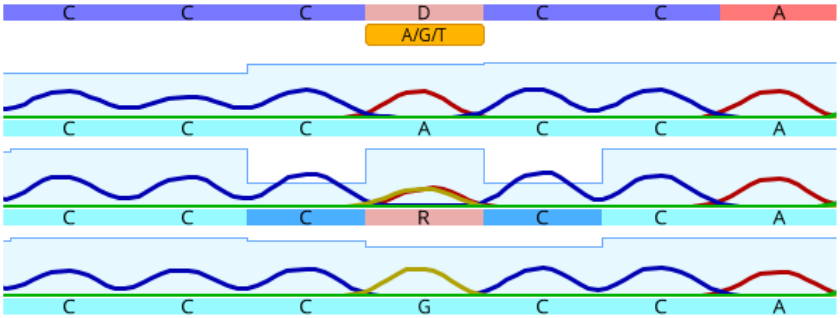


Figure 1: Sanger sequencing become used to analyze the IL37 gene's rs3811047 SNP. A unmarried "A" peak indicates the presence of an A homozygous allele. A unmarried "G" top indicates a G homozygous allele. The presence of the "A" and "G" top indicates the A/G heterozygous allele.

The analysis of the IL-37 gene polymorphism (rs3811047) revealed significant associations between specific genotypes and disease susceptibility. The homozygous AA genotype was notably more prevalent in patients than in controls (63.2% vs. 18%), suggesting a strong risk association (OR = 2.20, $p < 0.0001$). This indicates that individuals carrying the AA genotype may have a higher predisposition to develop the disease=

Similarly, the A allele frequency was higher in patients (40%) compared to the control group (44%), with a moderate risk association (OR = 1.40, p = 0.01), supporting its potential role as a contributing risk factor.

Interestingly, the homozygous GG genotype was also more frequent in patients (18%) than in controls (7%), indicating another risk association (OR = 2.69, p = 0.028). However, the G allele showed equal frequency between patients and controls (60% vs. 60%), with no observed risk effect (OR = 1.00, p = 1.000).

Collectively, these results suggest that both homozygous AA and GG genotypes might act as significant genetic risk factors in the pathogenesis of the disease, whereas the allelic distribution alone (especially for G) may not fully explain the observed associations. Further functional studies are warranted to clarify the biological mechanisms underlying these genetic effects.

4 DISCUSSION

The present day study was performed on 45 women with Trichomoniasis and (45) healthy uninfected women as a control group. Our results showed that the highest infection rate occurred in the age group (≤25) years and the lowest percentage of infections occurred among women in the age group 26-30 years, and this result agreed with Sutton et al [10] who showed a higher rate of infection among young women aged 18-25 years and also agreed with Tine et al.'s study [11] who showed that women under 25 were the most affected. However, our results did not agree with Younis and Elamami who observed that trichomoniasis was higher in the age group more than 40 years [12].

As for the emergence of high rates of infection in young women, it could be attributed to sexual activity and the high concentration of sex hormones, as well as the increase in glycogen, which causes the pH to shift to the alkaline side. Also, the high rates of infection in middle-aged women may have been due to previous infections that were cured and the antibodies to the parasite remained [13].

With regard to immunoglobulins (IgM and IgG), our results pointed out a relatively large increase in their levels in women infected with Trichomonas vaginalis. These results were in agreement with the study of [13], which showed the increased levels of IgM and IgG in women infected with trichomoniasis. Moreover, in the last forty years the presence of anti-T. Vaginalis antibodies (IgA, IgM, and IgG, and its subclasses) in serum and cervicovaginal secretions has been established by radioimmunoassay, ELISA, and immunofluorescence techniques [14]. Immune variables play an important role in maintaining human health. Infection with some parasites and changes in those variables can result in a variety of symptoms and signs, leading to increased production of immune cells such as B and T lymphocytes. This furthermore results in increased levels of antibodies such as IgG and IgM [15].

IgM is the first type of antibody secreted during the primary immune response, and therefore its presence in plasma is considered a diagnostic tool. It indicates the presence of a recent infection, and due to the multiple binding sites for antigens, it has a high capacity to agglutinate antigens [16]. IgG is the most abundant form of antibody in plasma, as it represents 70-80% of all antibodies. It is characterized by its ease and speed of movement in cells because it is smaller in size and more abundant. It protects against various types of disease causes such as bacteria, viruses, fungi, and toxins [17].

The understanding of the mechanisms involved in the interaction between T. Vaginalis and the host immune response may contribute to the development of new goals to combat the parasite [18].

The IL-37 gene is located on chromosome 2q12-13, close to the regulatory regions of the IL-1a and IL-1b genes. This specific location may be critical for the positioning of IL-37 as an inflammatory response inhibitor. The IL-37 gene is situated on chromosome 2q12-13, which is extremely close to the regulatory regions of the IL-1a and IL-1b genes. This particular position may be critical for the function of IL-37 as an inflammatory response inhibitor [19].

Among the cytokines is IL-37, and its immunological role in viral, bacterial, and fungal infections is receiving increasing attention [20]. Furthermore, accumulating data revealed that SNPs of the IL37 gene are conserved across humans through selection force, and their potential participation in the control of immune responses and susceptibility to human disorders has been hypothesized [21].

IL-37 functions as an anti-inflammatory cytokine, inhibiting inflammatory reactions by influencing the production of pro-inflammatory cytokines [22]. Because of this immunological

characteristic, IL-37 has been shown to play an important role in the pathogenesis of a variety of inflammatory and autoimmune illnesses, and dysregulated IL-37 expression has been reported under these conditions. Furthermore, the antiviral, antibacterial, and antifungal properties of IL-37 have also been identified [20].

To the best of our knowledge, the SNPs of IL-37 have not been examined in any human disease, therefore, limited information on their effects in human infectious diseases is available.

Genetic association studies have related several single nucleotide polymorphisms (SNPs) within the IL-37 gene to vulnerability to a number of autoimmune, inflammatory, and infectious diseases, including Behçet disease, systemic lupus erythematosus, periodontal infection, hepatitis viral infection, and tuberculosis [23].

A number of viral, inflammatory, and autoimmune diseases, including ankylosing spondylitis, rheumatoid arthritis, autoimmune thyroid disease, Behcet's disease, and Vogt-Koyanagi-Harada disease, have been linked to the polymorphism rs3811047 [24].

5 CONCLUSIONS

The present study identified a distinct epidemiological pattern, with the highest rate of T. vaginalis infection observed among women aged ≤25 years. From an immunological perspective, the infection triggered a pronounced immune activation, as evidenced by significantly elevated levels of IgM and IgG (indicating a robust humoral response) and IL-37 (suggesting a modulation of the inflammatory response) compared to healthy controls. Additionally, our investigation into the genetic variant rs3811047 of the *IL-37* gene revealed a distribution of AA, GG, and AG genotypes that implies a potential genetic predisposition to infection, although this requires validation in a larger cohort. In summary, these findings underscore the complex interaction between T. vaginalis and the host's immune system, involving both humoral and cellular mechanisms. This work contributes to the growing body of evidence on the immunology of trichomoniasis and opens avenues for further research into genetic factors influencing disease susceptibility.

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The Antioxidant Effects of Tamarind Fruit Extract Against Hepatic and Renal Function Disorders in Diabetic Rats

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Keywords: Tamarind Fruit, Antioxidant, Health-Promoting Effects.

Abstract: Tamarind contains a good amount of bioactive components with potential health benefits. Therefore, the current study was conducted to analyze the antioxidant effects of tamarind fruit extracts in alloxan-induced diabetic rats. The number of experimental rats was 49, which were distributed into seven groups: Group 0 (G0) as a control group, Group 1 (G1) as a diabetic group, Groups 2 and 3 (G2 and G3) as diabetic treatment groups that received aqueous (G2) and ethanolic (G3) tamarind extracts at a dose of 500 mgkg-1 body weight, and Groups 4 and 5 (G4 and G5) as non-diabetic groups that received (aqueous tamarind extract G4, at 500 mgkg-1 body weight) and (ethanolic tamarind extract G5, at 500 mgkg-1 body weight). Group 6 (G6), the diabetic, was treated with insulin at a dose of 6 IU/kg body weight, with all groups having access to food and water ad libitum, and the study period was 30 days. At the study's conclusion, the rats were euthanized, and their serum samples were analyzed for biomarkers, including liver enzymes (ALT, AST, ALP), kidney markers (creatinine and urea), antioxidant enzymes (SOD, CAT and GPx), and an indicator of oxidative stress (MDA). The results showed that administration of tamarind extracts (500 mgkg-1 body weight) led to a significant reduction in the liver enzymes levels, creatinine, urea, and MDA in the serum of diabetic rats (G2 and G3), compared to the diabetic group (G1), which exhibited elevated levels of these biomarkers. The results indicate that tamarind extracts possess antioxidant and hepatic and renal-protective properties, mitigating alloxan-induced oxidative stress and organ damage.

1 INTRODUCTION

Diabetes mellitus is a prevalent and progressing metabolic disorder, and its metabolic disturbances are directly associated with increased mortality and morbidity. Therapeutic use of natural substances may be more effective and safer than synthetic hypoglycemic agents in the therapeutic management of diabetes, providing a more cost-effective solution for various diseases and conditions. This growing interest in natural phytochemicals as potential treatments for many diseases, including diabetes, is driven by the high costs of medications and the adverse effects associated with synthetic drugs. Traditional therapies derived from natural substances have demonstrated significant efficacy in controlling diabetes [1].

Some studies suggest that an ethanolic extract of tamarind (Tamarindus indica) may mitigate the hepatotoxic and nephrotoxic effects of diabetic medications, as daily oral administration of tamarind extract for 30 days demonstrated significant improvement in pancreatic histological abnormalities, as demonstrated by histopathological analysis. Significant improvements were also observed in pancreatic tissue, suggesting potential therapeutic benefits. The proposed mechanisms behind these effects are likely attributable to tamarind's antioxidant properties and tissue protective effects. Therefore, the use of this extract as a therapeutic agent may hold scientific interest [2].

Experimental research has revealed that tamarind possesses anti-inflammatory, antioxidant, and protective properties against heart, kidney, and liver damage [3]. It has also been shown to reduce blood pressure and cholesterol levels, in addition to exhibiting antibacterial activity [4]. This study assessed the antioxidant characteristics of both aqueous and ethanolic extracts of tamarind in diabetic rats, demonstrating their potential therapeutic efficacy.

2 MATERIALS AND METHODS

2.1 Chemical Materials and Reagents

Ghaia Al-marafain of Babylon, supplied the chemicals employed in the study. Biomerieux, France, supplied the analytical kits for glucose GOD, aspartate transaminase (AST) (GOP), alanine transaminase (ALT) (GPT), alkaline phosphatase (ALP) (RandoxLa. (France)), creatinine, and urea (Biomerieux. (France)), while the local market provided the rat feed components. Insulin was sourced from a local pharmacy in Iraq, while alloxan monohydrate was procured from CDH (India). Elabscience, (Korea), supplies commercially available kits used for the assessment of rat glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD). Tamarind was obtained from local markets in Al-Najaf for the study.

2.2 Tamarind Sampling and Extract Preparation

Tamarind fruit samples were collected to prepare the extract. The plant material was dried. An accurate amount of the dry sample was weighed and placed in a beaker, then soaked in distilled water, ensuring that the sample was completely submerged. The mixture was incubated overnight and then filtered through Whatman No. 1 filter paper. The filtrate was concentrated to be more clear, centrifugation was performed to enhance filtration, and the filtrate was then allowed to dry completely in an oven at 40°C . The extract was then skimmed and collected, and the process was repeated until the desired amount was obtained. This process was used to prepare the aqueous extract [5]. For the ethanolic extract, the same procedure was followed, replacing the ethanol with distilled water.

The dose used in the study was 500 mgkg-1 for the aqueous extract and the same for the ethanolic extract. Based on the animal's weight, the dose was calculated using the following equation and prepared by dissolving the extract in 1 ml of distilled water.

$$Dose(mg) = 500(mg) \times Rat\ weight(g) / 1000g$$.

2.3 Acquisition of Animal Habitation and Management

Forty-nine euglycemic male mature rats, each weighing 215 g and aged 10 weeks, were obtained from the Kufa Institute of Sciences and adapted to a standard diet for 14 days in the institution's animal facility prior to the experiment. The plastic enclosures housed the animals, but the hardwood flooring was renewed regularly to uphold sanitation and avert infection. A temperature of 24 °C was sustained for 12 hours (12 a.m.–12 p.m.) in a climate-regulated environment for rats. Rats were given unlimited access to fresh water and a regular meal consisting of sunflower oil, dextrose, casein, starch, minerals, and vitamins. Body weight was assessed on the initial and concluding days of the experimental phase. The university's animal ethics board sanctioned all procedures and protocols necessary for the trial and analysis before the initiation of the investigation.

2.4 The Study Design

Forty-nine rats were allocated into into seven groups, each including seven , with the treatment groups, dosages, and administration methods as follows:

- **G0:** Control (Health rats (non- diabetic**).** Orally given 1 ml 0f 0.9% NaCl solution.
- **G1**: Diabetic control Rats administered a single dosage of alloxan (150 $mgkg^{-1}$, by intraperitoneal injection).
- **G2**: Diabetes + *T.indica* aqueous extract. Diabetic rats treated with (500 $mgkg^{-1}$/ B.W./day) *T.indica* aqueous extract gavage: *T.indica* was dissolved in distilled water just before use.
- **G3**: Diabetes + *T.indica* ethanolic extract. Diabetic rats treated with (500 $mgkg^{-1}$/ B.W./day) *T.indica* ethanolic extract gavage: *T.indica* was dissolved in distilled water just before use.
- **G4**: *T.indica* aqueous extract. Non-diabetic rats Orally given (500 $mgkg^{-1}$/ B.W./day) *T.indica* aqueous extract gavage.
- **G5**: *T.indica* ethanolic extract. Non-diabetic rats Orally given(500 $mgkg^{-1}$/ B.W./day) *T.indica* ethanolic extract gavage.
- **G6**: Diabetes + insulin. Insulin-treated diabetic rats (6 IU/kg/day). Insulin was delivered subcutaneously on a daily basis.

2.5 Haematological Chemistry Assessment

After collecting the blood sample, the serum was centrifuged and then left undisturbed for evaluation using a blood chemistry analyzer (Siemens, Munich,

Germany) to measure liver enzyme levels (AST, ALT, & ALP), creatinine , and urea.

2.6 Antioxidant Metrics and Malondialdehyde

Photometry was utilized to evaluate the activity of SOD, CAT, and GPx in rats, as specified by [6], using the Elabscience (Korea). The enzymatic activities of SOD, CAT, and GPx in the samples of blood animales were quantified in international units per milliliter (IU/L). A photometric method was utilized to determine the MDA concentration in erythrocytes, as cited in [7]. The MDA concentration was measured in millimoles per liter (mmol/L).

2.7 Induction of Type 1 Diabetes and Fasting Serum Glucose Levels

Rats were fasted for 12 hours overnight and then given an intraperitoneal injection of alloxan monohydrate (150 mg/kg/body weight dissolved in sterile normal saline) to induce diabetes [8].Fasting blood glucose was initially measured during diabetes induction, and a subsequent assessment was performed at the end of the experiment (after 30 days). To assess blood glucose levels, the animals' tails were sterilized with 10% alcohol, amputated using scissors, and blood was allowed to drain into contact with the test strip according to the methods described in [9].

2.8 Statistical Analysis

Data were reported as mean ± standard error (M ± S. E.) and analyzed using SPSS. Data were also analyzed using one-way analysis of variance (ANOVA) to assess the presence of statistically significant differences between the studied groups. This type of ANOVA was chosen based on the study design, which involved comparing multiple independent groups. Following ANOVA, a post-hoc LSD (least significant difference) test was applied to determine which groups differed statistically from each other. The LSD test was chosen because there were no statistically significant violations of the assumptions of ANOVA (such as normal distribution and homogeneity of variances). A P-value < 0.05 was set for statistical significance.

3 THE RESULTS

3.1 Effect of Tamarind Fruit Extracts on Liver Enzymes in the Serum of Rats

Table 1 indicates that the induction of diabetes resulted in a remarkable elevation ($P< 0.05$) in the effectiveness of AST, ALT, and ALP in male rats of group G1, with values of (157.4 ± 2.45, 172.4 ± 1.77, & 453.5 ± 2.97) IU/L in sequence, contrasted to the control group G0 (40.4 ± 3.49, 37.0 ± 2.79, & 108.8 ± 2.79) IU/L, respectively, as well as the other groups. The enzymatic activity in G2 and G3 considerably reduced ($P < 0.05$) compared to G1.

3.1 Impact of Tamarind Fruit Extracts on Renal Function in the Serum of Rats

Table 2 displays the results of several kidney parameters, including blood creatinine and urea levels, from rats administered tamarind fruit extracts in comparison to diabetic controls. The mean blood creatinine and serum urea concentrations in the control group (G0) were 0.43 ± 0.03 & 25.0 ± 2.25 mg/dL, respectively, whereas in the diabetic group (G1), its values were 0.85 ± 0.01 & 63.0 ± 1.35 mg/dL, respectively. The treated rats with tamarind fruit extracts exhibited improved results compared to the opposite diabetic group.

3.3 Influence of Tamarind Fruit Extracts on Anti-Oxidant Parameters

Reducing oxidative stress and improving organismal health are two of the primary functions of antioxidants including SOD, CAT, and GPx When comparing the diabetic group (G1) to the non-diabetic (G0), the levels of (SOD, CAT, and GPx) in the former were essentially lower (11.2 ± 1.41, 12.0 ± 1.31, & 17.7 ± 1.23 IU/L, respectively) ($p < 0.05$). The efficacy of the treatment group (G5) in terms of plasma SOD, CAT, and GPx was substantially different ($p < 0.05$). With comparison to G0 and other groups, they are relatively high at (55.8 ± 2.53, 65.5 ± 3.39, & 61.7 ± 3.06) IU/L. The diabetic rats in G1 had a considerably higher plasma MDA level (87.5 ± 2.19 mmol/L) than the non-diabetic rats in G0 (32.0 ± 2.98 mmol/L), according to the results in Table 3 and Figure 1.

Table 1: Liver enzymes activity in the serum of rats (n=7) ,following 30 days treatment with tamarind fruit extracts.

Groups	Liver enzymes IU/L		
	AST	ALT	ALP
	Mean ± S. E		
G0	40.4 ± 3.49 c	37.7 ± 2.79 c	108.8 ± 2.79 c
G1	157.4 ± 2.45 a	172.4 ± 1.77 a	453.5 ± 2.97 a
G2	45.7 ± 4.17 c	37.8 ± 3.38 c	116.4 ± 3.70 c
G3	41.1 ± 2.56 c	36.4 ± 2.77c	109.5 ± 4.80 c
G4	39.5 ± 0.71c	36.0 ± 3.52 c	109.0 ± 4.11c
G5	39.0 ± 1.74 c	33.8 ± 0.89 c	108.0 ± 3.28 c
G6	66.7 ± 3.44 b	54.8 ± 4.10 b	153.0 ± 1.96 b
LSD	8.31	8.49	10.1

The whole of the data is shown as mean ± standard error. Means designated by various superscript letters are remarkably different ($p < 0.05$).

Table 2: Kidney function in the serum of rats (n=7), following 30 days of treatment with tamarind fruit extracts.

Groups	Creatinine mg/dL	Urea mg/dl
	Mean ± S.E	
G0	0.43 ± 0.03 c	25.0 ± 2.25 cd
G1	0.85 ± 0.01 a	63.0 ± 1.35 a
G2	0.47 ± 0.02 c	27.7 ± 1.03 c
G3	0.45 ± 0.04 c	25.6 ± 2.12 cd
G4	0.42 ± 0.02 c	23.5 ± 2.18 cd
G5	0.40 ± 0.01 c	33.3 ± 0.63 d
G6	0.58 ± 0.04 b	41.3 ± 1.74 b
LSD	0.091	4.98

The whole of the data shown as mean ± standard error. Means designated by various superscript letters are remarkably different ($p < 0.05$).

Table 3: Antioxidant parameters and malondialdehyde levels in the serum of rats (n=7), following 30 days treatment with tamarind fruit extracts.

Groups of study	SOD (IU/L)	CAT (IU/L)	GPX (IU/L)	MDA (mmol/L)
	Mean ± S.E			
G0	42.5 ± 4.44 b	55.2 ± 2.87 b	44.8 ± 2.62 bc	32.0 ± 2.98 c
G1	11.2 ± 1.41 d	12.0 ± 1.31d	17.7 ± 1.23 e	87.5 ± 2.19 a
G2	41.0 ± 2.31 b	50.7 ± 4.34 b	40.6 ± 1.55 c	39.7 ± 3.08 c
G3	41.8 ± 2.75 b	52.2 ± 4.00 b	46.2 ± 3.50 bc	37.3 ± 2.20 c
G4	44.6 ± 1.48 b	57.9 ± 1.55 ab	48.9 ± 2.17 b	32.9 ± 2.28 c
G5	55.8 ± 2.53 a	65.5 ± 3.39 a	61.7 ± 3.06 a	21.4 ± 6.16 d
G6	23.9 ± 2.74 c	40.5 ± 3.52 c	31.8 ± 2.99 d	52.0 ± 4.06 b
LSD	7.80	9.24	7.44	10.2

The whole of the data shown as mean ± standard error. Means designated by various superscript letters are remarkably different ($p < 0.05$).

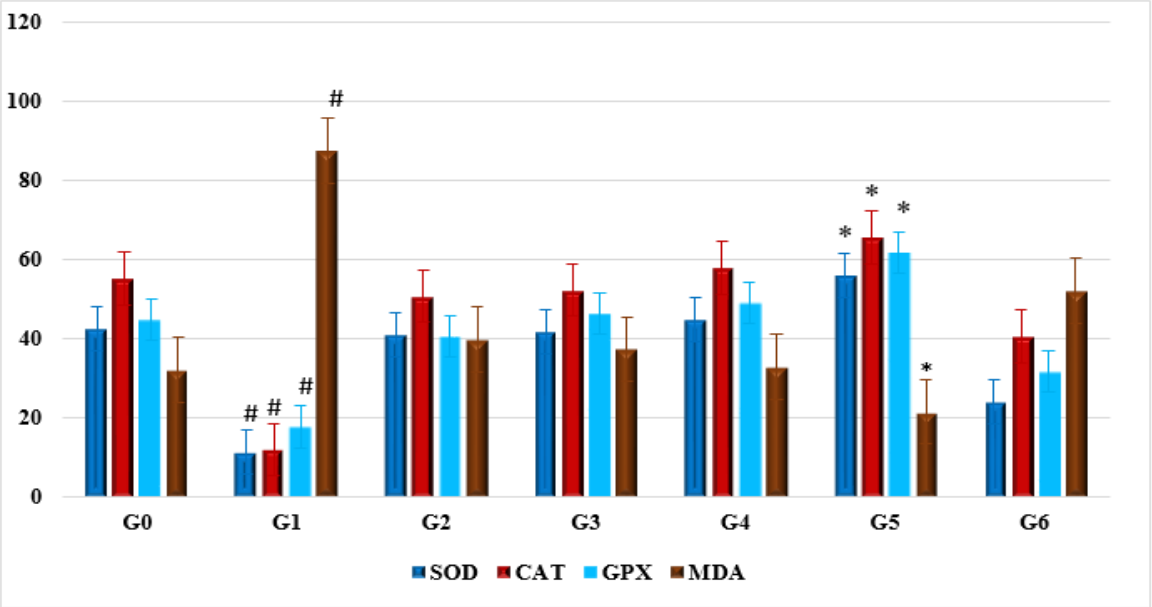


Figure 1: Antioxidant parameters (SOD, CAT & GPX) (IU/L) and MDA levels in the serum of rats (n=7), administered tamarind fruit extracts for a duration of 30 days, sig = # ,* at ($p < 0.05$).

4 DISCUSSION

The findings show that consumption of tamarind fruit affected liver enzymes, renal function, antioxidants, and the oxidative stress marker MDA. Compared with the control group and other groups, the diabetic group showed a significant increase in the activity of liver enzymes (AST, ALT, and ALP). Consistent with previous research, the current study found that injecting male rats with 150 mg/kg of alloxan induced diabetes [10]. The elevated levels of the oxidative stress factor (MDA) in this group indicate that increased free radical production resulting from drug toxicity likely leads to increased lipid peroxidation. When the selective permeability of liver cell membranes is impaired due to oxidative stress, a large amount of liver enzymes is released into the blood [11].

Diabetic groups treated with either an aqueous or ethanolic extract of the fruit showed significant improvements in liver enzyme activity compared to the diabetic group. In a previous study, rats were given a toxic dose of paracetamol (1 g/kg orally) for 7 days and were treated with aqueous extracts of various parts of the tamarind plant for 9 days, including 350 mg/kg orally for the fruit and leaves and 700 mg/kg orally for the seeds. This resulted in the plant's hepatoprotective effects; there was a significant decrease in liver weight and necrosis, and the results showed that serum bilirubin, ALP, and AST levels were significantly reduced [12]. The liver benefits observed in this study are believed to be due to the antioxidant polyphenolic chemicals present in the fruit. These may protect liver cells from necrotic damage caused by free radicals resulting from drug toxicity [13].

Furthermore, compared to the diabetic group, the insulin-treated diabetic group showed decreased liver enzyme activity, which is consistent with some studies [14] that found similar results in diabetic rats. This improvement, which explains the observed decrease in enzyme activity, is believed to be due to insulin's effects on liver glucose levels and lipid metabolism.

The findings showed that, compared to the healthy group (G0), creatinine and urea levels in diabetic male rats (G1) were significantly higher. Multiple studies [10, 15, 16] have found similar increases in these markers in the alloxan-injected group compared to the control group, and our results are consistent with those findings. This is likely due to vascular damage to the kidneys and nephrons caused by elevated blood glucose levels associated with diabetes [17]. Renal damage may occur as a result of non-enzymatic protein accumulation and excessive free radical formation resulting from high glucose levels [18]. The glomerular filtration process is impaired when the functional units are damaged,

reducing their ability to function. Kidney function is responsible for excreting creatinine (a product of muscle metabolism) and urea (a product of protein metabolism), with the kidneys excreting more than 90% of both. Renal damage reduces their excretion of creatinine and urea, leading to their accumulation in the blood and elevated serum levels [17].

In addition, the diabetic groups receiving tamarind fruit extracts, either aqueous or ethanolic (G2 & G3), showed significantly lower creatinine and urea levels compared to the diabetic group (G1). The kidney- and liver-protective effects of tamarind (T. indica) extract and its antioxidant properties are said to be due to its high concentration of polyphenols, which effectively neutralize intracellular free radicals [18]. Insulin may eliminate free radicals, thus reducing damage to the renal cortex, which showed lower kidney function indicators (creatinine and urea) (G6) compared to the control diabetic group (G1).

According to the results of [19], diabetic male rats (G1) showed decreased activity of SOD, CAT, and GPx, while malondialdehyde levels were increased, in contrast to the control group. The main reason for decreased antioxidant enzymes and increased malondialdehyde levels may be excessively high blood glucose levels, which produce large amounts of free radicals, leading to decreased antioxidant efficacy and, consequently, oxidative stress [20]. However, the results were opposite for diabetic rats treated with the aqueous extract (G2) and diabetic rats treated with the ethanolic extract (G3) of tamarind fruit at a dose of 500 mg/kg, as the antioxidant enzymes SOD, CAT, and GPx showed increased activity, while MDA levels decreased compared to the diabetic group. The antioxidant activity is likely due to the synergistic interaction of the many phytochemicals that the extract may contain. This interaction prevents free radicals, such as hydrogen peroxide, from initiating or stopping their chain reactions and may indirectly act as an antioxidant by increasing the activity of free radical-scavenging enzymes or enhancing their expression [21].

In comparison to the control and other groups, the rats that received ethanolic tamarind extract (G5) showed significantly higher levels of antioxidant enzymes and significantly lower levels of malondialdehyde (MDA). This effect is attributed to the presence of two phenolic compounds in the extract: orcinol (1-(2-furanyl)-1-propanone) and furyl hydroxymethyl ketone. Flavonoids in the extract, such as succinic acid, 3-methylbut-2-yl 3-heptyl ester, tartaric acid, 1,2-benzenedicarboxylic acid, and butyl acid, also have antioxidant properties [22]-[24]. The structural properties and hydroxyl groups of these compounds are likely responsible for their ability to neutralize free radicals by donating hydrogen atoms.

These findings highlight the therapeutic potential of tamarind fruit in improving liver and kidney function and reducing oxidative stress levels in diabetic patients. However, further clinical research is needed to confirm these effects and determine safe and effective doses.

5 CONCLUSIONS

The findings of this study demonstrate that T. indica extracts, especially the ethanolic extract at a dose of 500 mg/kg body weight over 30 days, significantly improved liver and kidney functions in alloxan-induced diabetic rats. These improvements were evidenced by the reduction of elevated liver enzymes (AST, ALT, and ALP) and normalization of kidney biomarkers (creatinine and urea levels), indicating recovery of organ function. The ethanolic extract showed superior efficacy compared to the aqueous extract, likely due to its higher retention of bioactive antioxidants such as polyphenols and flavonoids. These compounds appear to mitigate oxidative stress (MDA) and enhance the activity of endogenous antioxidant enzymes (SOD, CAT, and GPx). The protective effects observed suggest that the ethanolic extract of T. indica may counteract the hepatotoxic and nephrotoxic effects associated with diabetes or its pharmacological treatments. Therefore, this extract shows promise as a complementary therapeutic agent in preventing or reducing diabetes-induced organ damage. These results provide scientific justification for the traditional use of T. indica in managing chronic conditions and support further investigations into its potential clinical applications.

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Evaluation of Aqueous Flaxseed Extract on Liver Histological Structure in Diabetic-Induced White Mice

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Keywords: Linum Usitatissimum , Liver, Hepatic Cells, Diabetes Mellitus, Alloxan.

Abstract: This study aimed to evaluate the effectiveness of an aqueous extract of flaxseed on liver histology in diabetic rats. The results of the study showed that the liver of animals induced with diabetes had many histological changes, including the appearance of congestion in the central vein surrounded by hepatocytes that appeared irregular in shape. It was also noted that there was a loss of the radiographic appearance, irregularity in the venous sinusoids, expansion of the liver sinusoids, necrosis in the wall of the central vein, and the appearance of active transport vesicles. Nuclear death was also observed in some hepatocytes, and the cytoplasm of the hepatocytes was necrotic. The study also showed hypertrophy and thickening of the nuclei. Fatty degeneration was also observed with necrosis of the hepatocytes, indicating the occurrence of hepatocyte degeneration and focal destruction. Infiltration of inflammatory cells was also observed. The two groups of animals treated with the aqueous extract of flax seeds at a concentration of 0.2 showed that the central vein was surrounded by more regular cells than in the two previous groups, and that there was less blood congestion in the central vein of the liver. The shape of the venous sinusoids was more regular in most sections, and there was less accumulation of Kupffer cells in them, as well as the disappearance or less decomposition of the nuclei in some liver cells. It was also noted that there was an improvement in the level of radial organization of cells in the liver parenchyma.

1 INTRODUCTION

Diabetes is one of the most common chronic diseases in the world, with the number of people affected increase significantly due to various factors, including unhealthy lifestyle, obesity, and genetic factors [1]. This disease is characterized by high blood glucose levels due to insufficient insulin production or tissue resistance to it, leading to disturbances in the metabolism of carbohydrates, fats, and proteins [2] . Diabetes can be classified into two main types: Type 1 diabetes is an autoimmune disease that leads to the destruction of insulin-producing beta cells in the pancreas, making patients need lifelong insulin therapy [3]. Type 2 diabetes, the most common form, occurs as a result of tissue resistance to insulin, leading to an abnormal increase in blood glucose levels [4]. Since the discovery of this disease, humans have developed many pharmaceutical treatments used to treat diabetes. These drugs and medications rely on different mechanisms to stimulate insulin secretion, reduce insulin resistance, or slow down glucose absorption [5]. Among these well-known treatments is metformin, which is the first-choice treatment for type 2 diabetes and belongs to the biguanide group. This drug works by reducing glucose production in the liver, improving insulin sensitivity in muscles and peripheral tissues, and finally reducing glucose absorption from the intestine [6]. Other treatments used to treat diabetes are sulfonylureas. This class includes medications such as glibenclamide and gliclazide, which stimulate the beta cells in the pancreas to secrete more insulin, and also effectively lower blood glucose levels [7]. However, these medications may lead to some side effects, such as severe hypoglycemia and weight gain, or gastrointestinal disorders including nausea, vomiting, diarrhea, bloating, and abdominal pain, which makes their use limited in some cases [8]. Despite the significant developments in the field of drug treatments for diabetes, the search for highly effective natural alternatives with limited side effects is still of interest to researchers around the world [9]. Physicians, pharmacists, and drug manufacturers

resort to using and preferring herbal medicines and drugs over chemical medicines in some cases for several main reasons, including the high degree of safety from side effects, the acceptability of these treatments to the majority of patients, their low cost, their low resistance to bacteria unlike chemical medicines, the growing medical and pharmaceutical interest in them, their availability, accessibility, and sustainability, which makes them a more popular option, especially with their complementary use with other treatments[10]. Certain plants have shown scientifically proven effects in lowering blood sugar levels and improving the body's response to insulin. Prominent among these plants are fenugreek [11], cinnamon [12], Aloe vera [13], Moringa oleifera [14], Curcuma[15], garlic [16], mulberry leaves [17] and others. These plants contain soluble fiber that slows the absorption of carbohydrates, improves insulin sensitivity, and reduces glucose levels, thus potentially lowering blood sugar levels in people with type 2 diabetes [18]. Despite these medicinal uses, these plants and their wastes have many other uses through which they can preserve human health and the environment. Rice husk [19], watermelon [20], orange peels [21], buckthorn leaves[22], and waste tea leaves [23] can be used to treat various toxic pollutants such as heavy metals [24], water hardness [25], organic dyes [26] and other plants from water and soil. Among these plants that have many beneficial medical and environmental uses are flax leaves [18]. Flax has been used as a food and medicine for centuries due to its numerous health benefits and nutrient-rich ingredients. Flax is high in fiber, omega-3 fatty acids, and proteins, making it an important nutritional component that promotes digestion, supports heart health, and helps control blood sugar levels [27]. Medicinally, flax has been used since ancient times to treat digestive disorders, reduce inflammation, and support skin health [28]. Some recent medical studies have shown that flaxseeds may help lower blood pressure [29], reduce harmful cholesterol levels [30], and improve insulin sensitivity [31], making them beneficial for people with diabetes and heart disease. Flaxseeds also possess antioxidant and anti-inflammatory properties, making them a natural choice for promoting overall health and preventing certain chronic diseases [28]. Despite these many therapeutic benefits, the use of flax seeds in treating diabetes is not at the desired level, as many people in many countries of the world are still ignorant of the complementary health benefits of consuming these seeds and their potential applications as useful ingredients in foods and pharmaceutical products [32]. Therefore, the current study aimed to investigate the effect of aqueous flaxseed extract on the histological composition of the liver in diabetic mice.

2 MATERIALS AND METHODS

2.1 Induce of Diabetes Mellitus in the Mice by Alloxan

Diabetes was induced in mice weighing 23–32 g after overnight fasting by a single intraperitoneal injection of freshly prepared alloxan monohydrate ($C_4H_2N_2O_4 \cdot H_2O$) at a dose of 150 mg/kg body weight, according to the method described by [33] .

2.2 Synthesis of Aqueous Linum Usitatissimum Extract

Flax seeds (Linum usitatissimum) were obtained from local markets and washed several times by tap water and then by deionized water to get rid of suspended impurities. The clean seeds were dried and crashed in an electric grinder more than once, until fine powder obtained. After that, the aqueous extract of flax seeds was prepared using the method mentioned in the study of [34].

The required dose of flax seed aqueous extract was prepared based on the lethal dose (LD50) of 1.5 mg/g body weight in mice. Two concentrations (doses) of the extract were selected to test its effect, namely (0.1 and 0.2) mg/g. The calculated doses of the aqueous solution of flax seed extract were calculated and injected into the experimental mice according to their weights according to the following equation:

$$\text{Dose of Linum usitatissimum extract} = \text{LD50} \times \frac{\text{weight of mice}}{1000\ \text{g}}.$$

2.3 Preparation of Experimental Mice

Laboratory white male mice (24 mice) weighing 23-32 g and aged 7-8 weeks obtained from the College of Veterinary Medicine, Tikrit University, were used in this study. These mice were randomly divided into four groups, the details of which were as follows: the first group was the control group with six mice, and the second group was the experimental group with 18 mice. The mice were injected with alloxan to induce diabetes, then the experimental group was

divided into three groups: the first group was injected with alloxan with six mice, the second group was diabetic and injected with flax seed aqueous extract at a concentration of 0.1 mg/g, and the third group was diabetic and injected with the extract at a concentration of 0.2 mg/g of body weight) daily for 30 days. On the last day, the mice were anesthetized with chloroform, then the animals were dissected and the livers were removed from their site. Then the samples were fixed in formalin solution 10% for 24 hours and then washed by tap water and transferred to 70% alcohol for preservation. The tissue sections were prepared according to the method describe in [35] then stained using Hars hematoxylin and eosin (H&E) stain according to the method followed in [36]

3 RESULTS AND DISCUSSION

3.1 Histological Changes in MICE Live

The results of the current study showed that the induction of diabetes in male mice led to several histological changes in the liver of the treated animals. These changes include the appearance of congestion in the central vein surrounded by hepatocytes that appear irregular in shape. The loss of the radiographic appearance, irregularity of the venous sinusoids, bleeding within the sinusoids, expansion of the liver sinusoids, necrosis of the central vein wall, and the appearance of active transport vesicles are also observed, as shown in Figure 1.

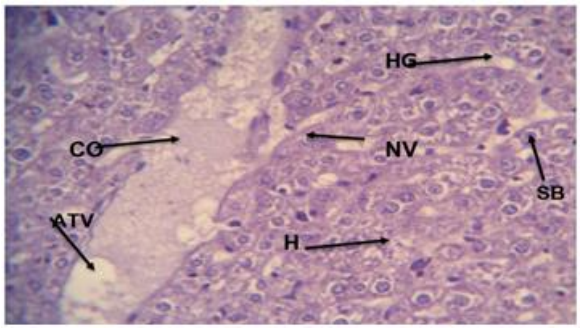


Figure 1: A cross-section of the liver tissue of male rats with alloxan-induced diabetes for 30 days shows central vein congestion (CO), central vein wall necrosis (NV), hepatocyte scattering (H), sinusoidal dilatation (SB), active transport vesicles (ATV), and sinusoidal hemorrhage (HG) (H&E 40X).

Nuclear death was also observed in some hepatocytes, and the cytoplasm of the hepatocytes was necrotic. The study also showed hypertrophy and thickening of the nuclei. Fatty degeneration was also observed with necrosis of the hepatocytes, indicating hepatocyte degeneration and focal destruction. Infiltration of inflammatory cells was also observed, as shown in the Figure 2.

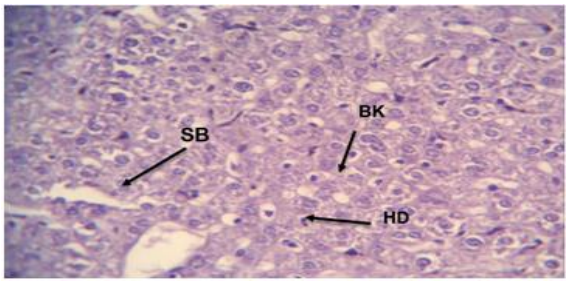


Figure 2: A cross-section of the liver tissue of male rats with alloxan-induced diabetes for 30 days shows the occurrence of severe necrosis and degeneration of hepatocytes (NC), degeneration of the nuclei of most hepatocytes (KA), expansion of the sinusoids (SB), enlargement of the hepatocytes (HT), thickening of the nuclei (BK), and degeneration of the hepatocytes (HD) (H&E 40X).

The current results also showed that the histological changes of the liver in the experimental group with diabetes were irregular in the hepatic parenchyma and around the central vein in the form of ropes, but rather irregular with the appearance of soft granules spread in the hepatic cells and the accumulation of glycogen granules inside the hepatic cells, the presence of a large number of dividing cells and cytoplasmic fatty explosion inside the hepatic cell, as shown in the Figure 3.

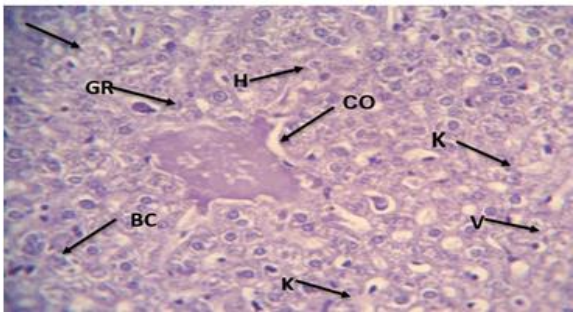


Figure 3: A section showing the irregularity of hepatocytes and expansion of blood sinusoids in the liver tissue of male mice with induced diabetes and the appearance of fine granules in the hepatocytes GR, the dissolution of most of the nuclei of the cells KA, Kupffer cells K. The explosion of dividing cells V BC, the scattering of hepatocytes H, the

occurrence of congestion of the central vein CO (H&E 40X).

3.2 Histological Changes in the Liver of Diabetic Rats Treated with Linum Usitatissimum

The results of the study showed congestion of the central vein in the diabetic group treated with flaxseed extract at a concentration of 0.1 mg/ml of body weight, as well as containing a blood clot, The results also showed irregularity in the rows of hepatic cells around the central vein in a radial manner, with the presence of a cluster of inflammatory cells and the appearance of active transport vesicles within the blood vessels, as shown in Figure 4.

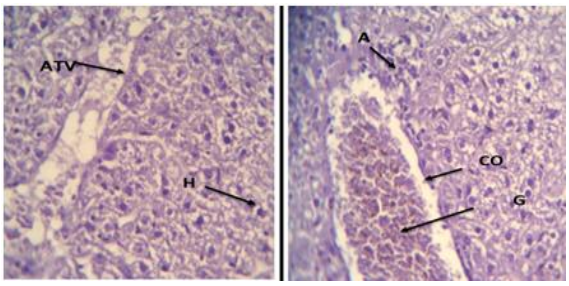


Figure 4: shows a cross-section of the liver tissue of male rats with induced diabetes and treated with flaxseed extract at a concentration of 0.1 mg/ml of body weight, showing congestion of the central vein (CO) and a blood clot (G), infiltration of inflammatory cells (A), irregularity of the rows of hepatocytes around the central vein (H), and active transport vesicles (ATV) (H&E stain 40X).

It was also noted that some hepatic cells contained more than one nucleus, and there was an enlargement of the hepatic cells and dilation of the sinusoids containing Kupffer cells and the occurrence of bleeding in them. Necrosis, rupture and infiltration of inflammatory cells were also found in the liver parenchyma, as shown in the Figure 5.

The tissue sections of the liver of the diabetic group treated with flaxseed extract 0.1 mg/ml of body weight showed nuclei disintegration, cell enlargement, and nuclei thickening in most sections, as shown in the Figure 6.

It is also noted that the basement membrane (inner) of the central vein was peeled off and there was clear damage to the liver tissue in the diabetic group treated with flaxseed extract 0.1 mg/ml, and there was degeneration and necrosis of the liver cells, as well as the rupture and thickening of the nuclei and the appearance of edema, as shown in the Figure 7.

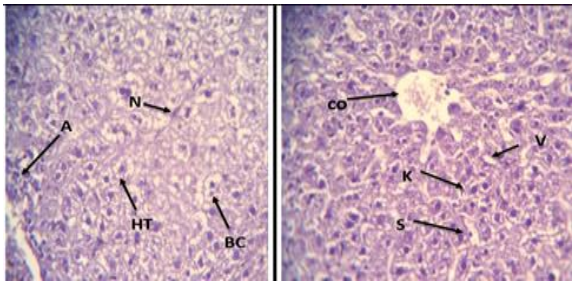


Figure 5: A cross-section of the liver tissue of male rats with induced diabetes and treated with flaxseed extract at a concentration of 0.1 mg/ml of body weight, showing: dilation of the blood sinusoids containing Kupffer cells (S), infiltration of inflammatory cells (A), necrosis of hepatocytes, (N) binuclear hepatocytes, (BC) hyperplasia of hepatocytes (HT), Kupffer cells (K), vacuolization (V), (H&E stain 40X.

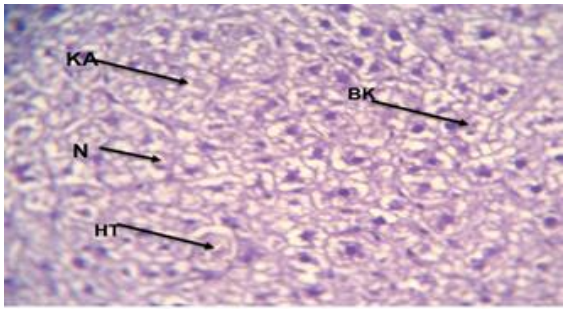


Figure 6: A cross-section of the liver tissue of male mice with induced diabetes and treated with flaxseed extract at a concentration of 0.1 mg/ml of body weight, hepatic cell hypertrophy (HT), degeneration of some nuclei (KA), necrosis of hepatic cells (N), and thickening of nuclei (BK) stained (H&E 40X).

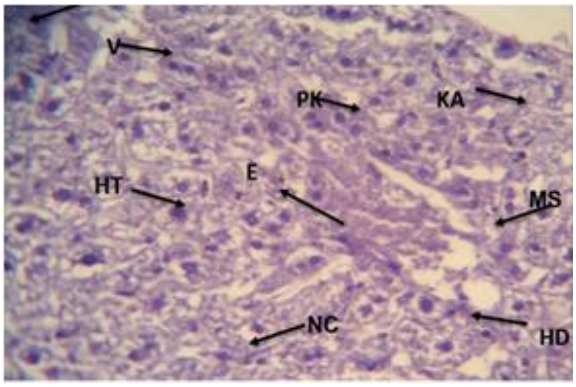


Figure 7: A cross-section of the liver tissue of male rats with induced diabetes and treated with flaxseed extract at a concentration of 0.1 mg/ml of body weight, sloughing of the central vein's inner membrane (MS), thickening of the

nuclei (PK), nucleolysis (KA), edema (E), necrosis of hepatocytes (NC), vacuolization (V), cell degeneration (HD), cell hyperplasia (HT) H&E 40X).

It was also noted in the sections that there was an accumulation of glycogen granules inside the liver cells, with the presence of some fluid accumulation (edema) inside the cells. It was also noted that there was a fatty cytoplasmic explosion inside the liver cell, as shown in the Figure 8.

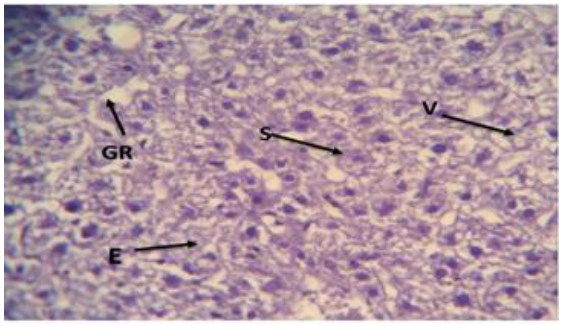


Figure 8: A cross-section of the liver tissue of male rats with induced diabetes and treated with flaxseed extract at a concentration of 0.1 mg/ml of body weight. Glycogen granules accumulated inside the hepatocytes (GR), (edema) E, cytoplasmic lipid vacuolation inside the hepatocyte (V), dilatation of the sinusoids (S H&E 40X).

3.3 Histological Changes in the Liver of Diabetic Rats Treated with Linum Usitatissimum

The results of the current study showed that the liver parenchyma of diabetic male rats treated with flaxseed extract at a concentration of 0.2 mg/ml of body weight for 30 days showed a central vein surrounded by more regular cells than in the two previous groups, as well as less blood congestion in the central vein of the liver, and the shape of the venous sinusoids was more regular in most sections, and there was less accumulation of Kupffer cells in them, as well as the disappearance or less death of nuclei in some liver cells, as shown in the Figure 9.

It was also noted in the diabetic group treated with flax extract that there was an improvement in the level of radial organization of cells in the liver parenchyma, which appeared almost normal, and the return of the organization of the liver cells in the form of hepatic cords. It was also noted that the division of the nuclei and liver cells occurred in a manner similar to the liver parenchyma in the control group, as shown in the Figure 10.

While some sections showed enlarged hepatic cells, degeneration of some cells and less nuclei degeneration were observed than in the two previous groups (Fig. 11).

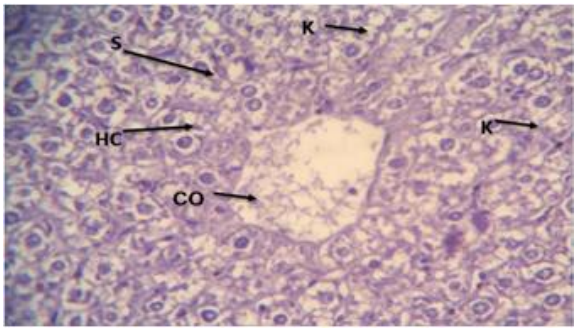


Figure 9: A cross-section of liver tissue in diabetic male rats treated with flaxseed extract at a concentration of 0.2 mg/ml for the central vein (CO), sinusoids, Kupffer cells (K), hepatocytes (HC), nuclei (K stained (H&E 40X).

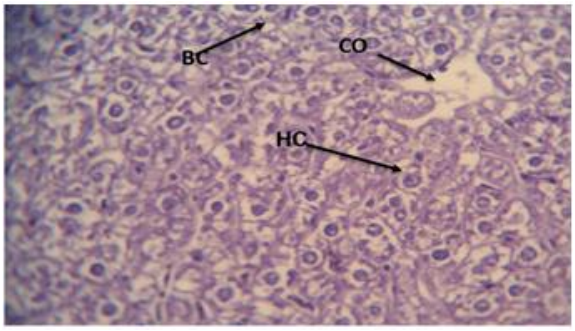


Figure 10: A cross-section of liver tissue in diabetic male rats treated with flaxseed extract at a concentration of 0.2 mg/ml. There was an improvement in the level of radial organization of cells in the liver parenchyma (HC), the central vein CO, and the dividing hepatocytes BC stained (H&E 40X).

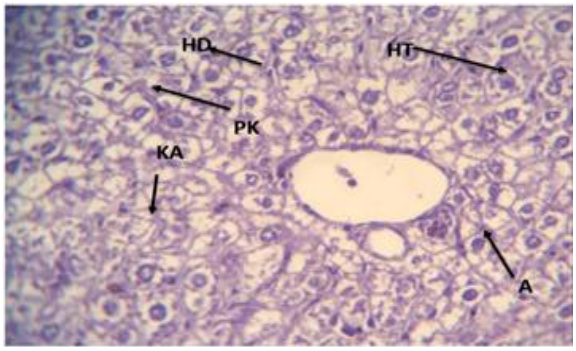


Figure 11: Transverse section of liver tissue in diabetic male rats treated with 0.2 mg/ml flaxseed extract. Note the presence of inflammatory cell infiltration and aggregation (A), nucleolar degeneration (KA), nucleolar thickening

(PK), cell hyperplasia (HT), and cell degeneration (HD). Stained (H&E 40X).

It was also noted in the results of the study in the group of diabetic male mice treated with flaxseed extract, infiltration of inflammatory cells and their accumulation around the central vein of the liver, as well as necrosis of the liver cells, and the enlargement of some liver cells, some of which were binucleated. This is illustrated in Figure 12.

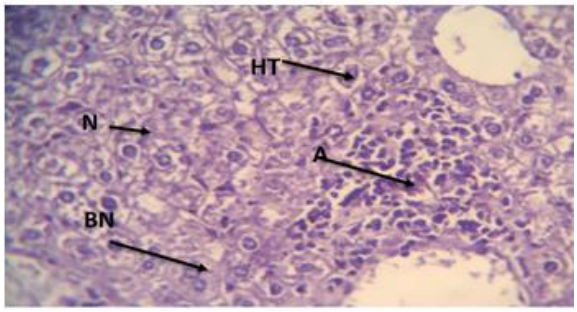


Figure 12: A cross-section of liver tissue in diabetic male rats treated with flaxseed extract at a concentration of 0.2 mg/ml Appearance of infiltration of inflammatory cells (A), hepatocyte necrosis (N), binucleated hepatocytes (BN), hepatocyte hyperplasia (HT), stained (H&E 40X).

3.4 Effect of Alloxan-Induced Diabetes on the Histological Structure of the Liver in Male Mice

The results showed that the induction of diabetes using alloxan led to changes in the liver tissue of diabetic mice compared to the liver of control mice. This is consistent with the study by [37], who attributed these changes to insulin deficiency and high blood glucose levels in mice.

It is known that alloxan is an analogue of glucose, which is what gives it the effectiveness in causing diabetes, as alloxan causes a deficiency in the body's supply of insulin after selective damage to the pancreatic beta cells (β-cells), and thus the cells will refrain from introducing glucose into the cell in the absence of insulin [38].

The results of the current study showed that the induction of diabetes with alloxan at a dose of 150 mg/ml in mice led to an increase in blood glucose levels during the experimental period compared to the glucose level in the control group animals.

This is because alloxan attacks the pancreatic beta cells, which inhibit the secretion of insulin, which leads to elevated blood glucose levels. Because insulin works to introduce glucose molecules into living cells, it balances and regulates blood glucose levels.

The results of the study are also consistent with what was stated by researchers [39], who concluded that rats with induced diabetes had an increase in blood sugar levels, which leads to severe, harmful changes in the liver, pancreas, and kidneys.

The results of our current study are also consistent with those of [40] in his study on rabbits, which found that alloxan injections lead to an increase in blood glucose levels and, consequently, dilation of the sinusoids. This may be due to weak venous flow at the hepatic vein or inferior vena cava level.

The results of the current study showed that induced diabetes in male albino mice led to the infiltration of inflammatory cells in the liver parenchyma, and the appearance of cytoplasmic rupture due to liver cell damage, which occurs for immune reasons or as a result of the toxic effect of alloxan, represented by oxidative stress resulting from the accumulation of free radicals that cause the destruction of liver cells, as well as the oxidation of lipids of the cell membrane or mitochondrial membranes, causing the appearance of an inflammatory and immune response, according to what was mentioned by [41].

The results showed the appearance of cytoplasmic fatty deposits within the liver cells, and that this accumulation of fatty materials reflects the activity of the enzyme (Insulin degrading enzyme) as a result of a decrease in the insulin hormone. Therefore, we notice the clarity of the cytoplasm and the clarity of the cell membrane [42].

While researchers [43] indicated that type 2 diabetes causes fatty degeneration within liver cells through the presence of fats in the liver parenchyma.

It was also noted through the tissue sections obtained in the current study that there was blood congestion in some areas. This is due to the weakness of blood drainage resulting from hepatic venous obstruction, which leads to a disturbance in blood flow through the hepatic parenchymal cells. This is what was indicated by [44], regarding the occurrence of blood congestion when diabetes occurs.

This is consistent with what the researcher [45] has concluded that diabetic rats suffer from hepatic cell necrosis due to poor blood supply to the liver as a result of arterial blockage and thrombosis in the hepatic artery, which leads to a lack of oxygen. This

causes the release of lysosomal enzymes and other secretory substances into the blood, which explains the occurrence of necrosis, and damage of hepatic cells.

The results of the current study also demonstrated a clear division in the parenchymal cells in this group, as demonstrated by the observation of a number of binucleated parenchymal cells. This division in hepatocytes may be attributed to an important adaptive response when the liver is afflicted with acute or chronic diseases, and when hepatocytes become inflamed or destroyed, resulting in cell division to replace these cells with new ones, thus maintaining the liver's adequate function [46].

It was also noted that some hepatic cells were enlarged and the hepatic cells were irregularly arranged in bands or cords around the central vein. This is consistent with what was stated by [47] (Farokhi et al., 2011), which confirmed the enlargement of hepatic cells, the lack of radial arrangement of hepatic cells, and the accumulation and infiltration of inflammatory cells.

An increase in the average diameter of hepatocytes was also observed. This increase is attributed to hepatocyte hypertrophy due to the accumulation of glycogen when blood glucose levels are high, coupled with a lack of insulin. This hypertrophy is then converted into glycogen by the liver cells, leading to decreased glycogenolysis due to metabolic imbalances associated with hyperglycemia, as indicated by [48].

Changes were also observed in the nuclei of some hepatocytes in the livers of male mice, such as karyomes, which occupied most of the cell volume. This is consistent with what [49] confirmed, who demonstrated in their study of the chronic toxic effects of lead on the livers of mice, that changes in the nuclei of hepatocytes occur due to increased cellular and nuclear activity in the detoxification process.

In some sections, nuclei were observed to be disintegrating, and in others, pyknosis of the nucleus occurred when compared to the control group. This result is consistent with the findings of Kumar et al., 2013, who confirmed that the cell undergoes necrosis after a toxic injury, which is characterized by degeneration, after which the cell condenses and thickens its chromatin.

In addition, karyolysis of some hepatocytes was observed in this result, as the cell appeared uniform in color due to the complete disappearance of the nucleus.

The results of this study also showed the appearance of granulomas or fine granules in the liver tissue in the form of a collection of inflammatory cells. This was indicated by [50], that granulation results from the collection of macrophages with other inflammatory cells, especially T-cells, as the appearance of these cells is evidence of a delayed immune reaction due to a toxic substance such as alloxan. The results of the current study showed that liver parenchyma in diabetic male rats treated with the extract provided protection to liver cells. Flaxseeds are one of the richest sources of the plant-based fatty acid alpha-linolenic acid (ALA), and consuming them may help prevent or treat a variety of diabetic complications, according to [51].

The results of the current study are consistent with experimental findings conducted on mice treated with flaxseed, which contains the antioxidant secoisolaricinole diglucoside (SDG). The study concluded that the development of type 1 diabetes can be prevented by approximately 71% and type 2 diabetes by 80% [52]. While a study [53] showed significant improvements in blood sugar control in type 2 diabetics who were treated for 12 weeks with flaxseed-derived lignin supplements.

Lignin may act as an antioxidant by directly scavenging free radicals and preventing lipid peroxidation, as indicated by [54].

One of the results reached by the current study is the radial rearrangement of liver cells and blood sinusoids, and this may be due to the flaxseed extract containing lignin and alpha-linolenic acid, as lignin works as an antioxidant by directly scavengers of free radicals and preventing lipid peroxidation, and this is what was reached [54].

This study is consistent with what Ventura et al., 2023 reported in their study on fish oil, eicosapentaenoic acid, and docosahexaenoic acid, where increased hepatic LDL receptor activity resulted in decreased plasma LDL levels.

As noted by Ahmed, 2011, peroxisome proliferator-activated receptor alpha (PPAR-α), a transcription factor that plays an important role in controlling carbohydrate and lipid levels, is significantly upregulated by a flaxseed oil diet [55].

This may be because alpha-linolenic acid is found in large quantities in flax seeds, so through a series of reactions, alpha-linolenic acid is metabolized into docosahexaenoic acid and eicosapentaenoic acid Zahid and Lamis, 2017.

The results showed the presence of some pathological histological changes, including small dilation of the sinuses. This is consistent with the findings of [56], who indicated that the reason for this is due to a decrease in venous blood flow and central vein occlusion, which may lead to an increase in venous pressure, which leads to dilation of the sinusoids. Or it may be due to chemicals that lead to a decrease in venous blood flow as a result of inflammation, which then leads to the expansion of the sinusoids [57], while [58] mentioned in their study that acidic liver infiltration with or without necrosis is due to a reaction to chemicals.

5 CONCLUSIONS

The results of this research clearly indicate that the daily intake of aqueous flaxseed Linum usitatissimum extract has a protective and healing impact on the liver tissue of diabetic mice induced by alloxan. Histological analyses indicated that untreated diabetic mice showed significant liver damage, characterized by congestion, necrosis, fatty degeneration, cytoplasmic rupture, and infiltration of inflammatory cells indicative of liver dysfunction associated with uncontrolled hyperglycemia and oxidative stress. Nevertheless, the diabetic groups receiving flaxseed extract, especially at the elevated concentration of 0.2 mg/ml, showed significant enhancements in liver structure.

These enhancements involved diminished central vein congestion, normalized sinusoidal structure, reduced Kupffer cell aggregation, and a reinstatement of radial hepatocellular organization. The extract's capacity to reduce liver damage could be linked to its abundant bioactive compounds like alpha-linolenic acid, lignans, and secoisolariciresinol diglucoside (SDG), recognized for their antioxidant, anti-inflammatory, and insulin-sensitizing effects. The noted hepatoprotective benefits indicate that flaxseed extract may act as a possible complementary treatment option in addressing diabetes and its liver-related complications. Its natural source, relative safety, and various mechanisms of action render it a strong contender for additional pharmacological advancement.

ACKNOWLEDGMENTS

The authors would like to thank Department of Biology, College of Education for Pure Science, University of Diyala, Iraq. for providing the laboratory facilities to conduct this study.

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Effect of Stimulating Garden Cress Seeds with Distilled Water, Ascorbic Acid, and Citric Acid on Seedling Growth and Germination under Salt Stress Conditions

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Keywords: Garden Cress, Ascorbic Acid, Citric Acid, Nacl.

Abstract: A factorial experiment with three factors was conducted in the laboratories of the College of Education for Pure Sciences, University of Diyala, following a completely randomized design with three repetitions. The experiment took place from January 15th to January 25th, 2025, to investigate the impact of soaking garden cress seeds in non-enzymatic antioxidants. The results demonstrated the superiority of soaking with distilled water, achieving the highest values in germination percentage, root length, shoot length, fresh weight of seedlings, and seedling vigor, recording 66.66%, 8.33 cm, 3.66 cm, 0.272 g, and 289 g, respectively. The most effective concentration was found to be 3NaCl combined with distilled water. Additionally, soaking in ascorbic acid significantly enhanced the germination percentage and dry weight of the seedlings, reaching 66.66% and 0.057 g, respectively, with irrigation using 3NaCl. On the other hand, soaking with citric acid and irrigation with 5NaCl resulted in the lowest values for all traits, indicating a negative impact on seedling development. These findings highlight the potential of using specific antioxidants in optimizing the growth and vigor of garden cress under saline conditions, providing insights for enhancing agricultural productivity.

1 INTRODUCTION

Garden cress seeds are considered ideal medicinal and nutritional seeds because they include medical components that protect the body, such as carotenoids, polyphenols, triterpenes, flavonoids, saponins, minerals, antioxidants, and various forms of sterols and alkaloids [1]. Garden cress belongs to the largest plant family, the Brassicaceae, which is found in many countries throughout the world, including the United States, Europe, and the Middle East. [3], [2]. Garden cress has several local names, including Cress, Cresson, and red seed, and it is scientifically known as (Lipidium Sativum). The primary benefit of garden cress is that it may grow at any time, in any location, and in any climate or soil condition. [4] Garden cress seeds can help avoid several ailments, including arthritis, liver disorders, bronchitis, high blood sugar, and high blood pressure [5]. Garden cress seeds can be used in a variety of cuisines.[6]. Its leaves are used in salads alongside other vegetables and as a spice to season foods [7]. It is also suitable as animal feed [8].

Ascorbic acid (vitamin C) is classified as an antioxidant sugar acid. It is also a vital vitamin for all living species, as well as a nutrient that the body requires in restricted quantities. It also helps limit flowering and the development of aging [9]. It comprises six carbon atoms produced from glucose sugar, and it plays a crucial function in photosynthesis in plants. Many fruits and vegetables, including lemons, oranges, peppers, and strawberries, contain high levels of this vitamin. Its solubility in water makes it an effective antioxidant [10].

It's also called the "vitamin of fresh food products" [11]. Vitamin C lowers the risk of many diseases since it is one of the body's mossoluble antioxidants, in addition to playing a key role in metabolic processes. It is also thought to function as a cofactor in the activation of numerous important enzymes in the body [12].

The Krebs cycle includes acids such as citric acid ($C_6H_8O_7$). It is a 6-carbon tricarboxylic acid produced by the condensation of oxaloacetate in mitochondria (TCA). It is an intermediary in the tricarboxylic acid (CS) cycle, which is mediated by

citrate synthase and acetyl CoA. It is one of the items with significant commercial value because of its wide application in foods, medical and pharmaceutical preparations, and others. [15], [14], [13].

Citric acid is used in a variety of applications, including chelation, derivatization, pH adjustment, buffer solutions, cosmetics, detergents, oil extraction, and more [16] The majority of citric acid is produced organically, primarily through fermentation, which includes molasses, coffee husks, wheat bran, and a variety of other fertilizer ingredients [19], [18], [17] It is the most efficient acid for energy production and plays an important role in plant physiological processes and functions [20].

Salt stress is the presence of excess amounts of ions in water and soil, as it reduces the percentage of water in the plant [21]. Salt stress is one of the most important physiological stresses that fundamentally affects seed germination and seedling growth, which in turn affects subsequent growth stages due to the accumulation or collection of dissolved salts in the soil, which leads to inhibition of germination as a result of the negative impact of the entry of some ions in large quantities that do not match the cell's needs, thus affecting the plant's vital processes [22]. Salinity has many negative effects on the germination process, as it reduces water absorption within the seeds due to the decrease in osmotic pressure within the seed [23]. The aim of the study is to demonstrate the effect of stimulating garden cress seeds with non-enzymatic antioxidants to reduce salt stress

2 MATERIALS AND METHODS

This study was carried out in the laboratories of postgraduate studies / Department of Biology / College of Education for Pure Science / University of Diyala from (15-1-2025) to (25-1-2025). The study used three elements to stimulate garden cress seedlings before planting: distilled water and non-enzymatic antioxidants (ascorbic acid and citric acid at 100 mg.L-1 for each treatment) for 24 hours. They were then placed in Petri dishes with a diameter of 12 cm and two opaque filter papers, each dish containing 15 garden cress seeds and irrigation with various sodium chloride (NaCl) concentrations (3 and 5 g. L-1). The Randomized Complete Design (CRD) was used with three repetitions for each treatment. After 10 days, the required characteristics for the study were studied.

2.1 Characteristics Studied

2.1.1 Standard Laboratory Germination Percentage(%)

The total number of natural seedlings was calculated after ten days of planting:

$$\textit{Germination percentage} = (\textit{number of germinated seeds} / \textit{total number of seeds}) \times 100 \quad (1)$$

2.1.2 Length of the Root and Shoot (cm)

The length of the root and shoot was measured at the start of the experiment, and the length of the root was measured after separating it from its point of contact with the seed, while the length of the shoot was measured after separating it from its point of contact with the stalk using a graduated ruler.

2.1.3 Dry Weight of the Seedling (g)

The same seedling used to measure the length of the root and shoot was used to examine the dry weight of the seedling, after which the seed coat was removed from each seedling and the embryonic axes were placed in perforated bags and dried in an electric oven at 80°C for 24 hours. After confirming that the weight was stable, they were weighed using a sensitive electric balance.

2.1.4 Fresh Weight of Seedling (g)

According to the fresh weight of the root and shoot using a sensitive balance on the tenth day from the beginning of the experiment.

2.1.5 Seedling Strength

Calculated using the following equation:

$$\text{Seedling strength} = \text{germination percentage (\%)} \times (\text{root length (cm)} + \text{shoot length (cm)}) \quad (2)$$

3 RESULTS AND DISCUSSION

3.1 Standard Laboratory Germination Percentage(%)

Table 1 shows that the soaking treatment with distilled water and ascorbic acid had a higher average germination percentage, with both at 66.66%, while the soaking treatment with citric acid had the lowest average germination percentage, at 36.66%. The

average salinity of irrigation water varied significantly, with the highest average being 83.33 in irrigation with distilled water and the lowest being 33.33 in irrigation water with a NaCl concentration of 5 g.L-1. We conclude from this that the higher the salt concentration, the more negatively it affects the plant, resulting in a decrease in the course of metabolic activities and an increase in osmotic potential, hence limiting water absorption by the seeds [24].

The table also shows significant differences between the soaking materials and the salinity of irrigation water in the averages of the interaction for the germination percentage, as the highest average of the interaction was in the treatment of soaking with distilled water and ascorbic acid and irrigation with distilled water, which was 100.0, while the lowest average of the interaction was in the treatment of soaking with citric acid and irrigation with 5NaCl g.L-1, which was 20.00.

Means with similar letters within the columns of single factors or the means of the interaction Duncan's multiple range test revealed that they are not substantially different at the 0.05 probability level.

3.2 Root Length (cm)

The results in Table 2 show that the soaking treatment with distilled water was superior in the average root length, as the highest average was 8.33 cm, while the least significant difference was in the soaking treatment with citric acid in the average root length, which was 2.03 cm. The average salinity of irrigation water showed a significant difference, with the highest average recorded in irrigation with distilled water (4.86) and the lowest average recorded in irrigation water at a concentration of 5 g. L-1 of NaCl (1.36). Because salt stress hinders the essential vital activities required for root growth, it also impacts the suppression of plant growth, which is why the high concentration of NaCl resulted in a decrease in root length. This impact is brought on by a decrease in the plant's ability to absorb water, which raises the salt concentration in the absorption medium [25].

The table also demonstrates notable differences in the averages of the interaction of the root length between the soaking materials and the salinity of irrigation water. The soaking treatment with ascorbic acid and irrigation with distilled water had the highest average of the interaction, while the soaking treatment with distilled water, citric acid, and irrigation with 5NaCl g.L-1 had the lowest average of the interaction, which was 1.00.

Means with similar letters within the columns of single factors or the means of the interaction Duncan's multiple range test revealed that they are not substantially different at the 0.05 probability level.

3.3 Length of the Shoot (cm)

The results in Table 3 show that the soaking treatment with distilled water was superior in the average length of the frond, with the greatest average being 3.66 cm,while the soaking treatment with citric acid had the smallest significant difference in the average length of the frond at 1.50 cm. The average salinity of irrigation water varied significantly, with the highest average being 4.26 in irrigation with distilled water and the lowest being 1.33 at a concentration of NaCl 5 g. L-1. This effect is caused by citric acid, which is an antioxidant substance whose action is similar to the natural auxins that stimulate growth inside the plant [26].

The table also shows significant differences between the soaking materials and the salinity of the irrigation water in the averages of the interaction for the length of the rue, as the highest average of the interaction was in the treatment of soaking with distilled water and irrigation with distilled water, which was 5.50, and the lowest average of the interaction was in the treatment of soaking with citric acid and irrigation with NaCl5 g.L-1, which was 0.50. Means with similar letters within the columns of single factors or the means of the interaction Duncan's multiple range test revealed that they are not substantially different at the 0.05 probability level.

3.4 Dry Weight of Seedling

The results in Table 4 show that the soaking treatment with ascorbic acid was superior in the average dry weight of the seedling, with the highest average being 0.057 g, while the soaking treatment with distilled water had the least significant difference in the average dry weight of the seedling at 0.018 g. The average salinity of irrigation water differed significantly, with the highest average in irrigation with NaCl3 g. L-1 being 0.059, while the least significant difference in the average salinity of water (irrigation with distilled water and irrigation with NaCl 5 g. L-1 was 0.044 for both). Ascorbic acid protects plants against environmental stressors like salt stress, Thus, it can be regarded an antioxidant in plants [27].

The interaction averages for the dry weight of the seedling also demonstrate notable differences between the soaking materials and the salinity of the irrigation water. The soaking treatment with ascorbic acid and citric acid and irrigation with 3NaCl g.L-1

Average Average had the highest interaction average (0.066), while the soaking treatment with distilled water and irrigation with 5NaCl g.L -1 had the lowest interaction average (0.026).

Table 1: The effect of stimulating garden cress seeds with non-enzymatic antioxidants on the average germination percentage% of the seedling and the interaction between them.

Average	Soaking materials			Irrigation water salinity concentration
	Citric acid 100 mg.L^{-1}	Ascorbic acid 100 mg.L^{-1}	Distilled water 100 mg.L^{-1}	
83.33 A	50.00 b	100.0 a	100.0 a	Distilled water
53.00 C	40.00 c	60.00 a	60.00 a	3NaCl g.L^{-1}
33.33 B	20.00 d	40.00 a	40.00 a	5NaCl g.L^{-1}
	36.66 B	66.66 A	66.66 A	Average

Table 2:The effect of stimulating garden cress seeds with non-enzymatic antioxidants on the average root length (cm) of the seedling and the interaction between them.

Average	Soaking materials			Irrigation water salinity concentration
	Citric acid 100 mg.L^{-1}	Ascorbic acid 100 mg.L^{-1}	Distilled water 100 mg.L^{-1}	
4.86 A	3.00 d	6.60 a	5.00 a	Distilled water
3.03 C	2.10 c	4.00 f	3.00 b	3NaCl g.L^{-1}
1.36 B	1.00 v	2.10 d	1.00 c	5NaCl g.L^{-1}
	2.03 A	4.23 C	8.33 A	Average

Table 3: The effect of stimulating garden cress seeds with non-enzymatic antioxidants on the average shoot length (cm) of the seedling and the interaction between them.

Average	Soaking materials			Irrigation water salinity concentration
	Citric acid 100 mg.L^{-1}	Ascorbic acid 100 mg.L^{-1}	Distilled water 100 mg.L^{-1}	
4.26 A	2.30 a	5.00 c	5.50 a	Distilled water
2.56 C	1.70 b	2.50 e	3.50 v	3NaCl g.L^{-1}
1.33 B	0.50 c	1.50 s	2.00 d	5NaCl g.L^{-1}
	1.50 B	3.00 C	3.66 A	Average

Table 4: The effect of stimulating garden cress seeds with non-enzymatic antioxidants on the average dry weight (g) of the seedling and the interaction between them.

Average	Soaking materials			Irrigation water salinity concentration
	Citric acid 100 mg.L^{-1}	Ascorbic acid 100 mg.L^{-1}	Distilled water 100 mg.L^{-1}	
0.044 B	0.033 a	0.053 b	0.046 a	Distilled water
0.059 A	0.066 a	0.066 a	0.046 a	3NaCl g.L^{-1}
0.044 B	0.053 a	0.053 c	0.026 a	5NaCl g.L^{-1}
	0.056 C	0.057 A	0.018 B	Average

Table 5: The effect of stimulating garden cress seeds with non-enzymatic antioxidants on the average fresh weight (g)of the seedling and the interaction between them.

Average	Soaking materials			Irrigation water salinity concentration
	Citric acid 100 $mg.L^{-1}$	Ascorbic acid 100 $mg.L^{-1}$	Distilled water 100 $mg.L^{-1}$	
0.259 A	0.222 a	0.110 b	0.446 a	Distilled water
0.208 C	0.132 a	0.246 a	0.246 a	3NaCl $g.L^{-1}$
0.116 B	0.111 a	0.111 c	0.126 a	5NaCl $g.L^{-1}$
	0.155 B	0.155 A	0.272 A	Average

Table 6::The effect of stimulating garden cress seeds with non-enzymatic antioxidants on the average strength of the seedling and the interaction between them.

Average	Soaking materials			Irrigation water salinity concentration
	Citric acid 100 $mg.L^{-1}$	Ascorbic acid 100 $mg.L^{-1}$	Distilled water 100 $mg.L^{-1}$	
91.6 C	42 d	183 d	50.00 a	Distilled water
330 A	85.5 r	242 c	665 b	3NaCl $g.L^{-1}$
86.0 B	20.5 x	85.7 f	152 c	5NaCl $g.L^{-1}$
	49.3 B	170 C	289 A	Average

Means with similar letters within the columns of single factors or the means of the interaction Duncan's multiple range test revealed that they are not substantially different at the 0.05 probability level.

3.5 Fresh Weight of Seedling

The results in Table 5 show that the soaking treatment with distilled water was superior in the average fresh weight of the seedling, with the highest average reaching 0.272 g, while the soaking treatment with ascorbic acid and citric acid had the least significant difference in the average fresh weight of the seedling, with both reaching 0.155 g. The average salinity of irrigation water varied significantly, with the highest average in irrigation with distilled water reaching 0.259 and the least significant variation in the average salinity of irrigation water with NaCl being 5 g.L-1, reaching 0.116. Increasing the salt levels (concentrations) reduced the seedlings' fresh weight.

The table also shows significant differences between the soaking materials and the salinity of irrigation water in the averages of the interaction for the fresh weight of the seedling, as the highest average of the interaction was in the treatment of soaking with distilled water and irrigation with distilled water, which was 0.446, while the lowest average of the interaction was in the treatment of soaking with ascorbic acid and citric acid and irrigation with 5NaCl g.L-1, which was 0.026=

Means with similar letters within the columns of single factors or the means of the interaction Duncan's multiple range test revealed that they are not substantially different at the 0.05 probability level.

3.6 Seedling Strength

The results in Table 6 demonstrate that the soaking treatment with distilled water was superior in terms of average seedling strength g, with the greatest average reaching 289 g, while the soaking treatment with citric acid had the least significant difference in average seedling strength, reaching 49.3 g. The average salinity of irrigation water varied significantly, with the highest average at 330 with 3NaCl g. L-1 and the lowest at 86.0 with 5NaCl g. L-1. The explanation for this is due to variances in average shoot and root lengths caused by citric acid soaking. The table also shows significant differences between the soaking materials and the salinity of irrigation water in the averages of the interaction in seedling strength, as the highest average of the

interaction was in the treatment of soaking with distilled water and irrigation with 3NaCl g. L-1, 665, while the lowest average of the interaction was in the treatment of soaking with citric acid and irrigation with 5NaCl g. L-1, 20.0.

Means with similar letters within the columns of single factors or the means of the interaction Duncan's multiple range test revealed that they are not substantially different at the 0.05 probability level.

4 CONCLUSIONS

This research effectively illustrated that treating garden cress seeds with non-enzymatic antioxidants, especially distilled water and ascorbic acid, notably reduces the negative impacts of salt stress on both seed germination and seedling growth. The experimental findings distinctly indicate that the maximum germination rate (100%), along with root and shoot lengths, seedling fresh weight (0.446 g), and seedling vigor (665 g), were observed in seeds soaked in distilled water and similarly irrigated, suggesting that water alone is critical for stimulating seed metabolism under non-stressed conditions

It was discovered that the higher the salt of irrigation water, the less germination of certain plants, including garden cress. The most effective type of irrigation is with distilled water. The results showed that seed stimulation with distilled water was the best in terms of average of all features except for the seedling's average dry weight, which had the lowest average of 0.018 cm, followed by ascorbic acid. Citric acid stimulation affected the majority of characteristics, including the germination %, where the lowest average was 36.66, The lowest average root length was 2.02 cm, and the best concentration of salinity in irrigation water after distilled water , it is irrigation with 3NaCl g. L-1. The use of antioxidants (ascorbic and citric acid) works to reduce salt stress on the plant, in addition to distilled water. Therefore, it is preferable to use other types of antioxidants to stimulate the seeds and at different concentrations before planting them in the soil.

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Detection of DNA Damage in Esophageal Cancer Cell Lines Using Edible Insect Extract

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Keywords: Esophageal Cancer Cell Lines, Insect Extract, Schistocerca Gregaria, Comet Assay.

Abstract: This study investigates the genotoxic effects of an insect extract rich in bioactive compounds with potential anticancer properties. An extract of insects was made. In the biology labs at the University of Diyala's College of Education for Pure Science. GC-MS analysis was conducted at the Basra Oil Company laboratories to identify the active compounds in the desert locust (Schistocerca gregaria). The genotoxic effects were analyzed in esophageal cancer cell lines, and in comparison, to normal cell lines using the Alkaline Comet Assay method and a green dye. The experiments were done at the Al-Mustansiriya University Iraqi Center for Cancer Research. The findings showed that esophageal cancer cells had DNA damage, which stopped their ability to divide and caused apoptosis. However, normal cells are not harmed by the desert locust insect extract. With flavonoids, phenolic compounds, omega-3 alpha-linolenic acid, omega-6 linoleic acid, cholesterol, and glucosamine, the insect extract shows promise for the development of a successful treatment that uses natural insect extracts to eradicate esophageal cancer or any other type of cancer.

1 INTRODUCTION

Mutations caused by environmental factors in genes that regulate cell growth are what make cancer a deadly disease. Its main feature is what it does best: uncontrolled cell growth, which leads to the destruction of tissues around it[1]. Esophageal cancer has claimed the seventh ranking as one of the most common cancers, alongside being the sixth leading cause of cancer death globally [2]. This type of cancer is very advanced because it is hard for one to diagnose it early, and it spreads very quickly [3]. Esophageal cancer is broken down into two common types: squamous cell carcinoma and adenocarcinoma. The former is often the case of alcohol drinking and smoking, while the latter is attributed to gastroesophageal reflux disease [4]. Over the last decade, there has been interest in using insects as an alternative source of protein and other types of nutrients. In addition, they may be useful for the experimental treatment of cancer because of the many cytotoxic substances with anti-tumor activity that they produce. [5]. The large number of biologically active compounds produced by different insect species, which are important for their biological functions, has already aroused interest in pharmacology. A number of studies have shown various harmful effects on human cancer cells [6]. One study indicated that the viability of the Caco-2 colon cancer cell line can decrease after treatment with different concentrations of extracts from Schistocerca gregaria and Gryllus bimaculatus [5]. In the past few years, insects have become increasingly appealing for sourcing new bioactive compounds, particularly for therapeutic applications like cancer treatment. One of the most appealing is the desert locust (Schistocerca gregaria) due to its high nutritional value and rich pharmacological properties. Sterols, alkaloids, peptides, and numerous enzymes with potential protective functions against different infections, including colorectal cancer, are present in the bioactive profile of this traditionally used locust species [7].

2 MATERIALS AND METHODS

2.1 Extract Preparation

Schistocerca gregaria was collected and the species was authenticated by the Natural History Museum. To get rid of any contaminants, distilled water was used to wash the insects, and subsequently, the lower portions of the body were removed and disposed of. The insects were then dried and ground into a fine powder. Insects were extracted using a Soxhlet apparatus. 100 gm of the powdered insects was wrapped in a filter paper cone that was sharply closed to prevent any leakages. To the cone, together with the filter, 500 ml of the solvent was added for 24 hours. Thereafter, A rotating evaporator dial was used to remove a portion of the solution, leaving the necessary solvent left. [8].

2.2 Identification of Active Compounds

The biological tissues of Schistocerca gregarious, the gregarious phase locust, were transported to the Basra Oil Company laboratories for analysis. The active constituents were identified using the gas chromatography-mass spectrometry (GCMS) technique. The equipment used to analyze the insect's alcoholic extract included Agilent flame ionization gas chromatography (FID), gas chromatography-mass spectrometry (MSD) with a selective detector (GC-MS). The device's separation parameters along with the volume of extract injection are presented in Table 1 [9], [10].

2.3 Genotoxicity Assay

The genotoxic effects of DNA from the cell lines and agarose gels were studied using the Alkaline Comet Assay as described in [11]. Agarose gel was used to embed all malignant and healthy cells that had been exposed to varying doses of the insect extract of 1x105 cells/ml and diluted 1:10 (v/v) at 37°C. After the comet slices were prepared, the cells were lysed for an hour in a lysis solution at 4°C. Then, the samples were electrophoresed in a NaOH EDTA solution with a current of 21 volts for half an hour. After 30 minutes, the cells were counterstained with Syber green dye. As imaging was performed utilizing a Comet Assay software, the profiling data were analyzed through specialized software developed for Comet Assays.

2.4 Statistical Analysis

I used an unpaired t-test to analyze the data with a significance value of $P<0.05$ and presented the results as mean± standard error of the mean (SEM) in triplicate experiments calculated using GraphPad Prism 6 software as documented in [12].

Table 1 Settings for Separation Conditions of Desert Locust Extract gas chromatography coupled with mass spectroscopy (GC–MASS)..

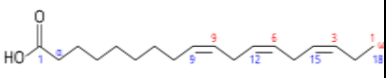
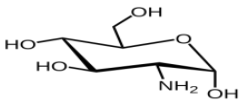
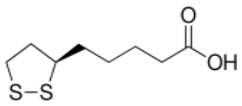
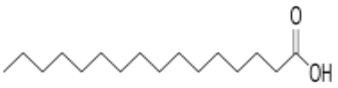
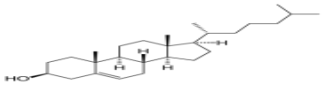
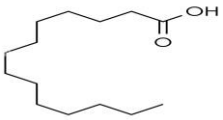
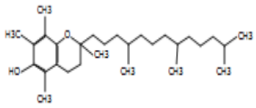
No	Device working	Device working information
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4	Ionization detector temperature	C 290
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6	Total flow	ml/min 19
7	Column flow	ml/min 1
8	Cleansing flow	ml/min 3
9	Column type	HP-5MS 5 % Phenyl methyl siloxane
10	Column dimensions: length x inner diameter	M30 x m 0.25 x m 0.25
11	Injection volume	Lµ1
12	the pressure	Psi 7.0699

3 RESULTS AND DISCUSSION

3.1 GC-MS Analysis Results

By comparing the spectra with commercial spectral libraries, such as the Wiley GC/MS Library, the different parts of the insect extract were considered using the MassFinder Library and the Baser Library for essential oil components containing more than 3,200 authentic compounds with mass spectra and retention information. According to Table 2 and Figure 1, the results confirmed the presence of bioactive compounds in the insect extract, which is in line with findings from other studies [13].

Table 2: The active ingredients in the insect extract are displayed using the GC-Mass method.

No	Compound name	Chemical formula	Formula for structure
1	Hexadecanoic acid, methyl ester	C17H34O2	
2	9-Octadecenoic acid, methyl ester, (E)	C18H30O2	
3	Glucosamine	C6H13NO5	
4	Linolenic acid	C6H32O2	
5	;Hexadecanoic acid , methyleste	C16H32O2	
6	Cholesterol	C27H46O	
7	Tetradecanoic acid	C14H28O2	
8	Vitamin E	C29H50O2	
9	9,17-Octadecadienal, (Z)-	C18H32O	

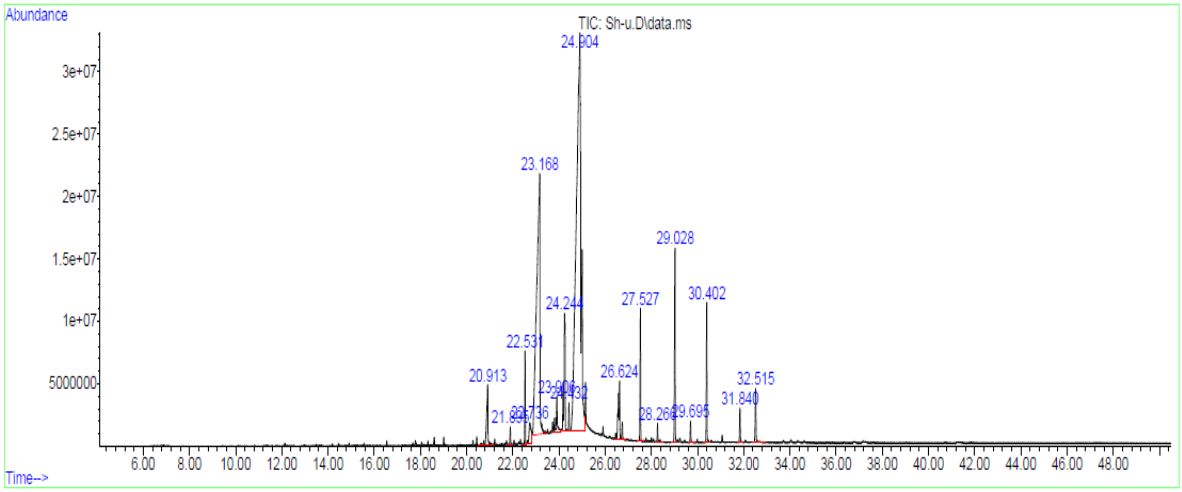


Figure 1: Gas chromatography-mass spectrometry of desert locust extract Schistocerca gregaria.

3.2 Genotoxic Effects of the Insect Extract on Cell Lines

Figure 2 demonstrates the genotoxic effects of the insect extract on esophageal cancer cell lines relative to normal cell lines., where: A) Represents esophageal cancer cells before treatment with the extract. B) Represents normal cell lines before treatment with the extract. C) represents esophageal cancer cells following administration of the extract at a 50 µg/ml dosage. D) depicts normal cell lines following treatment with the extract at a 50 µg/ml concentration. E) Represents an esophageal cancer cell in the apoptosis phase (programmed cell death). The Comet Assay demonstrated apoptosis in cancer cells treated with the insect extract, as indicated by the orange coloration of the cells, confirming their entry into the apoptotic phase. This apoptosis is likely due to the disruption of the mitochondrial electron transport chain, which is associated with reactive oxygen species (ROS) production within the cells [14]. Cell death and DNA damage may have been caused by caspase activity, especially caspase-3. The insect extract's free amino acids may be responsible for these effects.[15], [16]. No toxic effects were observed in normal cells treated with the extract. The extract's flavonoids and phenolic components probably caused cancer cells to undergo apoptosis by triggering antioxidants and preventing inflammation and migration. Consequently, these extracts may be utilized as adjuvants for anti-inflammatory, anti-oxidant, and anti-cancer medications [6]. The active compounds cause DNA damage or oxidation of nitrogenous bases, which leads to the death of cancer cells. In addition, these extracts work to prevent the division of cancer cells by inhibiting growth signaling pathways [17], [18]. Sterols like β-sitosterol, stigmasterol, and campesterol not only lower cholesterol levels, but also have possible effects in the modulation of cancer cells growth and differentiation [7]. Furthermore, the alkaloid trigonelline has displayed potent activity against tumor cells in colorectal cancer cell line by obstructing several important signaling pathways of cancer [19]. Schgr-AKH-II and other bioactive neuropeptides might control energy metabolism and cell proliferation, indicating further research could be warranted regarding cancer biology, Additionally, it has been demonstrated that a variety of proteins and enzymes derived from desert locusts include anti-inflammatory and antioxidant properties, which are crucial in regulating the growth of cancer. [20].

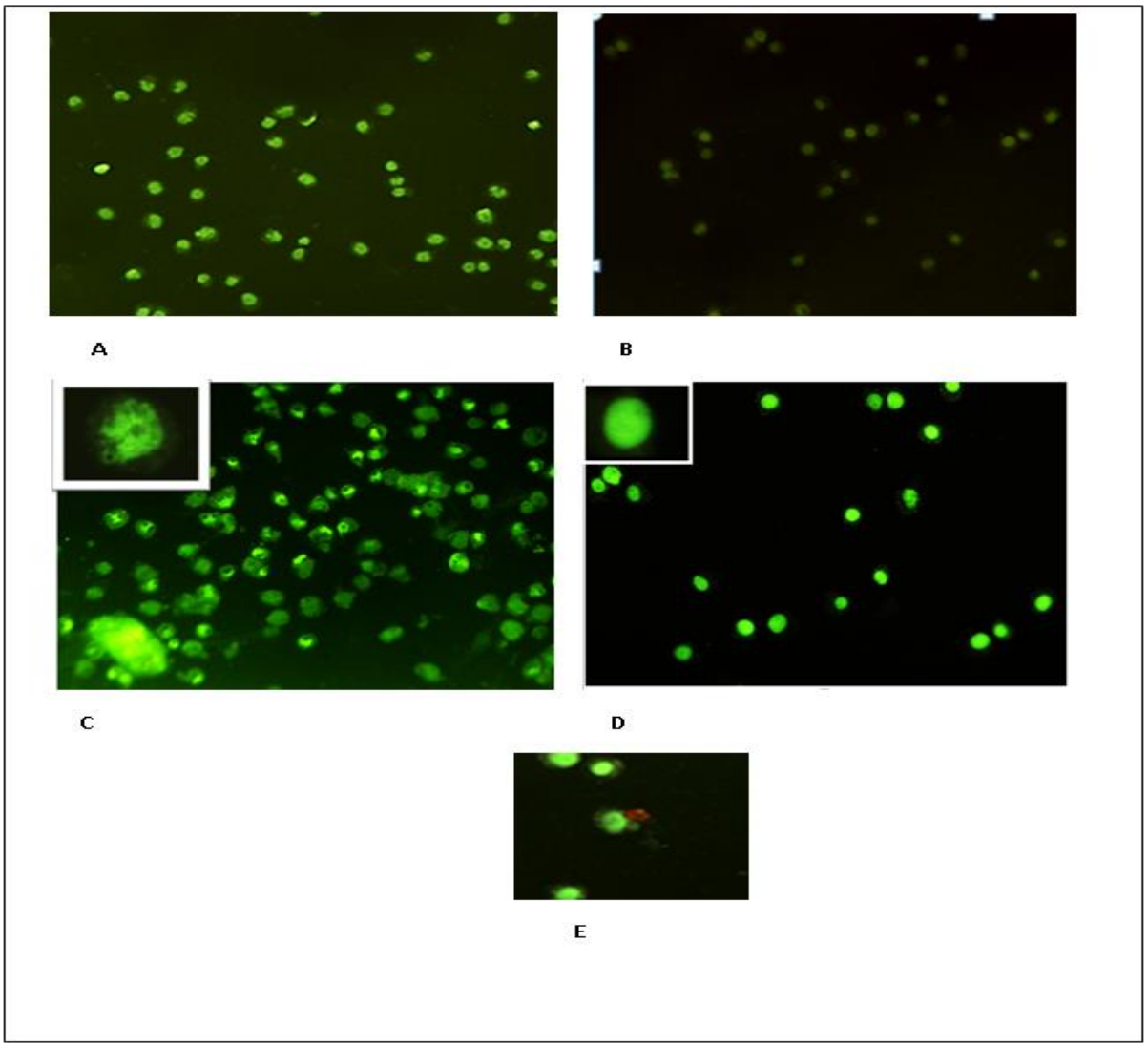


Figure 2: Illustrates the genotoxic effects of the insect extract on cell lines, where: A) Esophageal cancer cells before treatment with insect extract, B) Normal cells before treatment with insect extract, C) Esophageal cancer cells after treatment with insect extract, D) Normal cells before treatment with insect extract, E) A cancer cell in the apoptotic phase (programmed cell death).

4 CONCLUSIONS

The extract's treatment resulted in a promising reduction in esophageal cancer cell proliferation, suggesting the presence of bioactive compounds that may exhibit anticancer properties. This provides further evidence supporting the biological promise exhibited by the extract of the desert locust. The results also showed apoptosis induction in esophageal cancer cells, which further substantiates the hypothesis that the extract possesses properties of an antitumor agent. The extract also proved to show selective cytotoxic activity on malignant cells while leaving normal cells largely unscathed, reinforcing the notion that it could serve as a targeted therapeutic agent. More importantly, a decrease in genotoxicity markers was noted for the treated cells, suggesting that the extract may be free from direct genetic damage, which is an important factor in evaluating safety. Such evidence strengthens the rationale for isolating active extract constituents at the molecular level through further in vivo testing to assess efficacy, safety, and mechanisms of action. In conclusion, the evidence accumulated suggests further studies focusing on the desert locust are merited, especially considering the increasing demand for effective yet safe alternative cancer therapies. The presence of flavonoids, phenolic compounds, alpha-linolenic acid (omega-3), linoleic acid (omega-6), cholesterol, and glucosamine in the insect extract makes it a potential candidate for cancer treatment by inducing apoptosis, triggering antioxidants, and preventing inflammation and migration. These extracts might be added to anti-cancer medications as adjuvants , offering a natural alternative to chemical and radiation therapies that

also affect normal cells . These findings suggest that insect extracts may serve as promising adjuvants in anticancer therapies, offering a natural alternative to conventional treatments.

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Early Diagnosis of Fetal Sex and its Relationship to Some Biochemical and Physiological Variables in Pregnant Women in Salah Al-Din Governorate

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Keywords: SRY, DYS14, DAZ, Doppler, Real Time PCR, Testosterone, Estrogen, Iraq.

Abstract: Genes for sex determination are widely analyzed and used to predict fetal sex during different trimesters of pregnancy. However, their correlation with sex prediction based on ultrasound results, as well as with testosterone and estrogen levels in the first trimester, is quite limited. Therefore, the present study aimed to assess the degree of agreement between several sex-determining genes (SRY, DYS14, and DAZ) analyzed in the first trimester and Doppler-predicted sex, and to evaluate their correlation with hormone levels. Whole blood samples (5 mL) were collected from 110 Iraqi pregnant women between the 1st and 12th weeks of pregnancy. DNA was extracted from all samples, and the presence of the selected genes was confirmed using real-time PCR. The detection of amplification was considered a positive result for each gene, indicating male sex. The concentrations of testosterone and estrogen were measured using the ELISA technique. All pregnancies were monitored to confirm the Doppler results postnatally. The results of the present study showed no significant correlations between sex predicted by the SRY, DYS14, and DAZ genes and sex predicted by Doppler. Furthermore, the levels of both hormones did not significantly correlate with sex predicted by either molecular or Doppler methods. In conclusion, sex prediction in the first trimester of pregnancy frequently yields inaccurate results.

1 INTRODUCTION

The process of determining the sex of the fetus in the early stages of pregnancy is of great importance for the diagnosis of genetic disorders associated with the X chromosome, as more than 100 genetic diseases associated with the X chromosome have been discovered in humans, including muscular dystrophy, fragile X syndrome, and hemophilia [1].

Early determination of the sex of the fetus is precisely through targeted invasive procedures either by chorionic villus samples in the first trimester or by amniocentesis early in the second trimester pregnancy [2]. However, these procedures are associated with the risk of miscarriage of the fetus and are also expensive, making them less acceptable [3]. Therefore, studies have resorted to determining the sex of the fetus using 2D ultrasound technology, which provides 100% accurate results after 20 weeks of pregnancy [4]. Despite the high accuracy of ultrasound technology, its accuracy depends on gestational age and decreases to 70% in the first 11 weeks of pregnancy [5]. Therefore, the discovery of low-cost, non-invasive diagnostic methods with more accurate results in determining the sex of the fetus is especially important in the first trimester.

Testing for fetal DNA in maternal body fluids is one of the most reliable procedures for determining fetal sex in the first trimester [6]. and its presence can be demonstrated through non-targeted methods of peripheral blood of pregnant women as cell-free fetal DNA (cffDNA) or as free fetal DNA in the blood of pregnant women [7], or through targeted procedures through chorionic puncture or in chorionic villus samples [8], where the presence of fetal nucleated red blood cells (fNRBCs) is a circulating cell in the peripheral blood of pregnant women and contains the whole genome of the fetus, which is a source for determining the sex of the fetus [9].

The availability and level of fetal DNA is one of the necessary requirements to obtain accurate results, and the use of non-targeted methods results in obtaining low concentrations of DNA, which

requires the use of highly sensitive and specific techniques to determine the sex of the fetus [10], and many previous studies targeted the genes of the sex chromosome Y to determine the sex of the fetus using different genetic sequences of the Y chromosome In particular, single-copy sex determination region Y (SRY) [11], However, SRY is a single copy and is therefore not sensitive enough in the process of determining the sex of the fetus, so using a combination of sequences in the Y chromosome with multiple copies such as the deleted gene in Azoospermia (DAZ) and the testicular protein gene linked to the Y Testis Specific Protein Y-linked 1 (TSPY1) also known as (DYS14) gives more accurate and more sensitive results in determining the sex of the fetus in the early stages and more accurately through the use of advanced techniques such as Real time Polymerase chain reaction (qPCR).

At the beginning of pregnancy, most of the hormones secreted by chorioblasts are the pregnancy hormone human chorionic gonadotrophin (hCG), which stimulates corpus luteum to produce and secrete progesterone [12], the results of previous studies showed the relationship of fetal sex with some levels of hormones such as testosterone and progesterone, while the results of some studies such as a study [13] show low progesterone levels in pregnant women with female fetuses but more recent studies have shown no significant coefficient relationships between hormone level and fetal sex [14], 15], [12].

Through the results of previous studies, it is noted that the relationship of the fetal sex with hormone levels in the first trimester of pregnancy is not clear and the results of previous studies on the use of PCR techniques in determining the sex of the fetus are inconsistencies and there are no previous study correlates expected fetus gender with the results of doppler, the current study assumes that by relying on measuring the levels of sex-specific genes and linking them to hormone levels, it can give results with greater dependence and give clearer results for the relationship of hormones with the sex of the fetus, so the current study aimed to assess the degree of agreement in expectation fetus gender in first trimester of pregnancy between molecular technique and doppler results as will in correlation of concentrations of hormones.

2 MATERIAL AND METHODS

2.1 Subjects and Samples

Blood samples (5 ml) were collected from 110 of Iraqi women pregnancy during first trimester (from the 1st week to the 12th week), all women were approved for inclusion in the present study, and the study design was ethically approved by Ministry of Health / Salah El-Din Health Department.

2.2 DNA Extraction and Real-Time Reaction

Maternal DNA from blood was extracted using protocol recommended by commercial Blood mini kit DNA extraction (Qiagen, USA), and primers used of genes of this study (SRY, DYS14, and DAZ) are listed in Table 1 and qPCR reaction were done using ready 2×Ultra Sybr qPCR Mix (Tinzyme/China) that contain SYBR green, Taq DNA Polymerase, PCR Buffer, and dNTPs and final additions were 25µl of 2×Ultra Sybr qPCR Mix, 1µl for each forward and reverse primer, 2µl of template DNA, 50µl ddH_2O, and the reaction steps and amplification cycle are listed in Table 2 and designed according to [16].

Table 1: Primers used for sex determining genes.

Gene	Primer Sequence ('3 → '5)		Ref
SRY	Forward	AGTATCGACCTCGT CGGAAG	Khorshid *et al.*, 2013
	Reverse	TCTTGAGTGTGTGG CTTTCG	
DYS14	Forward	AGCCCTGATCACTG ACGAAG	
	Reverse	TGCAGAGATGAACA GGATGC	
DAZ	Forward	TACCTCCAAAGCAC CAGAGC	
	Reverse	AATCTACCCATTCC CGAACC	

Table 2: Reaction of qPCR.

Step	Temperature	Time	Cycles
Pre-denaturation	95˚ C	10 min	1
Denaturation	95˚ C	15 s	35
Annealing and Extension	60˚ C	1 min	
Melting curve	60˚C-95˚C	15 s	1

2.3 Statistical Analysis

Chi-square test and Kappa values were used to assess the degree of agreement between the expected gender by qPCR and Doppler expected results (a kappa value below 0 considered as poor agreement, between 0.00 and 0,20 is considered slight agreement, between 0.21 and 0.40 is considered fair agreement, between 0.41 and 0.60 considered moderate agreement, nearly 1 considered perfect agreement), then independent-sample T-test were used to show the correlation between levels of hormones and expected gender by both qPCR and doppler.

3 RESULTS AND DISCUSSION

Statistical analysis of the present study shows non-significant correlation between the expectation of fetal gender molecularly by assess presence of amplification of SRY, DYS14, and DAZ genes by real-time PCR, as the Table 3 show that the all maternal DNA were positive for SRY compared to doppler expected gender where only 67 of total pregnancy were bearing male gender while other were female gender, this was the different slightly for both DYS14 and DAZ, were show 106 and 108 positive amplification respectively from total 110 samples.

The degree of agreement between the two techniques was analyzed statistically using the Kappa value. The results showed a poor to slight agreement between the techniques, with a Kappa value of 0.032 for the comparison between DYS14 and Doppler results and 0.028 between DAZ and Doppler results; both values indicate slight agreement. In contrast, the agreement between SRY and Doppler results was poor because all SRY values were positive (Table 4).

Statistical analysis not computed due to the constant value of *SRY*.

Independent sample T-test was done between the expected fetal gender in the first trimester by sex determining genes, as well as doppler results and levels of both testosterone and estrogen, as shown in Table 5. The most (nearly significant) important results were between estrogen levels and DYS14 expected gender, while all other correlations were non-significant.

Table 3: Timing of samples collection.

Week of first trimester	Number of samples
1st	6
2nd	11
3rd	7
4th	11
5th	7
6th	11
7th	14
8th	11
9th	13
10th	12
11th	4
12th	3
Total	110

Table 4: Degree of agreement between the results of qPCR for SRY, DYS14, and DAZ genes and Doppler results in first-trimester pregnancy.

Gene	Expected gender		P-value	Kappa value	Degree of Agree-ment
	Male	Female			
SRY	110	0	*	0.000 *	Poor
DYS14	106	4	0.134	0.032	Slight
DAZ	108	2	0.075	0.028	Slight
Doppler	67	43			

Table 5: Correlation between the levels of Testosterone and Estrogen hormones and gender expected results by qPCR and Doppler results in first trimester pregnancy.

Hormones	*SRY*	*DYS14*	*DAZ*	Doppler	P-value
Testosterone	*	0.778	0.453	0.163	
Estrogen	*	0.068	0.879	0.338	

* Statistical analysis not computed due to constant value of *SRY*.

According to [2], real time results of sex determining genes should be negative for Y-chromosome genes (SRY, DYS14, and DAZ) to consider the female gender, and vice versa. This is one of the most applications of use of cffDNA analysis, however, several other applications were present, such as disorders in single genes [17] . were the first study relay the using of SRY and DYS14

due to presence of DYS14 in multicopy, and the study of [17] indicates its efficacy of presence of cffDNA in pregnancy blood.

Present studies show poor relationship and agreement between the results of real time PCR and doppler results, and this were contrary to most of the results of previous studies, as the results of [18] show perfect agreement between the results of DYS14 and ultrasound results during 12 week of pregnancy and this were also confirmed by several other previous studies [19], [20]. The results of this contrary in the present study may be due to sampling time of first trimester, as the present study collect the sample randomly from 1st week to 12th week of pregnancy while the other previous studies indicated that the accurate results were obtained at 12th week of pregnancy, and the present study as shown in Table 1 show that 35 sample were in 4th week and below and this may be the significant results of the poor agreement between the results of expected fetal gender by genes and doppler, and this approved by several previous studies, as the results of [6] which aimed to show optimal gestational age for sex determining and show that the accuracy of sex determination at 5th week of pregnancy were 50% compared to 80% at 7th week and 100 at 9th week of pregnancy, and this indicates that the accuracy of molecular determination of fetal gender affected greatly by gestational age, and this was also approved by several other studies [21], [22].

Although there is a strong relationship between the sex of the fetus with the levels of other hormones such as testosterone, this relationship with progesterone is not completely clear and the results of previous studies in this regard were few and varied significantly, while the results of a study [12]. were negative in the existence of a relationship between fetal sex and progesterone levels.

Some previous studies reported no significant differences in progesterone levels in pregnant women, whether the sex of the fetus was male or female [11], [23] but the results of a study showed that progesterone levels decrease in pregnant women carrying a female fetus, and this was later confirmed by the study [14], [24]. indicated a significant decrease in the levels of progesterone hormone in the blood serum of pregnant women in the female sex compared to its levels in pregnant women in the male sex.

The present study shows no correlation between the levels of both testosterone and estrogen and expected fetal gender by both doppler and genes, and the main reason for this may be due to gestational age of collected samples.

4 CONCLUSIONS

Determination of fetal gender in first trimester of pregnancy gives highly false positive results and contrary results with expected gender in doppler ultrasound, as well as estimation of concentration of testosterone and estrogen in this time will not give good indication for fetal gender. Using the SRY gene to detect fetal sex in the first trimester of pregnancy provides accurate and reliable results in determining fetal sex. The SRY gene cannot be used alone to detect fetal sex in the first trimester of pregnancy. It must be used in conjunction with the DYS14 gene, which has excellent support for male sex detection, especially when used with the SRY gene. The DAZ gene, used to detect fetal sex in the first trimester of pregnancy, produces unreliable results and has a relatively weak potential, making it insufficient for reliable detection. Results have shown a correlation between elevated estrogen levels and pregnancy with a female fetus. This can be used as a biological, non-genetic indicator for detecting fetal sex Testosterone levels were low in the first trimester of pregnancy and there were no significant differences between males and females.

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Polymorphism of Genes in Iraqi Females with Type 2 Diabetes Mellitus

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Keywords: Diabetes Mellitus Type 2, Polymorphism, Interleukin-1α, Interleukin-1β, Interleukin Receptor, Genetic Susceptibility.

Abstract: Diabetes Mellitus Type 2 (T2DM) is a global medical challenge, with cytokines playing a significant role in its developing. This study evaluates the frequency of polymorphisms in Interleukin-1α (IL-1α) at position $_{-889}$ C/T (rs1800587), Interleukin-1β (IL-1β) at positions $_{-511}$ C/T (rs16944) and $_{+3962}$ C/T (rs1143634) and compared to healthy controls, diabetic patients exhibited an increased risk associated with the T allele of IL-1RL1$_{-27609}$. In the study, which was conducted by Al-Yarmouk Teaching Hospital, 720 newly diagnosed Iraqi females with T2DM, aged 20-55 years, were included along with 240 age- and gender-matched healthy controls recruited from their own hospital. Genotyping was performed by using 49 patients and 40 normal after excluded others diseases and conducted the PCR-SSP assay, and results were validated by sequencing. Allele and genotype frequencies were analysed, and significant associations were identified. The findings revealed that the T allele and TT genotype of IL-1α $_{(-889}$ C/T) significantly increased the risk of T2DM, while the C allele and CC genotype were protective. For IL-1β$_{-511}$ C/T, the T allele acted as a protective factor, whereas the C allele elevated the risk of diabetes. No significant associations were observed for IL-1β $_{+3962}$ C/T. Interestingly, polymorphisms in the IL-1RL1 receptor $_{-27609}$ T/C showed that the T allele was related with increased susceptibility to diabetes, whereas the C allele was protective. Polymorphisms in IL-1α$_{-889}$ C/T, IL-1β $_{-511}$ C/T, and IL-1RL1 $_{-27609}$ T/C appear to contribute to the genetic susceptibility to T2DM in Iraqi females. These findings highlight the potential role of interleukin signalling pathways in diabetes development. Further studies with larger, more diverse populations are recommended to confirm these results and explore their clinical implications.

1 INTRODUCTION

Diabetes mellitus represents a worldwide health disorder characterized by chronic hyperglycemia because of one or more disturbances in insulin secretion or action-or even both. Further on, this metabolic disease may be followed by other complications like cardiovascular diseases, neuropathy, nephropathy, or even retinopathy. In the view of modern studies, inflammation has become underlined to play a key role during the development and progression of T2DM, in which cytokines seem to play an important role in the mechanism of inflammation. These are small proteins that modulate immune responses and transmit information among immune cells. In particular, pro-inflammatory cytokines, including IL-1, have been implicated in the development of insulin resistance and β-cell dysfunction, hallmarks of T2DM. [1]

Members of the IL-1 family are all important; however, IL-1α and IL-1β are unique in taking part in the processes of inflammation, immune activation, and regulation of glucose metabolism. Both IL-1α and IL-1β are encoded by different genes on the long arm of the human chromosome 2 at 2q14.2. These genes encode proteins constituting a gene cluster that is important for the regulation of both innate and adaptive immune responses, particularly in response to microbial infection or tissue damage. Elevated levels of IL-1 cytokines have been observed in various

chronic inflammatory diseases, including rheumatoid arthritis, autoimmune disorders, and polycystic ovary syndrome, suggesting that dysregulation of IL-1 production could contribute to the pathogenesis of these conditions, including diabetes [2], [3].

Mechanistically, there is evidence for the involvement of cytokines in T2DM pathogenesis by demonstration of IL-1 participation in insulin sensitivity and pancreatic β-cell function. To that respect, IL-1β induces the appearance of other pro-inflammatory cytokines associated with the development of a systemic inflammatory state that impairs insulin signaling and glucose metabolism [4]. Empirical studies have established that IL-1β, both directly and indirectly, contributes to the destruction of pancreatic β-cells via the activation of immune cells and induction of apoptosis within β-cells [5]. Moreover, IL-1β impacts insulin exocytosis; high levels of IL-1β may eventually reduce the capacity of β-cells to secrete insulin and thus worsen the hyperglycemic conditions of patients with diabetes [6].

IL-1α similarly play an important role in inflammation process and immune activation. It is produced mainly by tissue macrophages, but similar to the case for IL-1β, its effects are both local at the injury/infection site and systemic. The in vivo responses induced by IL-1α are the same as those triggered by IL-1β, including the activation of innate immunity, recruitment of immune cells into sites of inflammation, and induction of local tissue injury. In the setting of T2DM, increased levels of IL-1α have been detected in peripheral blood and pancreatic tissue from patients, thus indicating that it can play a role in β-cell inflammation and dysfunction [7].

The genetic polymorphisms can alter both IL-1α and IL-1β and thus may determine the level of their expression and the individual's susceptibility to diseases. A number of polymorphisms were identified within the IL-1 gene cluster including the IL-1α -889 C/T (rs1800587) and the IL-1β -511 C/T (rs16944). Some of these SNPs have been so far associated with variability in cytokine production and, hence, are likely to modulate susceptibility of an individual to develop T2DM or other inflammatory diseases. For instance, the IL-1α -889 C/T polymorphism was found to modulate the expression of IL-1α; it showed that the T allele indeed associates with increased levels of the cytokine and escalates the risk of inflammatory diseases [8], [9]. Similarly, the IL-1β -511 C/T polymorphism has been linked to increased IL-1β production, contributing to the chronic inflammation seen in T2DM patients [10].

Moreover, other genetic variants of IL-1β, such as rs1143634 occurring within exon 5, might influence the function of IL-1β itself, thus altering insulin sensitivity and β-cell function in diabetic patients [10]. These genetic variations point toward the possibility that IL-1 genetic polymorphisms may influence the individual's immune response and the pathogenesis of diabetes. Associations of these polymorphisms with susceptibility to T2DM have been evidenced in several populations, suggesting that genetic factors could modulate the inflammatory response and influence disease outcomes.

The aims of this work are to determine the frequency of gene polymorphisms concerning IL-1α and IL-1β in the case of Iraqi women with Type 2 Diabetes Mellitus. The respective study will particularly target the following polymorphisms: IL-1α -889 C/T (rs1800587), IL-1β -511 C/T (rs16944), and IL-1β +3954 C/T (rs1143634). By studying the frequency of such genetic variants in diabetic subjects, the investigation aims to establish whether they could be associated with the development of diabetes in the same subjects. The result could be highly instructive with regard to the genetic factors determining the inflammatory component of the disease and might contribute to identify those individuals who, due to their genetic background, are at particularly high risk for the disease.

2 MATERIALS AND METHODS

2.1 Sample Collection and DNA Extraction

In this study, 720 Iraqi females, ages ranging from 20 to 55 years old, attending NDC for checking and found to be newly diagnosed as having type 2 diabetes at AL-Yarmouk Teaching Hospital.. Two hundred forty Iraqi Arab females control subjects who were apparently normal in term non diabetic, non-hypertension and age, gender matched with the studied group were selected to be the control group. After that, from them Choosing 49 as patients and 40 as healthy control, who were free of acute illness and infection, had no history of hyperlipidemia, hypertension, renal disease, heart disease, smoking, alcohol use, or any other form of hyperlipidemia or hypertension.

1) Genotyping was conducted through experimental methods. Two milliliters of fasting peripheral cubical vein blood were collected and stored in an EDTA anticoagulant tube. Genomic DNA was extracted using the TIANGEN Biochemical Technology Co. kit, LTD (Beijing). Primers used in this study are listed in Table 1.

2) After extracting the DNA samples, they were stored at 20ºC and then amplification and purification were carried out. The purified PCR products were then sent to Macrogen Company in Korea for DNA sequencing, and their obtained sequences were aligned using (Mega-6) software [9]. Moreover, the nucleotide sequences were compared with the information on the NCBI website databases for SNPs and for any other differences in the PCR product segment by utilizing the BLAST search tool.

The PCR reaction was performed for the final 25 µl reaction volume by using 5 L of 2X Go Taq® bioneer master mix, 2 µl of 10M of each primer (forward, reverse), 5 µl of genomic DNA, and the volume was increased to 20 µl with nuclease-free distil water. As stated in Table 2, the Gene Amp® PCR System thermo cycler was used to carry out the reaction.

To conduct the present study, the University Clinic Heidelberg 3-Cytokine CTS-PCR-SSP Tray Kit was paired with PCR primers to identify alleles, genotypes, and some haplotypes in 22 positions of the promoter region of different types of interleukin. The Department of Transplantation Immunology at the University Clinic Heidelberg, Germany, designed these primers based on the WHO international nomenclature committee for cytokines. Each tray in the kit had primers that were ready for use for each allele, and they were composed of 48 PCR-dried lyophilized primer mixes that were placed in 96 wells to carry out cytokine genotyping for two individuals, with 48 wells per sample. Each well of the 96 well tray was identified by a combination of numbers and letters from H1 to A12. Numeric names were given to the PCR mix positions for each cytokine, which corresponded to locus specificities on the tray.

The kit included the master mix (CYT) that included ammonium sulfate, Tris buffer, magnesium chloride, glycerol (glycerin), cresol Red, and dNTPs.

2.2 Statistical Analysis

The direct gene counting method was utilized to calculate allele frequencies, and a significant deviation from Hardy-Weinberg (H-W) equilibrium was estimated using an H-W calculator for two alleles, which can be obtained for free on the website [1].

The WINPEPI computer programs for epidemiologists were used to calculate significant differences between alleles and genotypes displayed as percentage frequencies, with free online access at website [2].

3 RESULTS

The current study test the polymorphism for IL-1α $_{-889}$ C/T; rs1800587 and two SNPs including IL-1β$_{-511}$ C/T; rs16944 in promoter and IL-1 β $_{+3962}$ C/T; rs1143634 in encoding region exon 5 [8].

According to the presented results as shown in Table 3, the genetic polymorphism analysis of IL-1α-889 which was determined in promoter region -889 position, the result displayed a higher frequency of homozygous TT genotype in the patients' group than in the control group (40.82%vs.27.50%), that showed statistical significance. Conversely, the patients' group had a lower frequency of heterozygous TC genotype than the control group (26.53% vs. 32.50%), and the genotype frequency for both patients and the control group was higher than what HWE anticipated. This difference was statistically significant. However, the allele effect study, which included both genotypes carrying the T allele, revealed no significant association between the Diabetic group and the control group (Table 4).

While receptor gene SNP' -1970 and receptor alpha msp111000 showed significant devation from HWE with odd ration more than one for homozygous CC genotypes for both SNPs.

Table 1: Primer present researcher Design for IL1.

Primer	Sequence 5 -3	length	Tm Cº	Product length	References
Forward primer	GTTGCGCCATAGACCTGTTG	20	60	785bp	3ed Researcher design
Reverse primer	TCCAAAGTCACGTGGTGCTA	20	60		

[1] http://www.had2know.com/academics/Hardy-Weinberg-equilibrium-calculator-

[2] http://www.brixtonhealth.com.

Table 2: PCR reaction cycle.

Steps of reaction	Temperature C°	Time	Number of cycles
Initial denaturation	95	4min	1
Denaturation	95	45sec	40
Annealing	60	1m.	
Extension	72	1m.	
Final extension	72	5m.	1
Hold	4	α	α

Table 3: Genotyping frequency of present study Interleukins.

IL-s types	Genotype	No: Patients 49 control 40	Genotype Frequency (Observed% / Expected %)		HWE Patients control	OR (CI: 95%) Patients vs. Control	Etiological or prevention fraction	Fisher's exact proba-bility
			Patients	Control				
IL-1α-889 rs1800587	TT	20:11	40.82/29.25	27.50/19.14	*0.001**	1.82(0.74-4.56)	0.45	0.225
	TC	13:13	26.53/49.67	32.50/49.22		0.75(0.31-1.91)	0.25	0.564
	CC	16:16	32.65/21.08	40.0/ 31.64	*0.03**	0.73(0.30-1.76)	0.27	0.443
IL-Rα-msp111000	TT	14:18	28.57/21.08	45.0/42.25	*0.005**	0.49(0.20-1.19)	0.511	0.100
	TC	17:16	34.69/49.67	40.0/45.5		0.81(0.33-1.92)	0.20	0.586
	CC	8:6	36.73/29.25	15.0/12.25	*0.03**	1.11(0.34-3.72)	0.96	0.887
IL-1R-1970	TT	23/10	46.94/50.0	50.0/42.25	*0.005**	0.88(0.38-2.06)	0.115	0.833
	TC	14/12	28.57/47.48	30.0/45.50		093(0.37-2.38)	0.67	0.909
	CC	12/8	24.49/15.04	20.0/12.25	*0.03**	1.30(0.47-3.71)	0.229	0.710
IL-1β-511 (rs16944)	TT	12:6	24.49/28.15	15.00/14.06	NS	1.84(0.62-5.80)	0.456	0.243
	TC	28:18	57.14/49.81	45.00/46.88		7.56(2.69-22.50)	0.868	4.3E-5
	CC	9/16	18.37/22.03	40.0/39.06	NS	0.34(0.13-0.89)	0.663	0.025*
IL-1β+3962 rs1143634	TT	18/18	36.73/34.78	40.82/48.39	NS	0.71(0.30-1.68)	0.290	0.453
	TC	20/15	40.82/48.39	37.50/46.22		1.15(0.48- 2.75)	0.130	0.747
	CC,	11/7	2245/16.83	17.50/13.14	NS	1.36(0.47- 4.12	0.267	0.517
IL-1RL1 -27609	TT	15/8	30.61/25.84	20.00/55.06	Ns	1.76(0.66-4.92)	0.433	0.281
	TC	24/ 14	48.9 / 42.7	35.00/55.06		1.78(0.75-4.26)	0.439	0.167
	CC	10/18	20.41/31.46	45.00/55.06	*0.013**	0.31(0.12-0.81)	0.687	*0.017**

*P≤0.05 significant , **P<0.001 high significant

Table 4: Allele frequency of present study.

ILs type	Allele type	Number patients No.: control No.	Allele frequency ratio		Odds ratio	Etiology fraction	Prevention fraction	Fisher's exact proba-bility	CI (95%)
			Patients	Control					
IL-1α-889 rs1800587	T	53:35	54.08	43.75	1.51	0.45	0.25	0.155	0.87-2.75
	C	45:45	45.92	56.25	0.66			0.155	0.36-1.20
IL-Rα-msp111000	T	45:52	45.92	65.00	1.67	0.4	0.57	0.083	0.94-2.99
	C	53:28	54.08	35.00	0.94			0.854	0.50-1.77
IL-1R-1970	T	60:52	61.22	65.00	0.45	1.15	0.55	0.11	0.64-2.18
	C	38:28	38.78	35.00	1.18			0.588	0.64-2.18
IL-1β-511 (rs16944)	T	52:30	53.06	37.50	0.188	0.469	0.469	0.049*	1.03-3.45
	C	46:50	46.94	62.50	0.58			0.049*	0.29-0.97
IL-1β+3962 (rs1143634)	T	56:51	57.14	63.75	0.76	0.242	0.25	0.401	0.41-1.39
	C	42:29	42.85	36.25	1.32			0.40	0.72-2.43
IL-1RL1 -27609	T	54:30	55.10	12.50	8.59	0.88	0.019	0.0001*	4.0-19.2
	A	44:50	44.90	62.50	0.49			0.020*	0.27-0.93

*P≤0.05 significant , **P<0.001 high significant

There was no discernible difference between the observed and expected frequencies of the three genotypes in the current study of the IL-1β$_{-511}$ gene polymorphisms, which were identified in the promoter region at the $_{-511}$ position gene in patients with diabetes mellitus (a good agreement with Hardy-Weinberg equilibrium; HWE) (Table 3).

Whereas, the two alleles' frequency (T, C) revealed some significant difference (Table 4). Nevertheless, comparing patients to controls some differences were revealed in homozygous TT genotype for patients and control (24.49 vs 15.00), and the T allele was significantly increased in patients (53.06%) compared to controls (37.50%) respectively (Tables 3 and 4)

Patients with the heterozygous TC genotype did not differ significantly from control. However, compared to control (40.0 and 62.50%), patients' CC genotype and c allele frequencies were significantly lower (18.37 vs. 46.94%).

The fifth position in the promoter region +3962 (rs1143634) of IL-1β gene, for diabetic patients and control showed that the homozygous TT genotype was detected in (36.73 vs 40.82%), heterozygous TC genotype was detected in (40.82 vs 37.50 %). Homozygous CC genotype was detected in (22.45 vs 17.50 %) Table (3). The T allele frequency was (57.14 vs 63.75%) and the C allele frequency was 42.85 vs 36.25%). (Table 4).

There were no discernible variations in the genotype distribution or allele frequency between our study groups, and the above-observed genotype frequencies matched those anticipated by the HWE.

IL-1RL1 receptor genotype polymorphism (-27609 T/C), the genotype distribution, the frequency of the TT genotype was significantly higher in diabetic patients, 30.61%, compared with controls, 20.00%, thus showing strong association with increased risk for T2DM, On the contrary, CC genotype was highly expressed among the control group, 45.00%, compared with diabetic patients, 20.41%, indicating a protective role against the disease.

In the case of allele frequencies, the T allele was strongly associated with an increased risk of T2DM, with an odds ratio of 8.59. In contrast, the C allele showed a protective effect, as evidenced by the significant prevention fraction. These contrasting roles underline the differential impact of receptor allele variants on diabetes risk within the studied population.

In addition, the genotype frequencies of the IL-1RL1 receptor -27609 T/C followed HWE expectations, showing a stable genetic distribution in this cohort. This may indicate that the observed associations are not related to population stratification or sampling bias.

4 DISCUSSION

In the Iraqi population, IL-1 polymorphisms have been linked to the etiology of metabolic diseases, diabetes, and inflammatory disorders [5], [12] and until now, no local studies have illustrated the association between IL-1α -889, IL-1β -511, and IL-1β +3962 genotypes polymorphisms with diabetes risk.

In light of these findings, the current genetic analysis provides a more precise assessment of the relationship between the genotypes IL-1α -889, IL-1β -511, and IL-1β +3962 with the risk of diabetes in female Iraqi patients.

First of all, comparison of the results of IL-1α -889 polymorphism shows that the homozygous TT genotype and the T allele may be considered as risk factors for diabetes in Iraqi females that make them more susceptible to developing T2DM by modifying immune response for most inflammatory diseases [10], [11]; while TC, CC genotypes with C allele significantly decreased the risk of diabetes which can be considered as a protective factor from T2DM. The outcome is consistent with other research that demonstrated the risk allele has a larger role in the pathophysiology of specific diseases [12], with mutant allele C being mostly derived from the ancestral allele A [13].

Neutral progressive mutations are most likely the source of late-onset diseases. These mutations can result in the total replacement of ancestral alleles, creating a situation where the common (derived) allele is the dangerous one and the rare (ancestral) allele is the protective one [14], [15].

Moreover, current findings along with previous local maintained studies results showed that the IL-1α -889 is a strong indicator for the incidence of T1DM in the Iraqi population [12], [16], as well as in an Indian study supporting a positive association between aggressive periodontitis and the presence of the IL-1α -889 with two allele polymorphisms [17], [18].

The present study disagrees with a study on older people in Brazil, where allele T of IL-1α -889 protects against the occurrence and intensity of tinnitus in the elderly, while allele C has a role in the pathophysiology of the inflammatory response, making older people more tinnitus susceptibility

associated with a history of workplace noise exposure [19].

The current findings conclude that IL-1α -889 SNP might have a role in the developing mechanism of diabetes in Iraqi female patients.

The second SNP under investigation is associated with Interleukin-1β, IL-1β -511. SNP rs16944 has been linked to immune-mediated diseases like diabetes mellitus because it plays a vital role in controlling inflammatory and immunological responses [20]. The current results suggest that; although the present expected is compatible with observed according to HWE, the IL-1β -511 polymorphism for both alleles acts significantly as protected barriers against diabetes. The present findings contradict a prior study conducted in the Indian population that demonstrated a correlation between a higher risk of diabetes and the IL-1β TT genotype and T allele [21].

In contrary, an Egyptian study finds that there is a strong significant association of C allele with the diabetic mellitus risk. Environmental factors, genetic background, and ethnic variance could all be responsible for this disparity in risk allele [22]. This finding is consistent with a systemic assessment published by the Jiao team, which discovered a link between a lower risk of diabetes and the T allele of IL-1β -511 [23].

The genotype distribution and allele frequency of the IL-1β +3962 SNP (rs1143634) DNA genotyping results showed no discernible differences between the diabetic patients and control groups. The observed genotype frequencies were in line with the Hardy-Weinberg Equilibrium, indicating that no evolutionary mechanisms were performed on this locus in the female population of Iraq. Even though the T allele's relative risk (OR) was 0.76, suggesting that it might be a protective factor, and the C allele's was 1.32, suggesting that it might be a risk allele, the above variation was not statistically significant. Since the three genotypes (CC, CT, and TT) of the IL-1β +3962 SNP appear to have the same picture and there is no discernible difference in their frequencies between patients and the control group, these current results are consistent with local research regarding Iraqi patients with multiple sclerosis (MS). This suggests that IL-1β gene polymorphisms may not be relevant to susceptibility to MS in Iraqi patients [4]. Its functionality is still debatable, although IL-1β, rs1143634, is situated in a coding area on the fifth exon of the IL-1β gene and has a synonymous codon as a functional consequence. While some studies do not indicate any difference in IL-1β secretion, others indicate that the T allele is linked to greater IL-1β production [24]. An Arabian study concluded that T allele for both IL-1β SNPs can be used as a strong marker for susceptibility to develop chronic hepatitis B in the Tunisian population [25], [26].

Another article identified independent association of common IL-1β SNPs polymorphic with HBV-related hepatocarcinogenesis in a Caucasian population [27].

The results confirm that the IL-1RL1 receptor (-27609 T/C) polymorphism is significantly associated with susceptibility to T2DM in Iraqi females, with evidence from results for the importance of genotype variations in modulating susceptibility to diabetes. At the same time, the T allele was found to be a risk allele, whereas the C allele seemed to be protective, underlining the importance of interleukin receptor pathways in the genetic predisposition to T2DM.

This is likely different across populations for a variety of reasons, including sample size limitations, which are crucial for SNP studies, racial and ethnic diversity, which causes SNPs to correlate with gene pools in each population, the relevance of this gene's haplotype with other cytokine-encoding gene haplotypes, and environmental factors that disrupt genetics and may cause the population's selection machinery to evolve.

Lastly, for the cluster of IL-1 and receptor genes polymorphism more research is needed to shed light on this relationship so that researchers may assess genetic vulnerability to prognostic and diagnostic diseases. SNPs may play crucial roles when coupled with one another and have an impact on protein production.

5 CONCLUSIONS

The analysis of IL-1 genetic polymorphisms indicates that variations, particularly in the IL-1α $_{-889\ C/T}$ and IL-1β$_{-511\ C/T}$ genotypes and ILR$_{-1970}$, may contribute to the susceptibility to T2DM among Iraqi females. The findings suggest that the T allele of IL-1α$_{-889}$ is associated with an increased risk of T2DM, while the C allele demonstrates a protective effect. Similarly, the T allele of IL-1β$_{-511}$ acts as a protective females who carry it from T2DM, whereas the C allele increases the risk of developing diabetes.

In this population, there was Fisher's ratio analysis showed that the genotypes of both alleles for IL-1β$_{-511}$and IL-1RL1 were significant, making it important to follow up with T2DM

These results emphasize the necessity for additional research with advanced methodologies

such as haplotype analysis, and Real-Time PCR techniques to validate and expand upon these results.

6 LIMITATIONS AND FUTURE DIRECTIONS

This study provides valuable insights into the genetic factors influencing T2DM risk in Iraqi females, even that there are several limitations that need to be addressed in future research. First, the sample size of 49 patients and 40 controls may limit the generalizability of the findings. Larger cohort studies are necessary to confirm the association between IL-1 polymorphisms and T2DM risk. Additionally, exploring the role of gene-environment interactions, including factors such as diet, physical activity, and obesity, is crucial in T2DM pathogenesis as epigenetic factors.

Furthermore, functional studies examining the impact of these polymorphisms on IL-1α and IL-1β expression levels, as well as their role in immune modulation, could help elucidate the underlying mechanisms linking these polymorphisms to T2DM.

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Isolation and Identification of Bacteria Isolated from the Oral Cavity of Oral Leukoplakia

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Keywords: Oral Leukoplakias, Bacterial Isolation, Staphylococcus Aureus, Oral Microbiome.

Abstract: A potentially cancerous condition called oral leucoplakia (OL) is typified by white patches on the oral mucosa. Microbial colonization, especially by bacteria, has a role in the pathophysiology of OL by promoting inflammation and the advancement of the illness. In this study, bacterial species from the oral cavity of patients with OL will be isolated, identified, and compared to those found in healthy persons. From July to December 2024, samples were taken from dental clinics and specialty centers in the Diyala Governorate. Micrococcus luteus, Kocuria kristinae pneumonia, Streptococcus alactolyticus, Staphylococcus hominis, and Rothia dentocariosa were among the most commonly isolated bacterial species. The development and malignant transformation of oral leukoplakia may be facilitated by the presence of particular pathogenic microorganisms. Thirty of the 120 MDR bacterial samples showed antibacterial sensitivity, indicating a possible role in chronic inflammation and the development of disease. To better understand how these bacteria contribute to the pathophysiology of OL and create focused treatment plans, more investigation is needed.

1 INTRODUCTION

White spots that cannot be wiped off and cannot be linked to any other recognized condition are the hallmark of oral leucoplakia (OL), a frequent and possibly malignant disorder of the oral mucosa [1]. The aetiology of OL is complex and includes microbial infections, persistent irritation, alcohol and tobacco use, genetic predisposition, and artificial feeding of diabetic infants. [2]. Bacteria stand out among the microbial components because of their propensity to contribute to malignant transformation, change the oral microenvironment, and promote inflammation. The prevalence of oral leukoplakia is 4.11% worldwide [3].

The human oral cavity hosts a diverse microbiome, with both commensal and pathogenic bacteria playing significant roles in oral health and disease. Previous studies have suggested that bacterial dysbiosis in oral leukoplakia could be associated with increased inflammatory responses, oxidative stress, and immune modulation, which may facilitate the progression of precancerous lesions [4]. Oral leucoplakia has a malignant transformation rate of 7.5% based on clinicopathological investigations and 9.7% based on systematic review research. Further histological analysis revealed that some tissue portions had changed into malignant lesions, even though some leucoplakia instances exhibited clinically benign characteristics in clinical investigations. [3].

According to some research, leucoplakia is linked to an increased risk of upper gastrointestinal cancers since it is a precancerous lesion that, if ignored, can have systemic health repercussions. Identifying and isolating bacterial species from OL lesions can reveal their potential role in disease progression, aid in developing targeted therapies, and assess antibiotic resistance patterns for effective treatment planning and infection control [5]. Because oral leukoplakia has serious side effects, such as tissue abnormalities and pharmacological side effects, it must be treated as soon as possible. Traditional techniques such as local surgical excision and systemic medication application have been supplanted by cryotherapy, laser, and photodynamic therapy. Both men and women are more prone to have oral leukoplakias, but they are also more likely to develop into malignant transformations. Additionally, nonsmokers are more vulnerable. [6].

This study aims to isolate and identify bacterial species from the oral cavity of patients with oral

leukoplakia, compare them with bacteria present in healthy individuals, and assess their antibiotic susceptibility profiles. By doing so, we hope to highlight the potential microbial factors involved in OL pathogenesis and provide a foundation for future research in microbial-targeted therapies at follow.

2 MATERIALS AND METHODS

2.1 Sample Collection

The study was conducted at the Laboratory Biology Department, /College of Education for Pure Sciences/University of Diyala and the First Specialized Dental Center in the Ba'aqubah / Diyala Governorate. The time frame is September 2024–December 2024. Samples were taken from gingivitis patients. A cotton swab was used to gather them from both sexes' mouth cavities. Twenty percent of the samples exhibited no growth, whereas 120 (80%) of the samples showed positive growth. A total of 55 (46%) male and 65 (45%) female samples received the positive growth isolates. At the First Specialized Dental Centre in Baqubah/Diyala Governorate, they were between the ages of one and forty. The plates were incubated at 37 degrees Celsius for 24 hours while they were cultivated on a selective and differential medium, such as blood agar and MacConkey agar.

2.2 Isolation and Diagnosis

Direct culture of the materials was performed on a nutrient agar medium. Following purification on Deferential blood agar and MacConkey selective culture medium, the isolates were aerobically cultured for 24 hours at 37° C. Phenotypic and biochemical assays were then performed, and the Vitek 2 compact was used for confirmation

2.3 Microscopic and Biochemical Examination

Following the conclusion of the Gram staining procedure, a microscopy examination is conducted. A 100X objective is used to examine the slide. Following the identification of the gram stain-positive and gram-negative bacterial kinds, all biochemical assays were carried out and the bacteria were cultivated on diagnostic and differential media tailored to each type of bacteria [6].

3 RESULTS

150 gingivitis patients had their samples taken. Specialized dentistry facilities provided the samples. As seen in Table 1 and Figure 1, there was a significant difference ($p<0.05$) between the number of samples that showed positive growth (120, or 80%) and those that did not (30, or 20%).

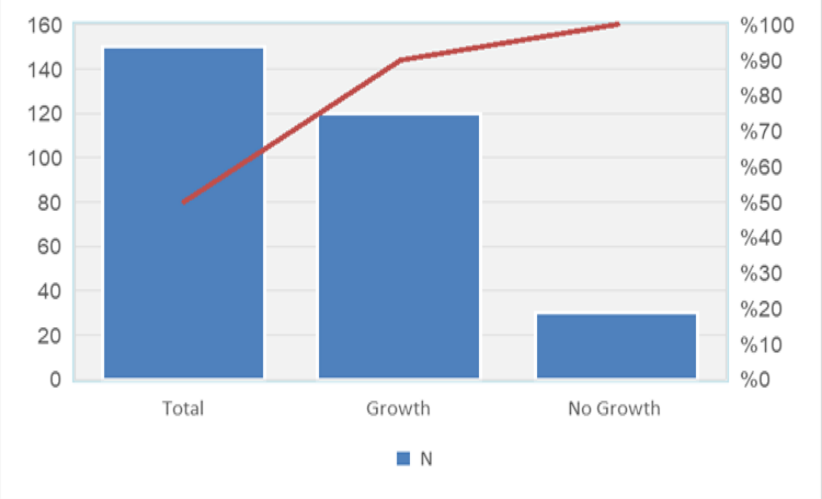


Figure 1: Participants' bacterial growth frequency and percentages.

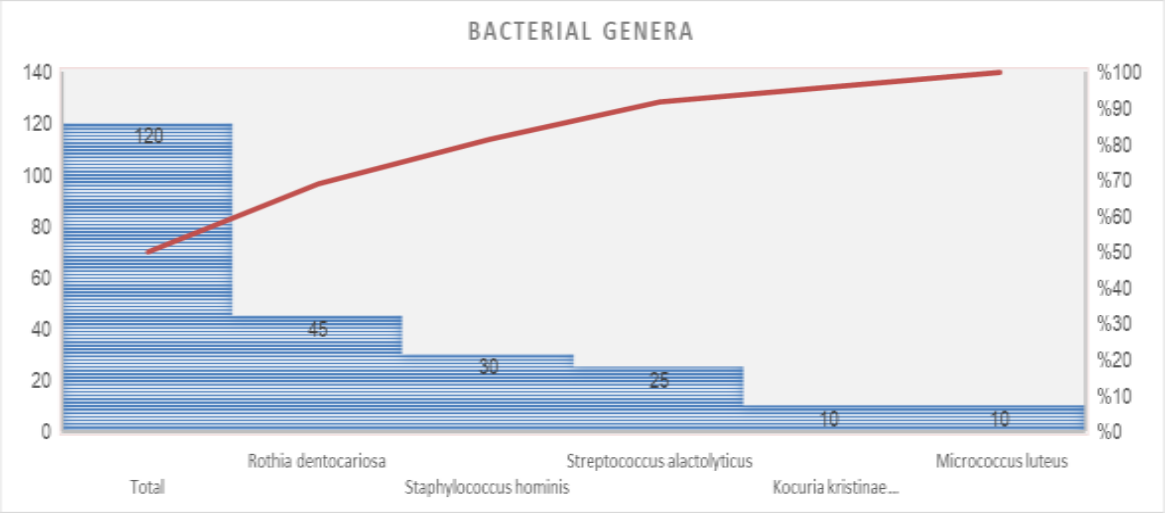


Figure 2: Participants' bacterial species frequencies and percentages.

Table 1: Frequency and percentages of bacterial growth in participants.

Bacteria growth	No.	%	P Vule
Growth	120	80%	P<0.001***
No Growth	30	20%	
Total	150	100%	

A range of age groups for both sexes were included in the sampling. The following is the distribution of the isolates that showed favorable growth: Table 2 displays 55 (46%) male samples and 65 (54%) female samples.

Table 2: Distribution of participants with bacteria growth according to gender.

Gander	N	%	P value
Males	55	46%	p>0.05
Females	65	54%	
Total	120	100%	

Present findings showed the most bacterial growth was in patients within age groups; 1-10 (32.5%) and >40 years (36.7 %) and a little of it was at 11-20 (10.0 %), 21-30 years (12.5 %), 31-40 years (8%). The differences in bacterial growth among age groups were significant(p<0.05) Table 3.

The following were the findings of the investigation that demonstrated the presence of bacterial growth in the samples: Micrococcus luteus 10 (8%), Kocuria Kristine pneumoniae 10 (8%), Streptococcus alactolyticus 25 (21%), Staphylococcus hominis 30 (25%), and Rothia dentocariosa 45 (38%). There were substantial differences across the types of bacteria (p<0.05). Figure 2 and Table 4.

Table 3: Distribution of participants with bacterial growth according to age groups.

Age group	No.	%	P Value
1-10	39	32.5%	*p<0.001***
11-20	12	10.0%	
21-30	15	12.5 %	
31-40	10	8.3%	
40<	44	36.7 %	
Total	120	100%	

Table 4: Frequency and percentages of bacterial species in participants.

Bacterial genera	No.	%
Rothia dentocariosa	45	38%
Staphylococcus hominis	30	25%
Streptococcus alactolyticus	25	21%
Kocuria kristinae pneumoniae	10	8%
Micrococcus luteus	10	8%
Total	120	100%
P Value	***P<0.001	

4 DISCUSSIONS

The oral cavity of the human is a mini ecosystem comprised of different niches colonized by an immense number of microorganisms, including fungi, several types of viruses and diverse bacterial

fauna. Around 1100 different taxa were discovered in the oral cavity and recorded in the Human Oral Microbiome Database [7]. The most prevalent potentially cancerous condition affecting the oral mucosa is oral leukoplakia. The role of bacterial colonization in the progression of leukoplakia and its possible development into oral squamous cell cancer. The isolation and identification of bacteria from oral leukoplakia lesions, as well as their possible role in the development of the disease, were the main objectives of this investigation [8].

This study investigating the microbial composition of oral leukoplakia lesions has identified several bacterial genera with increased prevalence. Notably, Rothia dentocariosa 45 (38%), Staphylococcus hominis 30 (25%), Streptococcus alactolyticus 25 (21%), Kocuria Kristine pneumonia 10(8%), and Micrococcus luteus 10(8%). The differences among bacterial species were significant ($p<0.05$) and were found in higher abundance in these lesions compared to healthy oral tissues. The highest percentage of bacteria isolated from loral leukoplakia cases was Rothia dentocariosa 45(38%). Tables 3-4. These findings suggest a potential association between these bacteria and the pathogenesis of oral leukoplakia. Gram-positive, spherical to rod-shaped bacteria, Rothia species are typically found in the respiratory and oral tracts. [9].

This study showed that the incidence rate is higher among 55(46%) males than 65 (54%) females. This study disagrees with the findings of many authors' studies, whose studies have shown that the incidence rate among males is higher than that of females, at a ratio of 2:1. These results are consistent with those of many researchers regarding the occurrence of bacterial isolates, but at varying rates. This depends on the patient's health status, as most of the isolated bacteria are opportunistic bacteria, which are found as normal flora in the mouth and then become pathogenic depending on the patient's health and immune status [10]. This study offers highlights of Rothia species, focusing on their identification, pathogenicity, clinical implications and the role of Rothia dentocariosa as both a commensal organism in the oral cavity and a potential opportunistic pathogen [11].

According to in silico research of R. dentocariosa entire genome and proteome, a number of proteins are thought to have both virulence and secretion potential. Only mild biofilms could be formed by R. dentocariosa. [12]. Depending on the stimulant being utilized, *R. dentocariosa's* capacity to produce distinct cytokines changed. Certain cytokines that were not produced by whole cells or biofilm supernatants were produced by biofilms and planktonic cultures.

Planktonic and biofilm cells produced IL-8 at almost identical levels, but only the planktonic cultures produced IL-10 at significantly greater levels ($P<0.05$). Compared to biofilm and planktonic cultures, the biofilm supernatant and whole cell stimulants produced lower quantities of cytokines. [13]. Antimicrobial susceptibility testing revealed varying resistance patterns among bacterial isolates, with some strains demonstrating resistance to commonly used antibiotics such as amoxicillin and tetracycline[14]. The presence of multidrug-resistant bacteria suggests that conventional antibiotic therapy may be less effective in managing secondary bacterial infections in oral leukoplakia patients. Given the increasing evidence linking microbial dysbiosis to oral potentially malignant disorders, future research should focus on characterizing bacterial virulence factors, host-microbiome interactions, and targeted antimicrobial or probiotic interventions to modulate the microbial environment in leukoplakia patients [15].

4 CONCLUSIONS

A wide variety of bacterial species that colonize the oral cavities of patients with oral leukoplakia were effectively identified in this investigation. Staphylococcus hominis, Micrococcus luteus, Rothia dentocariosa, Streptococcus alactolyticus, and Kocuria kristinae were the most commonly isolated organisms. Even though these bacteria are a typical element of the oral flora, they may be involved in the pathophysiology and persistence of oral leukoplakia lesions or chronic inflammation, particularly in cases of immune dysregulation or mucosal impairment. The polymicrobial character of the oral cavity and the significance of comprehensive microbial profiling in patients with premalignant oral lesions are highlighted by the discovery of both Gram-positive and Gram-negative organisms. Further molecular and antimicrobial susceptibility studies are recommended to better understand the virulence potential and resistance profiles of these isolates, particularly in the context of chronic oral mucosal conditions.

ACKNOWLEDGMENTS

For giving the resources and assistance required for this study, we would like to sincerely thank the University of Diyala College of Education of Pure Science. Additionally, we would like to express our gratitude to the patients who took part in this study, as their collaboration was crucial to accomplishing its goals. We would especially want to thank our mentors and colleagues for their wise counsel and support during the study process.

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Study of the Relationship between Myeloperoxidase Enzyme, Ghrelin Hormone, and Relaxin Hormone Levels in Blood Serum During Late-Stage Pregnancy

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Keywords: Antioxidants, Ghrelin, Relaxin, Amyloperoxidase, Pregnant Women.

Abstract: To study the effect of levels of amyloperoxidase, ghrelin, and relaxin on the late stages of pregnancy in pregnant women, and to study the correlation between the study variables. To identify potential factors that may lead to oxidative stress in pregnant women during the late stages of pregnancy. Amyloperoxidase is classified as an oxidoreductase enzyme, belongs to the peroxidase family, and contains a heme group. It is found primarily in neutrophils and lymphocytes, as well as in monocytes and macrophages. Approximately 30% of cellular amyloperoxidase is secreted as an active enzyme into the extracellular fluid, along with several antimicrobial factors at the site of inflammation, through the lysis of neutrophils. The study included collecting 90 blood samples, from patients and healthy individuals, during the period from 14/1/2024 to14 /2/2024 from pregnant women in the final stages of pregnancy. The samples were collected from Al-Yarmouk Teaching Hospital, Department of Obstetrics and Gynecology, Baghdad. The samples included 60 patient samples and 30 healthy samples representing the control group. The results were analyzed using an ELISA device, which went through several steps, including preparing the ready-made measurement kit containing antigens for the variables under study. Then, the blood serum containing the antibodies was added, where the reaction process was carried out. The serum was then washed several times, and the results were placed in the device for the purpose of reading the absorbance at a wavelength of 450 nm. Research results: The research results showed a significant increase in the level of the enzyme amyloperoxidase and a decrease in the levels of both relaxin and ghrelin hormones. The current results indicate that there is no correlation between the enzyme myeloperoxidase and both relaxin and ghrelin.

1 INTRODUCTION

The final stages of pregnancy include the third trimester. The mother's body and fetus undergo significant changes in preparation for childbirth. During this stage, [1]. the pregnant woman's weight increases, and she may feel more tired and exhausted. Oxidative stress may play an important role in the final stages of pregnancy, resulting in an imbalance between free radicals and antioxidants in the body [2]. The enzyme amyloperoxidase is primarily found in white blood cells and plays an important role in the immune response by producing free radicals to fight infection. [3]. It also contributes to oxidative stress if it is present in excess. Its levels may rise in the final stages of pregnancy due to increased immune system activity in preparation for childbirth. Amyloperoxidase is the only enzyme in the peroxide family capable of reducing chloride ions to hypochlorous acid, an oxidizing agent that plays an important role in the immune system. However, excessive production of hypochlorous acid is a risk factor because it can oxidize many biological molecules. [4]. The hormone relaxin plays a prominent role in the final stages of pregnancy, as it contributes to preparing the body for childbirth by relaxing and stretching the pelvic ligaments and joints, which contributes to facilitating the process of dilating the birth canal during labor.

It also helps soften and gradually dilate the cervix in preparation for childbirth [5]. The hormone ghrelin plays an important role in stimulating appetite and regulating food consumption. It is a peptide hormone known as the hunger hormone, secreted by glandular cells in the stomach, as well as in smaller quantities from the small intestine and pancreas. It affects the mother's appetite and energy needs, as the mother's

energy needs are essential in the final stages of pregnancy to support fetal growth, and ghrelin levels may change in response [6]. Ghrelin has prokinetic effects in the gastrointestinal tract and is believed to play an important role as an anti-inflammatory [7].

2 MANUSCRIPT PREPARATION

The current project was approved by the Scientific Committee at Tikrit University, College of Education for Pure Sciences, Department of Chemistry, in coordination with the Baghdad Health Department and Yarmouk Teaching Hospital, and conducted from 14 January to 14 February 2024. Blood samples were collected from 60 participants, including 30 pregnant women in their third trimester (study group) and 30 non-pregnant women (control group). From each participant, 5 mL of venous blood was drawn into gel tubes. Serum was separated by centrifugation at 3000 rpm for 20 minutes and stored at −20°C until analysis. Serum levels of myeloperoxidase enzyme and other study variables were quantified using a sandwich ELISA kit (SUNLONG Biotech, China). The assay procedure involved antigen-antibody binding, enzyme-conjugated detection, and reaction termination with an acidic stop solution. Absorbance was measured at 450 nm, and analyte concentrations were calculated against standard curves.

All data were analyzed statistically using (SPSS Statistical) using a computer and through the digital data program described. Microsoft Excel 2013 program and using 21.0 and the arithmetic mean and standard deviation values were obtained as well as the correlation coefficients were obtained.

3 RESULTS

3.1 Amyloperoxidase

This study assessed the relationship between pregnant women and the control group and their amyloperoxidase activity levels. Statistical analysis was then conducted on samples from the pregnant and control groups. The results of our study indicated a significant increase in amyloperoxidase activity in the pregnant group compared to non-pregnant women, as shown in Figure 1, Table 1.

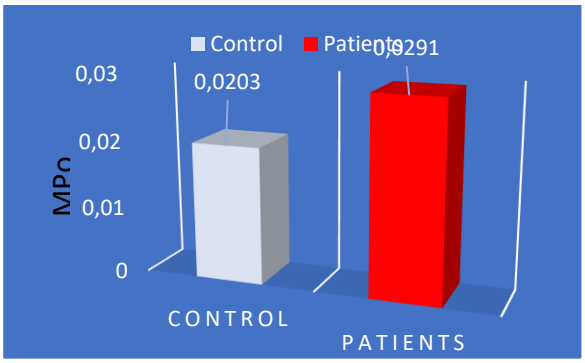


Figure 1: The activity of the enzyme amyloperoxidase inthe blood serum of healthy and sick pregnant women.

Table 1: The levels of study variables in the serum of pregnant and non-pregnant women.

Group Parameters	Control	Patients	P-Value
Amyloperoxidis	0.02±0.01	0.029±0.021	p<0.05
Relaxin	0.08±0.03	0.091±0.035	p>0.05
Ghrelin	225.26±25.10	226.40±27.27	p>0.05

3.2 Relaxin

When conducting a study on the level of relaxin hormone in pregnant and non-pregnant women, it was found that there were no significant differences in the relaxin hormone in pregnant women compared to non-pregnant women, as shown in Figure 2 and Table 1.

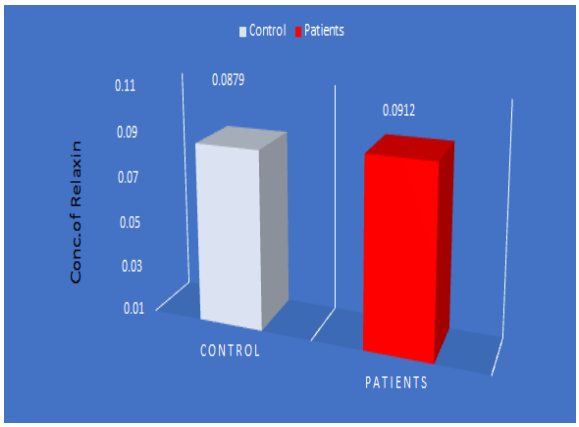


Figure 2: Level of relaxin hormone in the blood serum of healthy and sick pregnant women.

3.3 Ghrelin

The results of our current study indicated that there was no change in the level of the ghrelin hormone in pregnant women and non-pregnant women, as the results showed no significant differences in the ghrelin hormone in pregnant women compared to non-pregnant women, as shown in Figure 3 and Table 1.

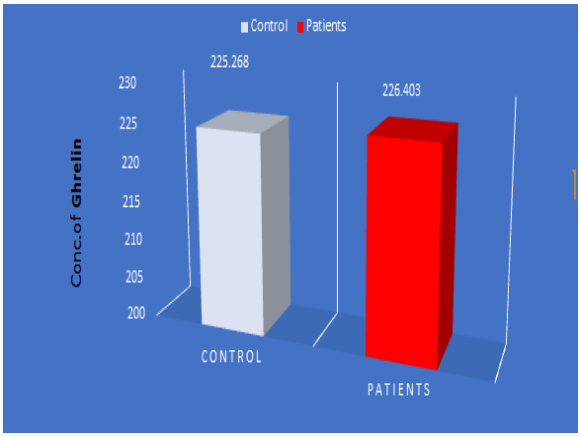


Figure 3: The level of ghrelin hormone in the blood serum of healthy and sick pregnant women.

3.4 Correlation Relationships

When studying the correlation relationships between the two enzymes, amyloperoxidase, our results showed that there were no significant correlation relationships between the enzyme amyloperoxidase and the hormone relaxin, as shown in Figure 4.

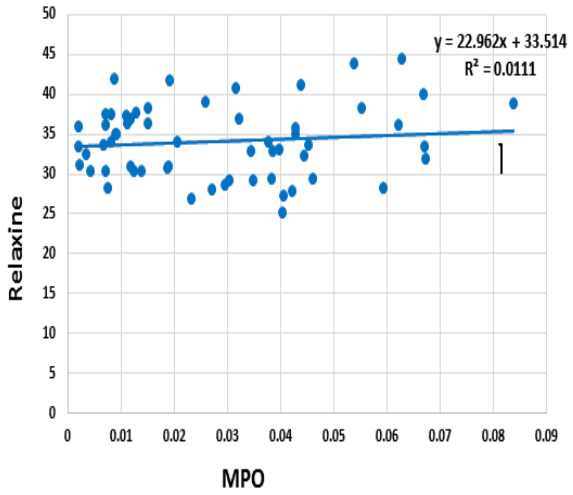


Figure 4: The relationship between the activity of amyloperoxidase and the hormone relaxin.

When studying the correlation relationships between amyloperoxidase enzymes, our results showed that there were no significant correlation relationships between the amyloperoxidase enzyme and the hormone ghrelin, as shown in Figure 5.

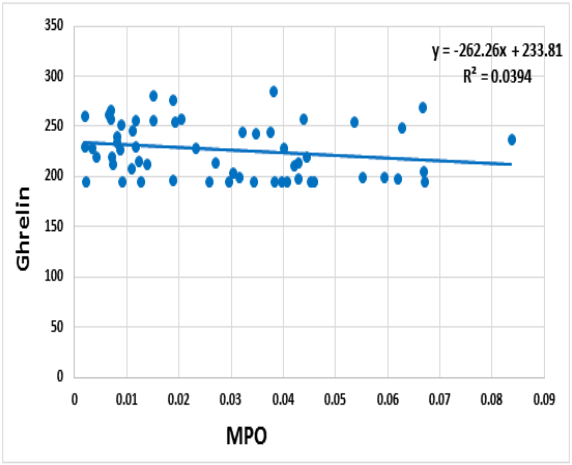


Figure 5: The relationship between the activity of amyloperoxidase and the hormone ghrelin.

The relationship between amyloperoxidase, an enzyme produced primarily by immune cells and acting as an antioxidant, and the hormone ghrelin, which regulates energy expenditure, and the hormone relaxin, which controls relaxation levels, has been studied. A study by Tsakonas [8] and Goodge et al. showed an increase in amyloperoxidase levels, which is consistent with our current study. The results showed that amyloperoxidase levels increase in pregnant women during the later stages of pregnancy and decrease in non-pregnant women. This is attributed to amyloperoxidase activity, which is under the influence of estrogen [9]. Amyloperoxidase is released outside the cells as an active enzyme into the fluids at inflamed sites. The hormone ghrelin is a peptide growth hormone and has been released. It has the nickname "hunger" [10]. Apart from its effect on growth hormone, it has many important biological actions such as regulating the autonomic nervous system Gupta et al. noted a decrease in ghrelin levels in pregnant women and suggested that there are factors controlling beta-glucose hyperplasia of the pancreas, and that changes in insulin and glucose sensitivity are responsible for the decrease in ghrelin. This study is inconsistent with our current study. A study by Jesse et al. indicated that relaxin levels increase in pregnant women in the seventh week of pregnancy and peak in the later stages of pregnancy and during labor. This is due to an increase in the corpus luteum, which is secreted in the final days of

pregnancy and helps relax the cervix and uterine ligaments during labor. This study does not resemble the results of our study [10].

A study by Duan et al. reported a significant increase in relaxin levels in the serum of pregnant women compared to the control group. There is evidence that maternal vasodilation persists after delivery and also reduces the risk of hypertension relaxin hormone in the serum of (patients) pregnant women compared to the control group. There is evidence that the expansion of the mothers' blood vessels continues after birth and also reduces the risk of developing high blood pressure in the next pregnancy and reduces the risk of developing cardiovascular diseases later in life. This study differs from our current study. There is a significant increase in the activity of the myeloperoxidase enzyme and a decrease in the hormone ghrelin and the hormone relaxin in the sera of pregnant women patients in the last stages of pregnancy compared to healthy people.

A study by Duan et al. reported a significant increase in relaxin levels in the serum of pregnant women compared to the control group. There is evidence that maternal vasodilation persists after delivery and also reduces the risk of hypertension [11]. in subsequent pregnancies and cardiovascular disease later in life. This study differs from our current study [12]. Gupta and others pointed out that there is a decrease in the hormone ghrelin in pregnant women and explained that there are factors that control the enlargement of the beta pancreas and changes [13] in insulin and glucose sensitivity that are responsible for the decrease in the hormone ghrelin. This study does not agree with our current study [14].

4 CONCLUSIONS

This study provides important insights into the biochemical changes that occur in the blood serum of women during the late stages of pregnancy. Specifically, the focus was placed on investigating the activity of myeloperoxidase (MPO), an enzyme associated with oxidative stress and immune activation, as well as two important regulatory hormones - ghrelin and relaxin - which are involved in metabolic balance and reproductive physiology, respectively. Through the clinical study of a number of vital variables that were conducted on the sera of pregnant women patients in the last stages of pregnancy, with partial purification of the MPO enzyme and a study of some of its kinetic properties, the following conclusions were reached in this myloperoxidase. Oxidative stress in pregnant women in the late stages of pregnancy leads to an increase in the level of the enzyme Amyloperoxidase. increases oxidative stress when elevated Ghrelin may have an antioxidant role, but its effect depends on the mother's metabolic state Relaxin may reduce oxidative stress, but its impact depends on its balance in the body.

ACKNOWLEDGMENTS

We sincerely thank the Scientific Committee at Tikrit University, College of Education for Pure Sciences, for their support of this research. We also gratefully acknowledge the staff of Yarmouk Teaching Hospital and all participants who contributed to the study.

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Broad-Spectrum Antibacterial Activity of the Buds Rosa Damascena Bud Extracts Against Pathogens Isolated Clinically

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Keywords: Rosa Damascene, Antibacterial Effects, Plant Extracts, Gas Chromatography-Mass Spectrometry.

Abstract: We present the chemical composition of the flower buds of Rosa damascena, as well as their inhibitory activity against pathogenic Gram-positive and Gram-negative bacterial strains. GC-MS analysis for the Detection of bioactive compounds in Rose Damascena buds and isolation of bacteria by using the Vitek 2 system. Evaluate the antibacterial activity of water and ethanolic extracts on multidrug-resistant strains. Assess the activity of both extracts also on bacterial growth inhibition. Methods: Preparation of Extracts: The flower buds (including calyx leaves, petals, stamens, and receptacles) were ground and extracted with water (aqueous) and ethanol (ethanolic). Antibacterial Assays: Tested extracts against seven bacterial strains using agar well diffusion method: Gram-positive: Staphylococcus aureus, Gram-negative: Escherichia coli, Klebsiella pneumoniae, Serratia, Pseudomonas aeruginosa, Proteus spp., Acinetobacter baumannii. Concentrations tested: Aqueous (15–50 mg/mL), Ethanolic (25–100 mg/mL). Bioactive Compound Profile: Bioactive compounds were identified by GC-MS. Results: The Inhibitory Activity: Ethanolic extract: Exhibited substantial inhibition against S. aureus, Serratia, and P. aeruginosa at all tested concentrations. Aqueous extract: More effective than ethanolic extract on K. pneumoniae and Proteus spp. (15–50 mg/mL). Strains that were not responsive: Both extracts did not affect A. baumannii. Isolates of E. coli were partially resistant, where one isolate was sensitive to ethanolic extract and another to aqueous extract. Chemical Composition: GC-MS analysis shows bioactive compounds such as 2,3-Butanediol, Oxirane,2,3-dimethyl and 6-Oxa-bicyclo[3.1.0]hexan-3-one are probably responsible for different antimicrobial activities. Conclusion: Rosa damascena buds are a reservoir of effective bioactive substances and have significant antibacterial potential, especially against S. aureus and P. aeruginosa. However, resistance seen in strains such as A. baumannii highlights the necessity for additional research to improve extraction strategies or to pair the active compounds with traditional antibiotics. The findings highlight the plant as a promising source of natural antimicrobials in the fight against antibiotic resistance.

1 INTRODUCTION

Medicinal plants play an important role in human life, as they are a rich source of active organic compounds and molecules that have been used as medicines or as important sources for drug discovery and design. Many studies have demonstrated the efficacy and safety of medicinal herbs in treating most human diseases, and some of these studies have revealed the molecular mechanisms behind the significant health benefits of medicinal plant drugs. However, some topics require further research, such as identifying and determining the active biological components, assessing the stability of these active substances, and evaluating their pharmacological efficacy [1]. The excessive use of antibiotics is a major problem that has led to the emergence of bacterial strains resistant to these antibiotics.

Therefore, it is necessary to find various methods, including referring to products manufactured from medicinal plants that have antibacterial efficacy and can reduce resistant bacterial strains [2]. Maintaining blood sugar levels is attributed to plant secondary metabolic products that are important in eliminating inflammation and have been used to treat many diseases such as coughs, colds, digestive system diseases, and pharyngitis, as these products serve as a

sedative, antispasmodic, and a significant immune stimulant [3]. Roses have been used for many purposes, including food, perfumes, and decoration. They have been cultivated throughout the ages. The most important of these roses is the Damask rose. It is distinguished by its pink or red colour and belongs to the rose family. It contains volatile oils that have been used in the perfume industry and the production of rose water [3]. The petals of the Damask rose contain gallic acid, flavonoids, phenols, anthocyanins, and beta-citronellas. Geraniol, in addition to antioxidants that are more than those found in green tea leaves, terpenes and glycosides, plants have antimicrobial, antibacterial, antiviral, anticancer and anti-inflammatory effects, used as a pain reliever, sedative and hypnotic, and if they contain terpenes and glycosides that have a beneficial effect on the body in treating eye diseases, it was also used in treating many diseases of the digestive system such as constipation as a laxative, and it showed an effect against depression, Alzheimer's and dysmenorrhea.

Before the introduction of antibiotics, the genes of bacterial resistance to antibiotics were very low. Over the past ten years, bacterial resistance to antibiotics has gradually accelerated since the first day of using these antibiotics. To reduce antibiotic resistance, the use of these antibiotics must be reduced, and other alternatives must be found, as the spread of infectious diseases poses a serious threat to public health, especially Gram-negative and Gram-positive bacterial species [4]. In the early 1940s, the miracle of penicillin had a remarkable impact on public health, saving many people from death due to infections and opening wide horizons for complex operations and the treatment of chronic diseases. However, the increasing resistance of bacteria, represented by Gram-positive and Gram-negative bacteria such as Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella spp., and others that cause various infections and diseases, necessitates finding solutions and understanding different ways to combat this increasing resistance. Various sources and media have sounded the alarm that we are returning to the pre-antibiotic era, with the possibility that antibiotic resistance could cause many deaths by the year 2050 [5].To find alternatives that reduce the spread of antibiotic-resistant bacteria while being effective and a natural substitute, the flower buds of the plant Rosa damascenes were chosen, hoping they would be a promising alternative to antibiotics.

This study aims to evaluate the antibacterial activity of aqueous and alcoholic extracts from Rosa damascena flower buds against clinically relevant Gram-positive (Staphylococcus aureus) and Gram-negative pathogens (Escherichia coli, Pseudomonas aeruginosa, Klebsiella spp., Serratia spp., Proteus spp., Acinetobacter baumannii) isolated from surgical and traumatic wounds, while identifying bioactive compounds via GC-MS analysis. Key objectives include determining the inhibitory efficacy of the extracts against these multidrug-resistant strains, characterizing phytochemical constituents, and assessing synergistic interactions between the extracts and commonly prescribed antibiotics to enhance clinical treatment strategies.

2 MATERIALS AND METHODS

2.1 Bacterial Samples

The Forty samples were collected from various sites and different parts of the body from patients at a private laboratory in Najaf Province after obtaining their consent. The samples were then cultured on media such as nutrient agar and MacConkey agar in Petri dishes, and incubated overnight at 37ºC in the incubator. After the incubation period, the plates were examined to identify bacterial growth, with distinctive growth patterns and colours recorded to assist in identifying potential microbial species. Additional tests were conducted to confirm the biological identity of the isolated bacteria [6]

2.2 Diagnosis of Bacterial Isolates

2.2.1 Culture Medium

To use the culture, Mueller-Hinton agar was used to isolate bacteria, and Nutrient broth was used to preserve and activate the isolated bacteria. All culture media were prepared according to the instructions of the manufacturers [7], and their pH was adjusted. The media were then sterilized with an autoclave for 15 minutes at 121°C and under pressure and used the Vitek 2 system (ANC Kit, bio Merieux) and aerobic control.

2.2.2 Microscopic Diagnosis

After examining the bacterial colonies growing in the solid Nutrient agar medium, casting them with a cram dye, and examining them with an electron microscope with a 1000 x oil lens, the microscopic field of the dyed slide showed cluster colonies dyed with crystal violet dye.

2.2.2.1 Preparation of Ethanol Extract from Rosa Damascenes' Flower Buds

Dried rose buds were processed in an electric grinder, ensuring that the entire bud - comprising sepals, petals, stamens, and pistil - was ground. A quantity of 250 g of the resulting powder was measured and placed in a Soxhlet extraction apparatus with a mixture of 75 ml of 99% ethanol and 25 ml of distilled water, maintained at a temperature of 45°C. After the extraction, the resulting solution was dried with a rotary evaporator, weighed, and various concentrations were prepared through dilution and stored in a refrigerator at 4°C.

2.2.2.2 Preparation of Hot Water Extract from Rosa Damascenes' Flower Buds

The entire flower bud (petals, sepals, stamens, and receptacle) was ground, and 30 grams of the dry powder was mixed with 200 millilitres of hot distilled water. The mixture was placed in a shaking water bath at 100 RPM at a temperature of 45°C for four hours.

Using sterile medical gauze, the plant extract was filtered, the filtrate was placed in a centrifuge for 10 minutes at a speed of 3000 rpm, then sterilized using a Milli Power Filter with a diameter of (0.45) micrometres, then diluted to different concentrations using the dilution law [5]. Which involve

$$C1*V1=C28*V2.$$

The inhibitory activity was studied by the diffusion method on the surface of Mueller-Hinton agar medium, and according to the McFarland tube at a dilution of (1.5×10^8) cells/ml, Gram-positive and Gram-negative bacterial isolates were cultured. Small holes with a diameter of 6 mm were made in the middle of the agar after leaving the cultured plates for ten minutes at room temperature. Different concentrations of Damask rose extract were added to the holes and the negative control hole containing distilled water. The plates were incubated for 24 hours at a temperature of 37 °C [8].

The Rosa damascenes used different concentrations of extract that were added to the wells, along with a positive control well containing distilled water.

3 RESULTS AND DISCUSSION

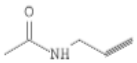
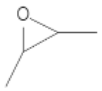
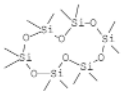
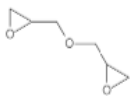
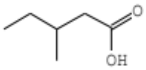
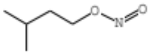
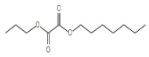
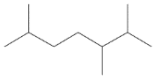
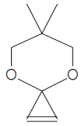
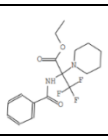
3.1 GCMS Analysis of Ethanolic Extract for Detection of Active Ingredients in Flower Buds of Damask Rose

Using a GCMS device, the active ingredients in the flower buds of the Damask rose were detected at the Environment and Water Directorate, Environmental Research Center. Ethanol was used as an organic solvent, and the results showed that the extract contained natural chemical substances represented by 2,3-butanediol, 1,5-heptadien-3-yne, acetamide, N-2-propynyl, oxirane, 2,3-dimethyl, and 6-oxa-bicyclo [3.1.0] hexan-3-one. Additionally, dodecamethy licyclohexasiloxane, 2-pentanol, 4,4-dimethylpentanoic acid, 3-methyl isoamyl nitrite, oxalic acid, heptyl propyl ester, heptane, 2,3,6-trimethyl, ethyl 2-benzamido-3,3,3-trifluoro-2-piperidinopropionate, and 4,8-dioxaspiro [2.5] oct-1-ene were also found, as shown in Table 1 and Figure 1.

Plants contain high concentrations of active biochemical compounds with antioxidant activity. It is known that free radicals cause autoxidation and that phenolic compounds with antioxidant activity depend on breaking the free radical chain by donating a hydrogen atom [9]. The petals of Rosa damascenes contain secondary metabolites that have antibacterial and antifungal effects and are considered oxidants against free radicals, as well as having soothing and anti-inflammatory properties. They are used as flavouring agents in food due to their content of various [10].

Essential oils [11]. It is used in the production of expensive rose oil, which is extracted through various commercial methods. Additionally, rose water has significant importance due to its soothing and comforting properties and is used on religious occasions in some countries [12]. Temperature is crucial for maintaining the quality of fruits and vegetables post-harvest, as it directly impacts their shelf life. Studies have shown that temperature fluctuations negatively affect plant quality [13].

Table 1: The natural components found in Rosa damascene rose buds.

	R.Time	Area%	
	2.94	2.75	Butanediol 2,3-
	7.61	1.84	1,5-Heptadien-3- yne
	8.151	20.66	Acetamide, N-2-propynyl
	8.7	2.18	Oxirane, 2,3-dimethyl
	10.135	3.54	6-Oxa-bicyclo[3.1.0]hexan-3-one
	10.761	1.62	Cyclohexasiloxane, dodecamethyl
	13.336	9.03	Oxirane, 2,2'-[oxybis(methylene)] bis
	15.042	3.42	Pentanoic acid, 3-methyl
	15.728	3.75	Isoamyl nitrite
	18.259	1.47	Oxalic acid, heptyl propyl ester
	20.572	7.83	2,3,6-Trimethyl - , heptane
	21.071	2.39	6,6-Dimethyl-4,8-dioxaspiro[2.5]oct-1-ene
	26.117	8.97	Ethyl 2-(benzoylamino)-3,3,3-trifluoro-2-(1-piperidinyl)propanoate

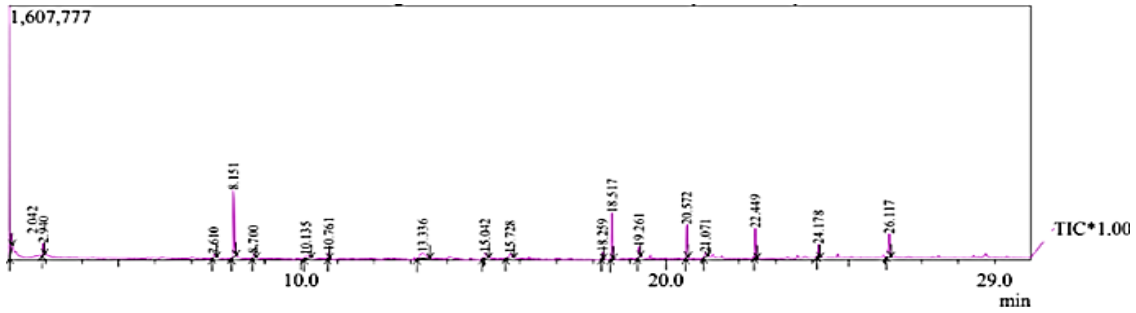


Figure 1: Chromatogram of extract of *Rosa damascenes'*GCMS (1-1).

Roses are among the most important ornamental plants, and their fruits and flowers have been used in a variety of traditional medicines and food products. They are considered significant oxidants due to their active compounds, particularly phenolics, with predominant quercetin and kaempferol glycosides [14]. A study conducted by [14, 15] showed a decrease in the viability of cancer cells when using *Rosa damascenes'* extract, depending on the concentration and duration of exposure, where the death of HeLa cancer cells was observed, indicating its potential as a promising therapeutic agent in the future. In addition to containing strong antioxidants, *Rosa damascenes* has shown activity against antibiotic-resistant bacteria, as the extracts were effective against a variety of Gram-positive and Gram-negative bacteria. The inhibitory activity is likely attributed to the presence of various phenolic and flavonoid compounds, which are believed to contribute to the antibacterial properties through bacterial growth inhibition tests. The numerous benefits resulting from the use of natural products rich in biologically active substances have enhanced the growing interest in the pharmaceutical, food, and cosmetic industries.

3.2 Spectrophotometer Ultraviolet (UV)

To analyze the UV spectra of the nanomaterial, a UV-Vis spectrometer (UV-1800, Shimadzu, Japan) was used. With a resolution of 1 nm, the absorption spectrometer was used in the laboratory.

Tests were conducted on the UV absorption spectra in the range of 200-300 nanometers for the alcoholic and aqueous extracts of the Damask rose plant. The preliminary study showed that the aqueous and alcoholic extracts of the Damask rose plant have a great ability to absorb ultraviolet rays effectively. This is because these extracts contain flavonoids, which are important substances used to protect the skin from the sun, as they are used in the manufacture of sunscreens.

2,3 Butanediol is an important industrial chemical found in the alcoholic extract of the Damask rose plant after detection of the natural components using GCMS. It is used in the production of pharmaceuticals, agricultural products and cosmetics. 2,3 Butanediol is a major secondary metabolite of many microorganisms, including both Gram-positive and Gram-negative bacteria. It exhibits anti-freezing, antibacterial, and anti-inflammatory properties and enhances natural defences [16].

3.3 The Effectiveness of the Aqueous Solution of Rose Flower Buds in Inhibiting Various Isolates of Gram-Negative and Gram-Positive Bacteria was Investigated by the Drilling Diffusion Method

The results showed that the aqueous solution of the flower buds of the rose plant "Rosa damascene" affected bacterial isolates, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella*, *Serratia*, *Proteus* spp. and *Acinetobacter Baumann*. After testing the efficiency of the isolates using the drill diffusion method, it was observed that the aqueous extract could inhibit the selected isolates, where Serratia bacteria recorded the highest inhibition rate, which ranged between 20-23 mm. Followed by the Staphylococcus aureus-1 isolate if the inhibition zones were 21-15 mm and in the *Pseudomonas aeruginosa* isolate, the inhibition zones were 12-17 mm and the *Escherichia coli*-2 isolate ranged between 0-17 mm. As for the remaining *Escherichia coli* isolates, the aqueous extract did not show any effect. *Klebsiella*-1, *Klebsiella*-2, *Proteus* and *Staphylococcus aureus*-2 showed varying effects ranging from 10-15 mm, 18-13 mm, 0-12 mm and 15-11 mm respectively, as the Inhibition zone increased with increasing concentration, except for

one isolate, Klebsiella-2, which was affected by the low concentration, as shown in Table 2, Figure 2 and Figure 3.

The results of this study are similar to a previous study that indicated the aqueous extract of Rosa damascenes' flower buds has inhibitory activity against Staphylococcus aureus, while Escherichia coli was not affected by the aqueous extract of Rosa damascenes. Plants are a rich source of active compounds, which has led to increased interest in research related to them, especially due to the continuously growing popularity of treatments using plants with significant biological activity. A study shown by [17]showed that the aqueous extract of the Rosa damascene plant contains active compounds of great importance as natural antioxidants and antibacterial agents due to their inhibitory ability against many bacteria, including Micrococcus luteus, Staphylococcus aureus, Bacillus subtilis, Shigella Flexner, Proteus vulgaris, and Escherichia coli.

Staphylococcus aureus is a pathogen of soft tissues and skin causing bacteremia, bloodstream infections, arthritis, and pneumonia. It is a Gram-positive bacterium. Escherichia coli is a Gram-negative bacterium that contributes to the pathogenesis of intestinal and extraintestinal infections. It is an opportunistic pathogen, with some strains showing resistance to antibiotics due to the acquisition of virulence factors [18].

Pseudomonas aeruginosa is one of the most widespread bacteria and is the focus of intense research due to its prominent role in pathogenesis due to its relatively large genome, flexible metabolic capacity, and presence in diverse environments, it is an opportunistic bacterium that infects humans when natural immune defences are compromised, being particularly lethal in patients with cystic fibrosis and causing issues in patients with wounds, burns, chronic wounds, and those with pulmonary obstruction, especially in hospitalized patients. It is considered one of the antibiotic-resistant bacterial species, particularly to beta-lactam and aminoglycoside antibiotics. Its high ability to encode a range of resistance genes makes it difficult to treat [19].

Acinetobacter baumannii bacteria have shown resistance to drugs and pose a public health threat associated with high mortality rates due to acute infections, particularly hospital-acquired infections. Bacterial resistance to many drugs is linked to overuse, poor supervision, and overuse of antibiotics. Many bacteria are resistant, such as Gram-negative bacteria [15, 20]. Among these bacteria is Klebsiella pneumoniae, which has been shown by many studies to be particularly resistant to antibiotics, especially penicillin and others, as penicillin is not currently used. It is an opportunistic species that causes clinical infections, especially in hospitalized patients [21].

Table 2: The inhibition zones of the aqueous extract of Rosa damascenes' flower buds.

Concentrations Type of bacterial isolation	Inhibition zones (mm) of aqueous extract of Rosa damascenes' buds (Mean ± S.D.)			
	50 (mlg/ml)	25 (mlg/ml)	23 (mlg/ml)	15 (mlg/ml)
Acinetobacter Baumann	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a
Escherichia coli-1	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a
Escherichia coli-2	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a
Escherichia coli-3	17 ± 1 a	11 ± 1 b	9 ± 1 c	0 ± 0 d
Klebsiella pneumoniae -1	15 ± 1 a	12 ± 1 bc	13 ± 1 b	10 ± 1 c
Klebsiella pneumoniae -2	13 ± 1 b	12 ± 1 b	12 ± 1 b	18 ± 1 a
Proteus	12 ± 1 a	10 ± 1 b	0 ± 0 c	0 ± 0 c
Pseudomonas aeruginosa	17 ± 1 a	12 ± 1 b	17 ± 1 a	12 ± 1 b
Serratia	23 ± 1 a	20 ± 1 b	21 ± 1 b	20 ± 1 b
Staphylococcus aureus-1	21 ± 1 a	15 ± 1 c	18 ± 1 b	15 ± 1 c
Staphylococcus aureus-2	14 ± 1 ab	13 ± 1 b	15 ± 1 a	11 ± 1 d

Groups with different letters are statistically different (p-value <= 0.05).
In each row:

- a: denote to the highest level,
- b: denote to the second highest level,
- c: denote to the third highest level,
- d: denote to the lowest level).

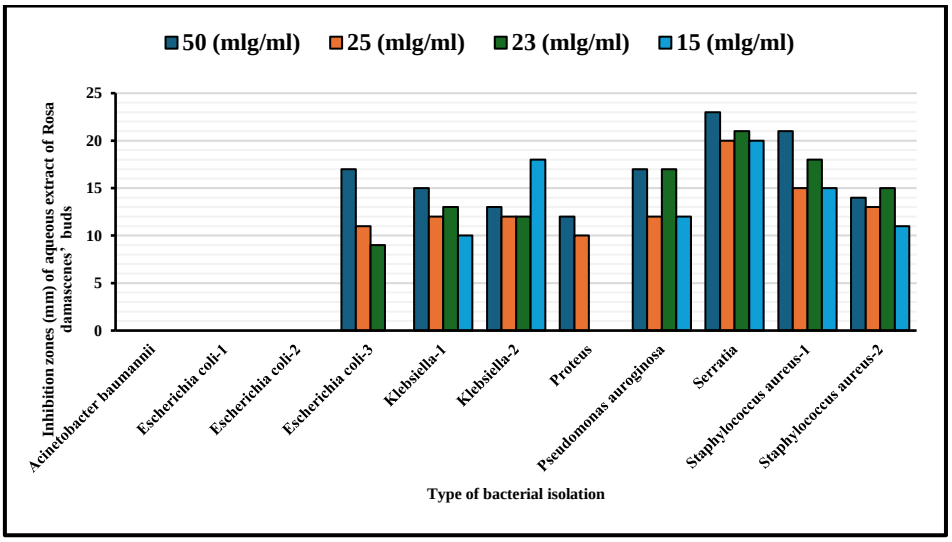


Figure 2: The inhibition zones of the aqueous solution of the flower buds of the Rosa damascene plant**.**

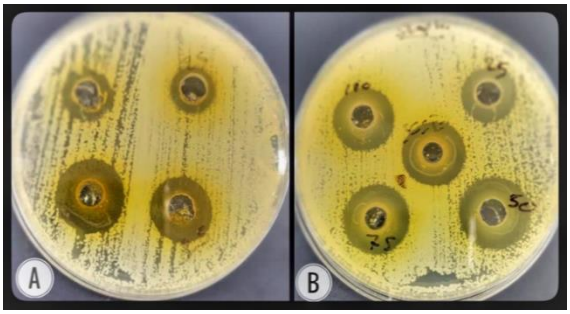


Figure 3: The effect of the plant extract on the flower buds of the Rosa damascene plant: a) is the effect of the aqueous extract on Staphylococcus aureus bacteria, and b) is the effect of the ethanolic alcoholic extract.

Also, among the types of bacteria is Serratia bacteria, which is classified as a common and harmful cause of diseases in hospitals and private clinics, as its presence is linked to medical devices and some methods of care provided to patients hospitalized in these places. This explains the large number of hospital infections resulting from these bacteria, especially S. marcescens. Hospital workers must be vigilant to prevent the spread of these diseases, which are considered opportunistic pathogens that affect insects, humans and animals. Given the significant resistance of bacteria to antibiotics. It is usually diagnosed in the clinical laboratory, but other rare types cannot be easily identified in laboratories [22]. Proteus.spp. Is a Gram-negative rod-shaped bacterium of the Enterobacteriaceae family that mainly causes urinary tract infections in humans. This bacterium can form biofilms in addition to primary bacteremia that may cause complicated bloodstream infections leading to infective endocarditis in addition to other diseases such as wound infections and respiratory tract infections [23].

3.3 Detection and Investigation of the Inhibitory Activity of Ethanolic Solution of Flower Buds of Rosa Damascene Against Different Isolates of Gram-Negative and Gram-Positive Bacteria by the Drilling Diffusion Method

This study showed that the inhibitory activity against *Staphylococcus aureus* bacteria was caused by the ethanolic extract of the flower buds of *Rosa damascene*, which ranged between 17-25 mm. This result was similar to what was mentioned by [24]. The highest effect of the extract was against Serratia bacteria with inhibition diameters ranging between 20-27 mm. As for *Pseudomonas aeruginosa* bacteria, it showed an effect of the extract, and the inhibition diameters ranged between 13-23 mm. The alcoholic extract did not show any effect against any of the bacteria *Proteus*, *Acinetobacter baumannii*, *Escherichia coli*-1, *Escherichia coli*-3, and *Klebsiella*-1, except for the *Escherichia coli*-2 isolate and the Klebsiella-2 isolate, which showed an effect, as shown in Table 3, Figure 4 and Figure 5.

Table 3: The inhibition zones of the ethanolic extract of *Rosa damascenes'* flower buds.

Concentrations Type of bacterial isolation	Inhibition zones (mm) of alcoholic ethanol extract of *Rosa damascenes'* buds (Mean ± S.D.)			
	50 (mlg/ml)	25 (mlg/ml)	23 (mlg/ml)	15 (mlg/ml)
Acinetobacter baumannii	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a
Escherichia coli-1	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a
Escherichia coli-2	20 ± 1 a	15 ± 1 b	10 ± 1 d	12 ± 1 c
Escherichia coli-3	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a
Klebsiella pneumoniae -1	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a
Klebsiella pneumoniae -2	10 ± 1 a	9 ± 1 b	0 ± 0 c	0 ± 0 c
Proteus	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a
Pseudomonas auroginosa	23 ± 1 a	20 ± 1 a	18 ± 1 c	13 ± 1 d
Serratia	27 ± 1 a	23 ± 1 b	21 ± 1 c	20 ± 1 c
Staphylococcus aureus-1	25 ± 1 a	22 ± 1 b	22 ± 1 b	18 ± 1 c
Staphylococcus aureus-2	24 ± 1 a	21 ± 1 b	20 ± 1 b	17 ± 1 c

Groups with different letters are statistically different (p-value <= 0.05).
In each row:

- a: denote to the highest level,
- b: denote to the second highest level,
- c: denote to the third highest level,
- d: denote to the lowest level).

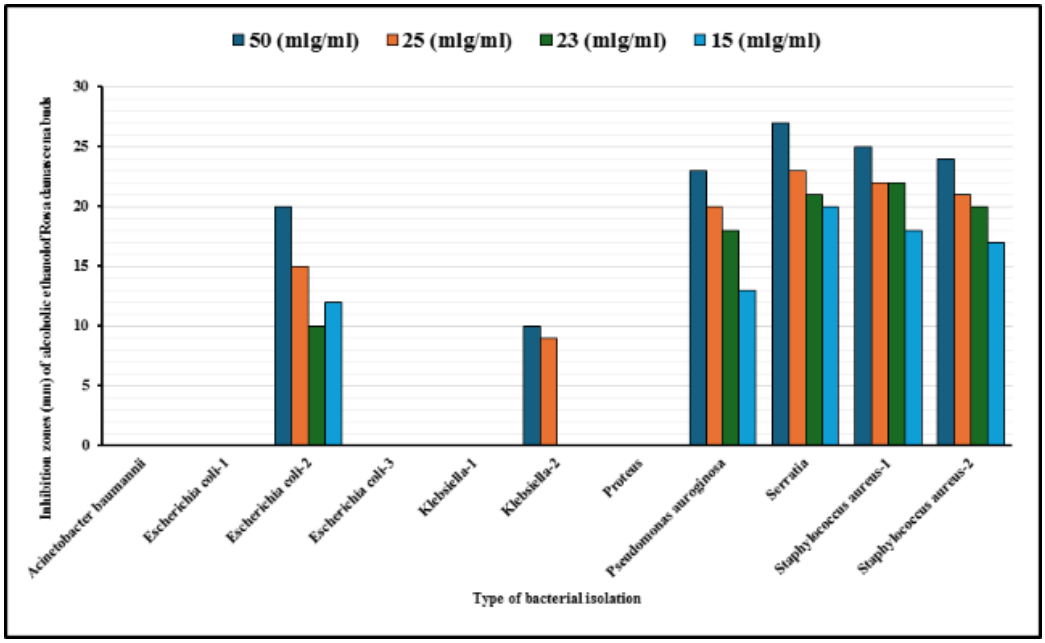
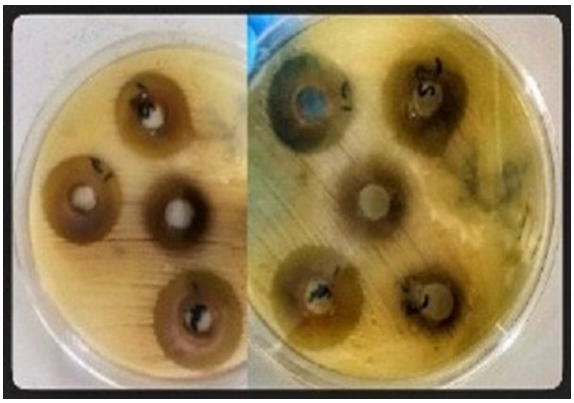


Figure 4: The inhibition zones of the ethanolic extract of *Rosa damascenes'* flower buds.

Part of the flower, which is a modified adjacent plant leaf called the petal, has an inhibitory effect and is a natural antibacterial against many Gram-positive and Gram-negative pathogens [25]. Because it contains active ingredients and secondary metabolites that have an antibacterial effect and is a rich source of natural antioxidants and anti-inflammatory agents [26]. A study of different aqueous and alcoholic extracts conducted by [27] showed that these extracts affect Gram-positive and Gram-negative bacteria. The aqueous and alcoholic extract has an inhibitory effect on the growth of many pathogens, especially antibiotic-resistant bacteria, including *Staphylococcus aureus* and *Staphylococcus*

epidermidis, which are methicillin-resistant and did not show any effect on *Escherichia coli* bacteria [28]. R. damascene is an ornamental plant and the flower is the important part that contains fragrant essential oils.

Many pharmacological properties have been reported, as it is considered an important antibacterial agent, an anti-HIV agent, and a cough suppressant. The ethanolic extract exhibits strong antibacterial and antifungal activity against a wide range of microorganisms and shows superior inhibitory effectiveness compared to the aqueous extract. No toxic effects of the ethanolic extract of *Rosa damascene* on living cells or hemolysis were observed when tested against human blood cells.



(a) (b)

Figure 5: The effect of the plant extract from the flower buds of *Rosa damascenes'*: a) shows the effect of the aqueous extract on Pseudomonas aeruginosa, while b) represents the effect of the ethanolic extract.

Studies have shown that the essential oils in *Rosa damascenes* can reduce pain and anxiety resulting from the dressing and cleaning of burns through the use of aromatherapy. Burns caused by various bacteria are a major cause of disability and death worldwide. Burns can be accompanied by physical, psychological, physiological changes or serious illnesses, with pain, redness and inflammation being the main side effects. It can also lead to mental and psychological illnesses such as depression and other psychological problems[29]. In addition, the essential oils found in Rosa damascene can improve sleep quality. A study has shown that it reduces anxiety and improves sleep quality, especially in patients in the cardiac care unit [30]. The pharmacological, inhibitory, antidepressant and anti-inflammatory properties of Rosa damascene are due to the presence of a group of phenolic compounds [31].

Studies have shown that plants are a vital source of a variety of secondary metabolites, which are used in the use and manufacture of different types of medicines. Medicinal plants in particular play a vital role in the health of the ecosystem in general and humans and society in particular, which necessitates the study, knowledge and identification of many of the compounds produced by these plants and the study of their composition to facilitate their use in the pharmaceutical industry. Among these main secondary compounds are phenols, alkaloids and terpenes. Medicinal plants containing alkaloids have been used for a long time, as the first alkaloids were isolated in the nineteenth century. Their medical and pharmaceutical uses were studied directly and their uses are still in various pharmaceutical applications. As for terpenoids, which are also used in medical fields, they are found in every natural diet [32]. In ancient times, plants were used to treat many diseases that affect humans.

All worlds, especially the plant kingdom, are valuable because they contain many compounds that play an important role in the pharmaceutical industry. In addition, natural products are less harmful than manufactured chemical products. Plants contain phenolic compounds that are important for human health and are used to prevent many diseases. These compounds are important because of their antioxidant, anti-inflammatory, antibacterial and anticancer effects, and they have protective effects on the heart and enhance immunity [32], [33]

4 CONCLUSIONS

The findings of this study demonstrate that Rosa damascena flower bud extracts—particularly the ethanolic and aqueous formulations—exhibit considerable broad-spectrum antibacterial activity against several clinically significant Gram-positive and Gram-negative bacterial isolates. The ethanolic extract, in particular, showed pronounced inhibitory effects against Staphylococcus aureus, Serratia, and Pseudomonas aeruginosa, indicating its potential as an effective alternative to conventional antibiotics, especially in treating drug-resistant infections. The aqueous extract also displayed significant activity against Klebsiella pneumoniae and Proteus spp., suggesting that different solvent systems may extract distinct active compounds with varying antimicrobial targets. The Rosa damascene plant, especially the aqueous and alcoholic extract of the flower buds of the plant, has an inhibitory effect against Gram-negative and Gram-positive bacteria.

ACKNOWLEDGEMENTS

We would like to thank the Department of Environment and Water, the Environmental Research Center in Baghdad, the Department of Microbiology and College of Medicine at Jabir Ibn Hayyan Medical University, the Clinical sites, and the patients for their participation.

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Bioremediation of Organic Contamination Using Molecularly Identified Bacillus Subtilis Bacteria

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Keywords: Bacillus, Bioremediation, Electrical Conductivity.

Abstract: Soil bioremediation primarily depends on the enzymatic activity of living microorganisms, to facilitate the degradation of contaminants or their conversion into less dangerous forms. Ten soil samples were collected from different locations in the city of Sharqat and the Rumana area of Qayyarah district. The Physical (concentration and Electrical Conductivity) and chemical tests (pH) conducted on soil samples. The *B. subtilis* were cultured and identified by Gram stain and VITEK2 compact system, also DNA extraction and Genome sequencing of *16S RNA* ribosome gene of bacteria was carried out to complete the diagnosis of *B. subtilis. Bacillus subtilis* is a characteristic bacterium that is rod-shaped. When cultivated on standard nutritional agar, the shape of this bacterium's circular colony is rough, opaque, fuzzy white or slightly yellow, exhibiting jagged edges. Sequencing revealed that most of the isolated strains were *B. subtilis*. The result of PCR showed that U1 gene has found in 100% of the *B. subtilis* isolates. BLAST results show 98% nucleotide sequence similarity and similarity for the *16RNA* ribosomal gene of *B. subtilis* with no gaps. The sequence for *16RNA* ribosomal gene was sub-mitted to NCBI, Gene Bank with accession No. KY962959.1. The alignment was done through Clustal Omega that recognized partial variations on some bases. The concentration of spontaneous wastes was decreased with bioremediation (3.864) for controlled temp. and (3.21) with un controlled temp. the Electrical Conductivity also decreased (3647) for controlled temp. and (2265) with uncontrolled temp. The pH increase in controlled temp. (8.699) and uncontrolled temp. (8.23). *B. subtilis* has a large efficiency in bioremediation process which had a benefit in ecosystem. It has a high effect in uncontrolled environmental condition(temperature) which lead to decrease the concentration of Spontaneous wastes and decease the electrical conductivity and increase the pH to more alkaline pH.

1 INTRODUCTION

Organic pollution of the environment is a critical concern that impacts the planet's soil, water, and air, presenting a substantial risk to ecosystems and living organisms, therefore there is an increased focus on formulating solutions to address environmental pollutants via physical, chemical, and biological methods. Conventional soil remediation techniques, including excavation, transportation to specialist waste facilities, incineration, stabilization, coagulation, or ion exchange, are complex, disruptive, and frequently lead to abrupt alterations in the soil's chemical, physical, and biological characteristics. Bioremediation provides an alternate approach by employing biological processes to detoxify, degrade, or convert contaminants into a harmless form [1], [2]. Bioremediation is typically used microorganisms, to remediate polluted environments[3]. This method offers several advantages over physical and chemical treatments, chiefly due to its lower cost and greater environmental sustainability. Furthermore, organic pollutants can be entirely mineralized or biodegraded into simple inorganic compounds, e.g. CO_2, H_2O, and Cl using bioremediation, while physical and chemical processes, such as vaporization, adsorption, and extraction, compromise soil structure and disrupt normal environmental processes, frequently merely relocating contaminants from one medium to another, for instance, from soil to the atmosphere [4]. Microorganisms are employed in bioremediation, an attractive, novel, cost-effective, and environmentally friendly method, to remove petroleum hydrocarbon pollutants from water or soil.

The predominant microorganisms employed in bioremediation include bacteria, fungi, and yeasts. Bacteria are reported to be the most effective microbial community in decomposing petroleum hydrocarbons[5]. Using the soil's native microbial populations' inherent capacity to biodegrade and remove pollutants, natural attenuation is a type of organic bioremediation.

The success of bioremediation was highly dependent on the type of soil, the pollution level, and environmental factors that includes pH, moisture level, temperatures, conductivity of electricity, and nutrient availability. Soil contamination samples must be defined and analyzed before biodegradation experiments may begin[6]. *Bacillus subtilis (B. subtilis)**:*** One species that is often used in bioengineering and biotechnology is *Bacillus subtilis (B. subtilis)***,** which is also available for purchase. The fact that *B. subtilis* can produce biofilms and has growth-promoting characteristics in plants suggests that it could replace fertilisers and pesticides made from petroleum [7]. It has been demonstrated that the matrix of biofilm of *Bacillus subtilis* mainly comprises three separate components, and that the formation of biofilm is controlled by complex regulatory processes.

The first part is an exopolysaccharide, which is both a signalling molecule and a moisture retainer in the biofilm. The non-wetting qualities of the biofilm are caused by the second component, the protein BslA. The biofilm's structural integrity, particularly its attachment to plant roots, is ensured by the third element of the matrix, the protein TasA, in conjunction with the accessory protein TapA [8]. *B. subtilis* have developed strategies such as active efflux or sequestration utilizing proteins or isolates genes, insoluble substances, via which they can resist, detoxify, or metabolize these contamination from soil and water samples [9].

2 MANUSCRIPT PREPARATION

2.1 Sample Collection

Ten soil samples were collected from different locations in the city of Sharqat and the Rumana area of Qayyarah district (see Fig. 1). This study is considered the first of its kind in this area because it is rich in organic pollutants, especially (hydrocarbons). The samples were collected from (November 2023 - June 2024), where the samples were taken from different depths ranging between (5-8 cm). The samples were collected in sterile plastic bags with information such as (sample number - date - size) written on them. They were transferred to the laboratory for the purpose of dilution and isolation of microorganisms present in this contaminated soil.



Figure 1: The locations for collecting soil samples.

2.2 Physical and Chemical Tests Conducted on Soil Samples

2.2.1 Measurement of Physical Characteristic

2.2.1.1 Temperature

A digital cooking thermometer was used to directly check the temperature while taking samples from the places and the temperatures measured by this device range between (-50-300°C).

2.2.1.2 Electrical Conductivity

The soil's electrical conductivity was assessed utilizing the suspension method (1:5). Subsequent to the preparation of the suspension and filtration, the electrical conductivity of the filter was measured using a conductivity meter, taking three measurements, and the average was calculated to determine the error rate following the calibration of the device with standard solutions**.** As for water samples, the ability of water to conduct electricity

was tested by immersing the electrode in water after placing it in special glass bottles for a minute until the reading was fixed, then it was recorded in microsemens/cm (APHA, 2003). The final value of conductivity was then extracted

2.2.2 Measurement Chemical Characteristic

The pH was measured using a Professional Benchtop pH meter type BP3001 and the device was pre-calibrated before measurement using solutions with a pH of (9-7-4) and tested immediately within the sampling time of the same day in the laboratory.

2.3 Preparation of Soil Samples for Microbiological Culture

One gram of soil contaminated with organic matter (hydrocarbons) was taken and sieve it using a sieve with a diameter of 2 mm. place the samples in 10 ml tubes. Place 9 ml of D.W in tube. In addition to the soil sample. The samples were mixed well using a Vortex mixed device for one minute to obtain the colony forming unit CFU/ml. Then, a series of successive decimal dilutions were made until we reached dilution number 10^6.

Then 0.1 ml of the 10^4, 10^5 and 10^6 dilutions were taken using a Micropipette and then spread on the following culture media: nutrient agar - MacConkey agar - potato dextrose agar - starch casein agar. They were spread on the surface of the culture media using a clean and sterile glass spreader. The plates were incubated for 24 hours at 37°C for bacterial growth.

2.4 Isolation and Identification of Bacteria From Soil

After preparing the series of dilutions in the previous paragraph in order to isolate and purify bacteria from soil , 1 ml of each dilution, which is 10^4-10^6-10^5, which we prepared from soil was transferred to the surface of the culture media, which are nutrient agar and MacConkey agar and blood agar Starch casein agar-, distributed by spreading method and the plates were incubated at a temperature of 37°C for 24-48 hours for bacteria [10].

2.5 Diagnosis of Isolated Bacteria

2.5.1 Microscopic Examination

A gram stained bacterial slides were examined using an oil lens with a magnification power of (100X) in light microscope, the shapes of the bacteria were identified and whether they were positive or negative.

Also the biochemical test was performed on the isolates and the diagnosis were confirmed by VITEK 2 Compact system.

2.5.2 DNA Extraction

The analysis kit provided by (mepep biosoence) was relied upon to extract DNA from samples of bacteria and filamentous bacteria.

2.5.3 Estimation of the Concentration and Purity of the Extracted DNA

The concentration and purity of the extracted DNA from the bacterial isolates belonging to the unknown bacteria were measured using the Nano-drop spectrophotometer device equipped by the BioDrop Company.

2.5.4 Electrophoresis of DNA in Agarose Gel

To transport and detect DNA, agarose gel is prepared at a concentration of 1%. To obtain this level of concentration, 0.35 g of agarose powder is mixed in 35 ml of Sodium Borate (S.B), and 3 microliters of red safe dye are included. This is accomplished by employing a heat source with constant agitation until boiling, then allowing it to cool to a temperature of 50-60 degrees. The gel solution is subsequently poured into the transport device's tray following the installation of a specialized comb to create wells at the gel's edges, ensuring that the pouring is executed gently to prevent bubble formation. If they develop, they are extracted using a pipette. The gel is allowed to solidify, after which the tray is positioned in the electrophoresis tank holding a suitable volume of X1 TBE solution. The comb is thereafter elevated with care. The transport samples are created by

combining 7 microliters of the DNA sample with 3 microliters of loading buffer. Subsequently, the relay device is activated by transmitting an electric current with a voltage differential of 5 volts/cm, and the procedure lasts between 1.5 to 2 hours. Subsequently, the gel is imaged under ultraviolet light utilizing a UV transilluminator to visualize the DNA bands and the outcome of the PCR reaction.

2.5.5 PCR Reaction Kits for Determining the 16srRNA Gene to Diagnose Unknown Bacteria

2.5.5.1 Primer

To determine the 16srRNA gene, a universal primer of bacteria was used in this study, prepared by the manufacturer in lyophilized form. To prepare the working solution, the primer was dissolved in nuclease-free water to obtain a concentration of 10 picomoles/microliter. The primer used in this study is shown in the following Table 1. The multiplication reaction conditions for PCR additives are summarized in Table 2.

Table 1: Sequence of the primer used in the study.

Primer	Sequence	Size bp
U1 Forward (21)	CCAGACTCCTACGGGAGGCAG	180
U2 Reverse (62)	CGTATTACCGCGGCTGCTG	

Table 2: Multiplication reaction of PCR additives.

No	Cycle number	Time	Temperature	Stage
1	1 cycle	5min	94	Initial denaturation
2	30 cycle	35min	94	Denaturation
3	30 cycle	1min	55	Annealing
4	30 cycle	1min	72	Extension
5	1 cycle	7min	72	Final extension

2.5.5.2 DNA Extraction from Agarose Gel

The bands obtained from the PCR reaction were excised from the gel for purification and subsequent nucleotide sequencing, based on various analyses conducted by the company. (Geneaid).

2.5.5.3 Nucleotide Sequence Determination of the Amplified Fragments Using DNA Sequencing

The nitrogenous base sequence of the samples was ascertained by dispatching the PCR reaction products, along with the primer from the resultant package, to North Korea, where the gene sequence was analyzed using the Hitachi 3130 Genetic Analyzer. The gene sequences were compared with those recorded in the National Center for Biotechnology Information (NCBI), and the results were assessed utilizing the BLAST program.

3 RESULTS AND DISCUSSION

3.1 Isolation and Identification of Bacillus Subtilis

The result of cultural growth with biochemical test and Vitek result revealed the species of *B. subtilis*. *Bacillus subtilis* is a characteristic bacterium that is rod-shaped. When cultivated on standard nutritional agar, the shape of this bacterium's circular colony is rough, opaque, fuzzy white or slightly yellow, exhibiting jagged edges, Figure 2. The results was presented in Figure 3, showed that that this bacterium belong to the Gram-positive class by morphological observations and was identified as Gram-positive *bacillus*, which can form spores.



Figure 2: Bacillus subtilis on nutrient media.

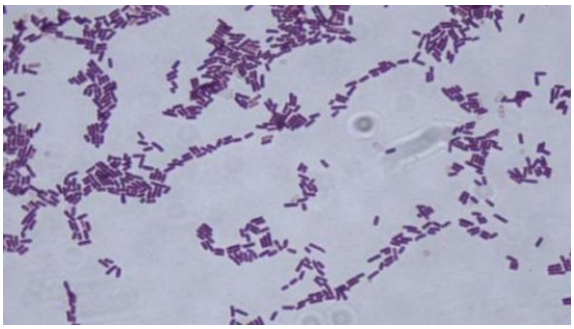


Figure 3: Gram positive B. subtilis under microscope.

3.2 Molecular Identification of B. Subtilis

The result of PCR showed that *U1* gene has found in 100% of the *B. subtilis* isolates as showed in Figure 4.

The number and location of genetic mutations of the *U1* gene (180 bp) in isolate of *B. subtilis* was showed in Figure 5. The gene was registered at the American International Bank of Genes NCBI under the serial number (KY962959.1). Sequencing revealed that most of the isolated strains were *B. subtilis* (Fig. 4). BLAST results show 98% nucleotide sequence similarity and similarity for the 16RNA ribosomal gene of *B. subtilis* with no gaps (Fig. 6). The sequence for 16RNA ribosomal gene was sub-mitted to NCBI, Gene Bank with accession No. KY962959.1. The alignment was done through Clustal Omega that recognized partial variations on some bases.

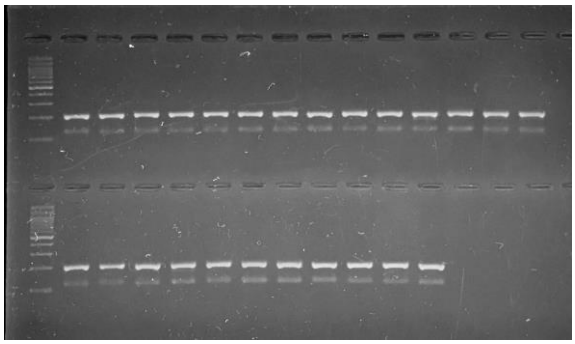


Figure 4: PCR products for the amplified product of U1 gene in B. subtilis after electrophoresis isolates on agarose gel (1%) at 5 V/cm for 60 min. M: DNA ladder marker; Lane (1-25): B. subtilis isolates.

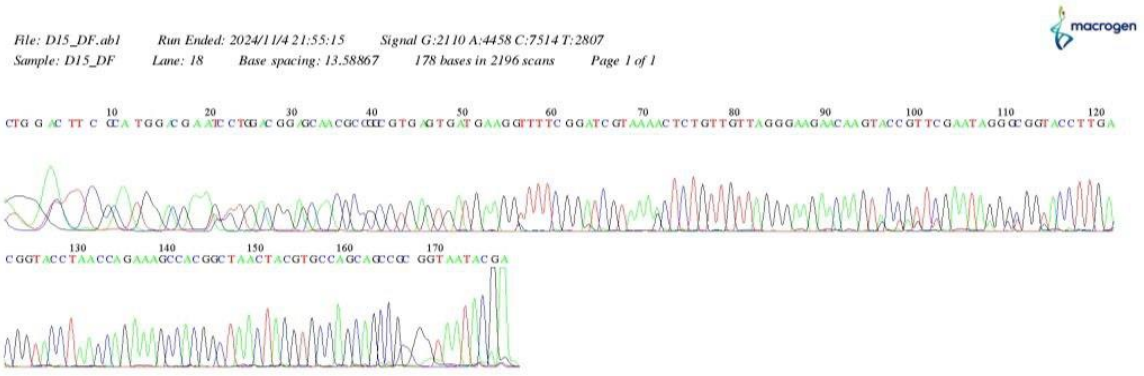


Figure 5: DNA sequencing of B. subtilis gene.

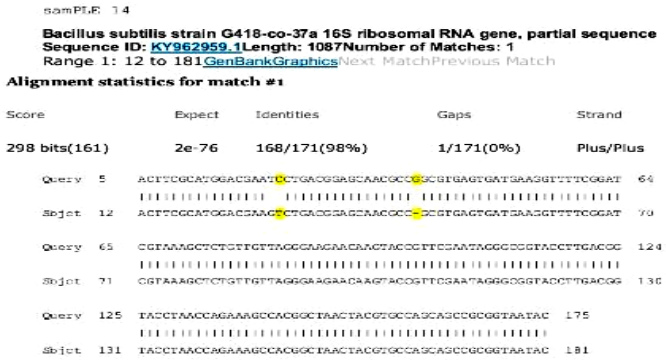


Figure 6: Confirmation of *B. subtilis* strain 16SrRNA gene sequence through BLAST.

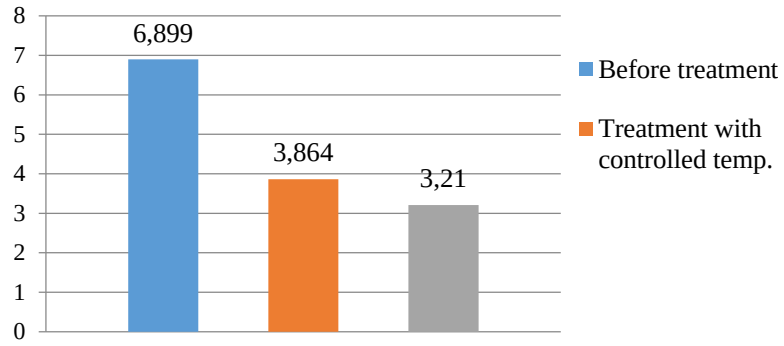


Figure 7: Organic contamination concentration during bioremediation with B. Subtilis.

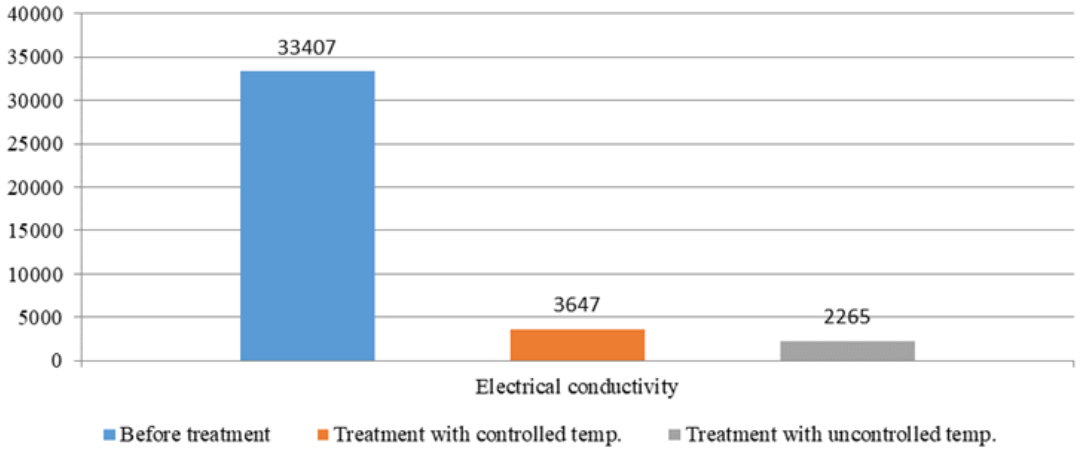


Figure 8: Electrical conductivity during bioremediation with B. Subtilis.

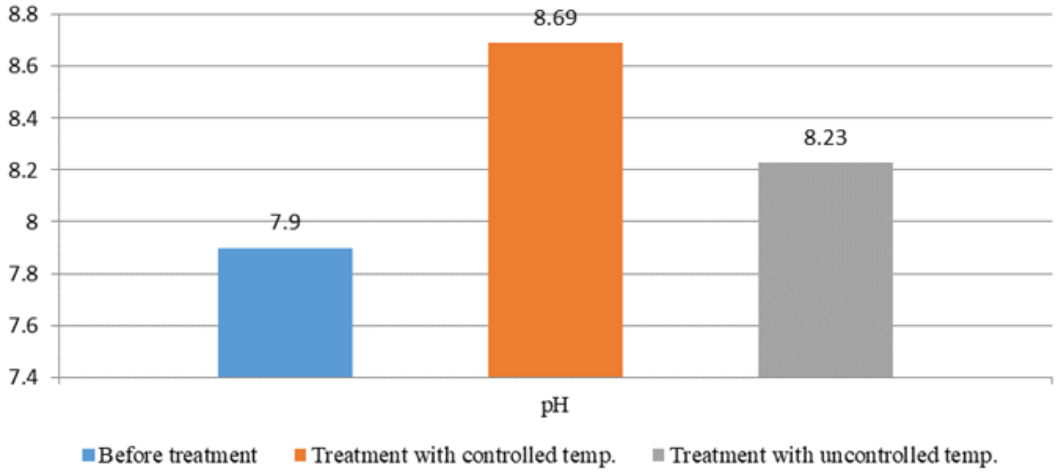


Figure 9: pH during bioremediation with B. Subtilis.

3.3 Bioremediation with B. Subtilis

The bacterium were used for spontaneous wastes bioremediation, the process were carried out in two condition, controlled temperature and uncontrolled temperature. The concentration of spontaneous wastes were decreased with bioremediation 3.864 for controlled temp. and 3.21 with un controlled Temp. Electrical Conductivity also decreased 3647 for controlled temp. and 2265 with uncontrolled temp. The pH increase in controlled temp.8.699 and uncontrolled Temp.8.23, which mean that bacteria has an effective role in bioremediation, Figures 7, 8, and 9.

Bioremediation has garnered significant scholarly attention over the past decade due to the superior environmental sustainability and cost-effectiveness of microorganisms compared to physical and chemical approaches for the removal of pollutants from soil and water samples [11]. Many studies have proven that most types of bacteria, such as Pseudomonas aeruginosa, Bacillus subtilis, and Bacillus melanogaster, are effective in decomposing many dangerous pollutants.

Bacillus subtilis emerged as the most extensively researched species within the Bacillus genus owing to its inherent competence for the uptake of extracellular DNA, which enables straightforward genetic manipulation and the occurrence of sporulation, one of the earliest investigated processes of bacterial cell development [12]. The current investigation identifies *B. subtilis* as gram-positive rods, characterized by a rough, opaque, fuzzy white or slightly yellow appearance with jagged edges on culture media [13].

The species of Bacillus spp. most well characterized for their bioremediation capability are *B. subtilis*, *B. cereus*, and *B. thuringiensis*. This bacterium genus employs many bioremediation techniques, such as biosorption, EPS-mediated biosorption, bioaccumulation, and bioprecipitation. Consequently, Bacillus spp. strains can diminish the concentrations of metals in the environment. Furthermore, Bacillus spp can promote growth of plants and enhance the bioaccumulation of pollutants in soil, making them one of the more effective sustainable alternatives for pollutant remediation in diverse habitats, especially soil. This makes them a promising candidate for phytoremediation [14].

Molecular detection of Bacillus species has only been documented in a few studies that have reported amplification, sequencing, and sequence analysis of the 16S rRNA gene [15], [16]. In their groundbreaking investigation, Geetha et al. [17] successfully used a sequencing analysis of the 16S rRNA gene to identify and categorize seven native strains of *B. subtilis* based on their taxonomy. We were able to identify the Bacillus species and two more subspecies using this molecular technology. Some problems have emerged in the Bacillus generation, despite the *16S rRNA* gene sequence's great success in bacterial species identification [18].

By comparing twenty-six strains having *16S rRNA* sequences with forty-eight incomplete Bacillus subtilis sequences from GenBank, Sabie et al. were able to build a phylogenetic tree. While the GenBank sequences for these 26 isolates ranged from 1153 to 1559 nucleotides in length, the in silico study showed that their lengths varied from 995 to 1233 nucleotides. The percentage of GC content ranged from 55% to 56% across all 68 sequences of *16S rRNA* genes [19].

Akinsemolu *et al*. [20] emphasised the potential importance of *B. subtilis* in environmental cleanup, in addition to its significant contributions to biotechnology, agriculture, and medicine. The more we learn about *B. subtilis*, the more important its role becomes in finding long-term answers to global problems.

Elenga-Wilson *et al*. [21] proven that Bacillus subtilis have remarkable bioremediation capabilities, particularly in the metabolism and destruction of various organic pollutants. Hydrocarbons, PAHs (polycyclic aromatic hydrocarbons), pesticides, and industrial chemicals are all part of this category. Decomposition and conversion of these pollutants into less hazardous compounds are made possible by *B.subtilis*'s extensive metabolic capabilities. Restoring and reviving ecosystems that have been polluted need it, thus.

JALILZADEH et al. [22] proved that *B. subtilis*' biodegradative potential stems from its capacity to create several biosurfactants, enzymes, and biodegradative chemicals. By utilising their complex metabolic and enzymatic interactions, *B. subtilis* is able to break down complex organic pollutants into their smaller, less dangerous components. The waste treatment and management processes are greatly improved by *B. subtilis*. A bacterium's enzymes may break down even the most complicated chemical compounds into simpler, less harmful ones. When these two processes work together, they reduce garbage volume and eliminate unpleasant odours. Using *B. subtilis* improves the efficiency and longevity of waste management processes by encouraging the creation of

ecologically friendly methods of trash disposal and recycling [23].

Soil type of soil, pH, and temperatures, the presence of oxygen or other electron acceptors, nutrients, and the presence of microbial populations that are good at degrading pollutants are some of the environmental variables that have a significant impact on the control and enhancement of biological treatment processes. Another factor is the accessibility of pollutants to these communities [24].

B.subtilis is more effective in bioremediation when exposed to unregulated temperatures, according to the present study. Sakthipriya et al. [25], showed that temperatures, salinity, and pH, affected the stability of the biosurfactant made by *B. subtilis* for improved oil recovery, therefore our results are in line with theirs.

4 CONCLUSIONS

The present study demonstrated the high efficiency of Bacillus subtilis in bioremediation of organic contaminants from soil environments with hydrocarbon pollution. The bacterium was successfully isolated and molecularly identified through morphological characteristics, biochemical tests, VITEK2 system, and 16S rRNA gene sequencing, with confirmed identification (NCBI accession: KY962959.1). B.subtilis has a large efficiency in bioremediation process which had a benefit in ecosystem. It has a high effect in uncontrolled environmental condition(temperature) which lead to decrease the concentration of organic contamination and decease the electrical conductivity and increase the pH to more alkaline pH. The study highlights that *B. subtilis* performs well even in fluctuating environmental conditions, making it a reliable candidate for large-scale bioremediation projects, particularly in hydrocarbon-contaminated zones. Its ability to produce biofilms and biosurfactants further enhances pollutant degradation efficiency while contributing positively to the ecosystem.

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Determining Optimal Conditions for Lactobacillus Paracasei Bacteria and Testing its Ability to Produce Conjugated Linoleic Acid

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Keywords: Conjugated Linoleic Acid, CLA, Lactobacillus Paracasei.

Abstract: The study included testing the ability of some bacteria to produce conjugated linoleic acid (CLA) and identifying the most efficient isolates for production. The results showed that seven species of the Lactobacillus genus were isolated, namely (Lactobacillus paracasei, Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus helveticus, Lactobacillus rhamnosus, Lactobacillus reuteri). The results indicated that all bacterial isolates had the capability to produce CLA when cultivated in MRS Agar medium. Each isolate was subjected to a screening process to determine the most efficient in producing CLA under various conditions. The results showed that the isolate Lactobacillus paracasei was the most efficient in producing CLA. Additionally, the optimal conditions for producing CLA by Lactobacillus paracasei were determined, and its ability to produce CLA under different conditions was tested. The results indicated that the optimal pH for production by Lactobacillus paracasei was 6, and the optimal temperature for producing CLA was 37°C. The incubation period for producing CLA showed that 24 hours was the best duration for fatty acid production. A concentration of 250 µg/ml of CLA resulted in the highest yield of the conjugated acid for all isolates, with the isolate Lactobacillus paracasei showing superior production of CLA.

1 INTRODUCTION

Conjugated linoleic acid (CLA) is a polyunsaturated fatty acid (PUFA) from the w-6 series, consisting of a variety of geometric and positional isomers (cis or trans) of linoleic acid (LA; cis-9,12-octadecadienoic acid, 18:2) with a conjugated double bond, and has garnered increasing attention in recent decades due to the discovery of many health benefits associated with it [1], [2].

CLA is found in various natural foods, such as vegetable oils like safflower oil, sunflower oil, corn oil, soybean oil, and sesame oil. It is also present in the meat and milk of ruminant animals [3].

Researchers, after identifying the ability of certain lactic acid bacteria and probiotics to secrete the enzyme Linoleic Acid Isomerase, have turned to producing CLA outside the living organism using microorganisms with various nutrient media supplemented with linoleic acid, as well as alternative sources of pure linoleic acid, such as safflower oil, sunflower oil, soybean oil, and others due to their high content of this fatty acid. Following the success achieved in producing CLA outside the living organism and from alternative sources rather than pure acid, a significant step has begun in incorporating these techniques into foods, including dairy products and cheese, to create dietary systems that serve as a benchmark against other diets due to their content of probiotics, in addition to CLA and the benefits provided by the product [4].

2 MATERIALS AND METHODS

Bacteria (Lactobacillus paracasei, Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus helveticus, Lactobacillus rhamnosus, Lactobacillus reuteri) were obtained from laboratories affiliated with the Holy Hussain Shrine in Karbala, packaged in sterile, airtight containers.

2.1 Activation of Bacterial Isolation

1 g of samples was taken, and a series of tenfold dilutions 10 - 10 - 7 was performed in peptone water. 1 ml of it was transferred to the culture medium MRS Agar, and the plates were incubated under anaerobic conditions using an Anaerobic jar at 37°C for 48 hours, following the method used by [5].

2.2 Test for the Ability of the Studied Bacteria to Produce Linoleic Acid

The screening of the initially identified isolates was conducted based on their ability to convert linoleic acid into CLA, according to the method described by [6]. As follows: Bacterial isolates were activated in MRS Broth medium containing 1% Tween 80 (w/v) and 1 µg/ml of pure linoleic acid, and the tubes were incubated at 37°C for 24 hours.

2.3 The Standard Curve for CLA

It was prepared according to the method followed by [8]. Different concentrations of the standard CLA (Cis9 Trans11) ranging from 0 to 12 micrograms/mL dissolved in hexane, were made, and the absorption was measured at a wavelength of 233 nanometers (Fig. 1).

2.4 Calculation of the Conversion Ratio of Linoleic Acid to CLA

The percentage of conversion capability was calculated according to the method followed by [7]. From the following (1):

$$\text{CLA acid \%} = \frac{\text{Amount of CLA obtained from the standard curve}}{\text{Amount of added linoleic acid (ml or g) standard}} \quad (1)$$

2.5 Determining the Optimal Conditions for the Production of CLA

In the broth medium, CLA optimal conditions were determined to test the bacteria's ability to produce CLA. 10 mL of the growth medium 10 microliters of 80 Tween, MRS microliters from safflower oil, and 500 microliters of the extracted bacteria were used and incubated in the shaker device for 4 days [9]:

- The effect of pH. The used ranges of PH are (4,5,6 and 7).
- Effect of temperature. For culturing liquid MRS medium, the temperatures (20,30,37,40°C) were used contained linoleic acid 1 µg/ml and inoculated with 2% of the active cultures to determine the optimal temperature.
- Effect of Safflower Oil Concentration. Different concentrations of safflower oil (50,100,200,300,400) µ /ml were used.
- Effect of incubation duration. Different incubation periods (6,12,24,48,72)hours were applied.

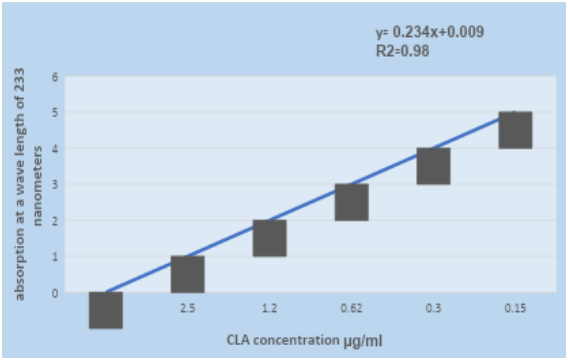


Figure 1: The standard curve for the measured CLA.

3 RESULTS AND DISCUSSION

3.1 Quantitative Screening to Identify Isolates with Highest Production of CLA

Many researchers have used spectroscopic methods for the detection and quantification of CLA due to their numerous advantages. They do not require the esterification process as in chromatography, have a low cost, and allow for the screening of a large number of samples. Furthermore, some researchers have used this method [12] and [8].

A screening process was conducted on the studied bacteria to evaluate their ability to produce CLA and the quantity produced. Table 1 illustrates the variation in the isolates' ability to produce CLA. The highest concentration of CLA reached 113.15 µg/L for the isolate Lactobacillus paracasei, followed by the isolates Lactobacillus casei, Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus helveticus, Lactobacillus rhamnosus, and Lactobacillus reuteri, with concentrations of 98.45, 79.11, 77.48, 69.93, 57.12, and 53.56 µg/L, respectively. The conversion percentages were

11.31%, 9.84%, 7.91%, 7.74%, 6.99%, 5.71%, and 5.35%, respectively.

The achievement of the highest production of CLA from the isolate Lactobacillus paracasei under static anaerobic conditions may be attributed to the microaerophilic nature of this isolate. Furthermore, the static culture flask provides an almost unlimited number of variable fermentation conditions, ranging from nutrient enrichment to depletion, and from abundant oxygen supply to partial anaerobic coexistence.

A study has shown that the process of screening and selecting the best organisms for the production of CLA revealed that Lactobacillus bacterial strains exhibit a production range of CLA between 20 and 4900 µg/L [15].

Table 1: This caption has one line so it is centered.

Bacterial isolation	Quantity of CLA product µg/L	CLA% percentage
Lactobacillus paracasei	113.15	11.31
Lactobacillus casei	98.45	9.84
Lactobacillus acidophilus	70.11	7.91
Lactobacillus plantarum	77.48	7.74
Lactobacillus helvticum	69.93	6.99
Lactobacillus rahmanosus	57.12	5.71
Lactobacillus reuteri	53.56	5.35

In a study conducted by [7], the results of isolating Lactobacillus rahmanosus showed the lowest production of CLA, amounting to 46.2 µg/L, with a conversion rate of 4.62%. On the other hand, [16] indicated the ability of lactic acid bacteria to produce CLA in skim milk medium prepared with linoleic acid at a concentration of 200 µg/mL. The bacterial isolate Lactobacillus acidophilus 6 gave the highest production, reaching 116.53 µg/mL, with a conversion rate of 58.26%, while the bacterial isolate Lactobacillus casei had the lowest CLA production, amounting to 71.36 µg/mL, with a conversion rate of 35.68%. The study also pointed out that using concentrations higher than 200 µg/mL resulted in a decrease in the amount of CLA, attributing this to the inhibitory effect of linoleic acid on the bacterial isolates.

3.2 Determination of the Optimal Conditions for the Production of CLA in the Reaction Medium.

3.2.1 The Effect of pH

The results in Figure 2 showed that when using pH values (4, 5, 6, 7) in the production of CLA from the L. paracasei producing isolate, the optimal pH for CLA production was 6, where the amount of linoleic acid reached 168.11 µg/L with a conversion rate of 16.2% for the producing isolate.

The results shown in Figure 2 when using pH values (4, 5, 6, 7) in the production of CLA from the producing isolate L. paracasei indicated that the optimal pH for the production of CLA is 6, as the amount of linoleic acid reached 168.11 µg/L, with a conversion rate of 16.2% for the producing isolate."

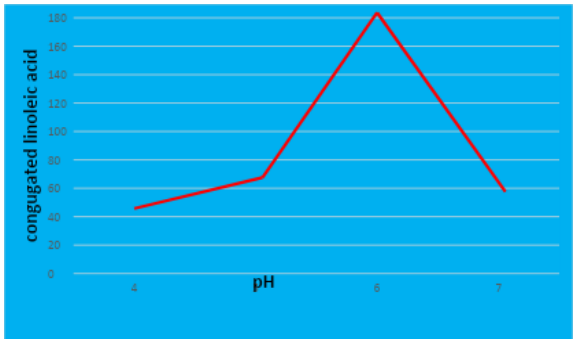


Figure 2: Effect of different pH levels on the production of CLA.

The pH of the medium is crucial for the growth of living organisms and its impact on metabolism. Changes in pH are significant for the effectiveness of enzymes in living organisms, as well as for intermediate products, their solubility, and decomposition. These changes have an effect on the yield of metabolic products of living organisms.

pH affects metabolic enzyme function and nutrient transport in the cell [10]. In a study conducted [18], at pH 6, in the production of CLA, it was possible to produce it from L. acidophilus bacteria, which achieved the best growth and the best production of CLA.

Maintaining a narrow pH range is essential for optimizing productivity in culture media. pH regulation is a critical factor, and therefore, compounds that act as pH buffers are added to culture media to maintain the desired pH. These buffers also serve as nutrient sources for microorganisms. Calcium carbonate is commonly used to maintain a neutral pH in the medium. When

the pH decreases, the carbonate dissolves, neutralizing the acidity. Conversely, when the pH increases, acids released into the medium by microorganisms help to lower the pH back towards neutrality (pH 7).

3.2.2 Temperature Effect

The results in Figure 3 showed that the highest production of CLA for the L.paracasei isolate when using different temperatures (20, 30, 37, 40 °C), was at 37 °C, where the CLA production reached 161.47 µg/L, with a conversion rate of 16.1%. Compared to the other temperatures, the amount of CLA production was (112.73, 97.47 µg/L) at 20 and 40 °C, respectively.

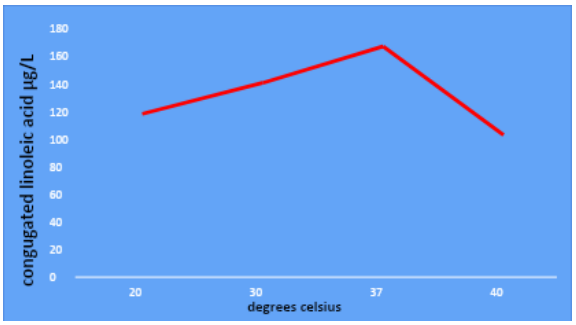


Figure 3: Effect of different temperatures on CLA production.

The increase in CLA productivity with rising temperature may be attributed to making the reaction medium conducive to the growth of microorganisms and the secretion of isomerase enzyme without causing any effect or change in the enzyme's function, while providing the substrate on which the enzyme acts. However, the decrease in CLA productivity when incubated at 40°C can be attributed to the effect on enzyme activity due to thermal inhibition of the enzyme [19]. These results are consistent with those obtained by [11] in his study, where he found that the optimal incubation temperature is 37°C, and that an increase in incubation temperature caused a sharp decrease in conjugated acid productivity. As a result, the increase in temperature affects hydrogen migration during the formation of isomers, and stopping enzyme activity causes hydrogen migration to stop completely. These results also agree with what [8] found, that the optimal incubation temperature ranged between 35 and 37°C, and that an increase above this temperature caused a sharp decrease in the production of conjugated acid, explaining the reason as the enzyme being sensitive to temperature, and that the increase in incubation temperature caused the enzyme structure to be destroyed. On the other hand, a study [16] showed that the best incubation temperature for producing CLA in MRS broth medium was at 37°C, and confirmed a sharp decrease in the production of conjugated acid upon reaching 40°C, explaining the reason for this decrease as a result of the decrease in enzyme activity.

3.2.3 The Effect of Linoleic Acid Concentration

The concentration of linoleic acid is considered one of the key factors that significantly influence the production of CLA in the reaction medium [8]. Due to its importance, it was studied under optimal conditions to determine the best production of CLA. Figure 4 illustrates the effect of different concentrations of linoleic acid on the production of CLA in MRS broth supplemented with concentrations of (50, 100, 200, 300, 400) µg/mL and inoculated with the local bacterial isolate, Lactobacillus paracasei. It was observed from the same figure that there was a gradual decrease in the concentration of CLA with the increase in the concentration of linoleic acid added to the reaction medium. The concentration of 50 µg/mL outperformed, reaching (181.15) µg/mL with a conversion rate of 18.1%. The concentrations of (100, 200, 300, 400) µg/mL resulted in (171.66, 164.11, 158.13, 36.22) µg/mL, respectively, in CLA production. The reason for the increase in CLA production at low concentrations may be attributed to the unaffected structural conformation of the isomerase enzyme secreted by the microorganisms, which is responsible for CLA production, thus increasing the surface area for enzyme binding with the substrate in the reaction medium. The decrease in CLA production in the reaction medium may be due to the gradual decrease in bacterial counts, as well as the effect of increased fatty acid concentration on the secretion of the isomerase enzyme, or due to the reduction in the surface area for enzyme binding with its substrate, leading to a decrease in acid productivity. This is supported by [8], who found that the concentration of CLA was significantly affected by microbial cell concentrations and linoleic acid concentration in the reaction medium, and that high concentrations of the substrate cause changes in the enzyme structure, as well as reducing the surface area for enzyme binding with its substrate in the reaction medium.

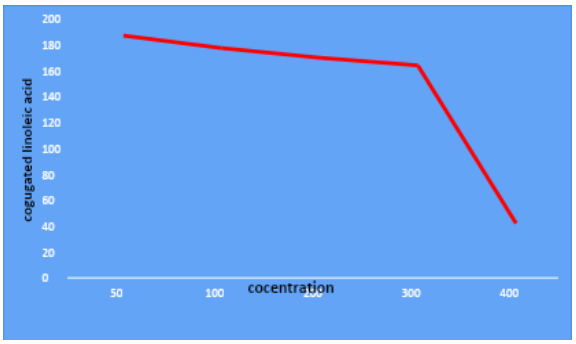


Figure 4: Effect of different concentrations on production.

3.2.4 Effect of Incubation Period

Figure 5 illustrates the effect of different incubation periods, ranging from (12, 24, 48, 72) hours, on the ability of the selected bacterial isolate L. paracasei to produce CLA using MRS broth, incubated at 37°C. The results for the different periods were (0, 14.92, 16.157, 32.140, 76.122) µg/mL, respectively. The results showed that at the initial incubation period, no CLA was formed, accompanied by a decrease in microbial counts. This is attributed to the microorganisms entering a lag phase to prepare the necessary enzymes for their vital activities.

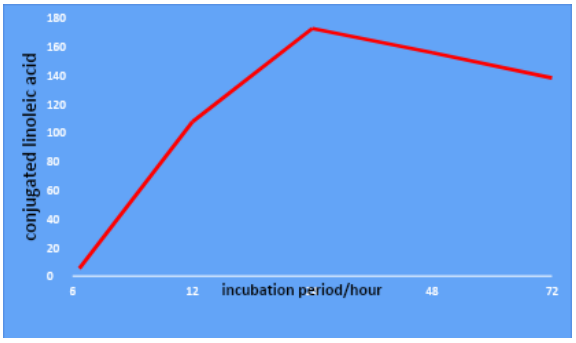


Figure 5: Effect of incubation periods on CLA production.

During this period, a decrease in microbial counts may occur due to the effect of linoleic acid in the production medium, as it is one of the factors that hinder microbial growth. The highest production of CLA was achieved at 24 hours, with a conversion rate of 27.15%. These results are consistent with several researchers who indicated that the optimal time for CLA production is at the end of the logarithmic phase and the beginning of the stationary phase, and they showed that the optimal incubation period for CLA production is 24 hours [13], [16]. In a study conducted by [6], [21], the optimal incubation period using MRS broth supplemented with 1 µg of CLA was 24 hours. The decrease in the amount of CLA with prolonged incubation periods is due to oxidation reactions, as well as oxidation resulting from microbial metabolism. During his study [9], found that the optimal incubation period is 48 hours. This was confirmed by [13], who found that the optimal incubation period for producing CLA was 48 hours. He also stated that the optimal period for producing CLA was during the stationary phase.

4 CONCLUSIONS

The current study demonstrates that Lactobacillus bacteria, in general, have the ability to produce linoleic acid when cultured on various growth media. Changes in pH, incubation time, or concentration can result in the production of varying amounts of the acid. This study successfully demonstrated the potential of various *Lactobacillus* species to biosynthesize CLA from linoleic acid in vitro, with *Lactobacillus paracasei* emerging as the most efficient producer. The isolate produced the highest concentration of CLA (113.15 µg/L) with a conversion rate of 11.31%, outperforming other species under standardized conditions.

These findings highlight the critical role of environmental conditions in modulating microbial CLA biosynthesis, primarily due to their influence on enzyme activity and microbial metabolism. Elevated linoleic acid concentrations beyond the optimal threshold were found to inhibit CLA production, likely due to enzyme feedback inhibition or toxicity.

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Effect of Green Selenium Nanosynthesis on Staphylococcus Aureus

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Keywords: SeO Nanoparticles, Anti-Bacterial, Antibiofilm, Staphylococcus Aureus.

Abstract: Selenium nanoparticles (SeNPs) represent a promising natural compound for modern medical treatments, offering a potent new weapon against pathogenic bacteria. This study aimed to synthesize SeNPs using sodium selenite (Na_2SeO_3) as a precursor and dried Camellia sinensis (green tea) leaf extract as a reducing and stabilizing agent through an eco-friendly biosynthesis approach. The synthesis was performed via a simple, cost-effective, and environmentally friendly method. The resulting nanoparticles were characterized using UV-visible spectroscopy, field-emission scanning electron microscopy (FE-SEM), and energy-dispersive X-ray spectroscopy (EDX). FE-SEM analysis confirmed the formation of spherical SeNPs with varied shapes and an average particle size of 20.5 nm, alongside a narrow size distribution. While the antibacterial evaluation against Staphylococcus aureus showed no significant inhibitory activity against planktonic cells, the SeNPs exhibited a strong antibiofilm effect, inhibiting biofilm formation by 54.7%. These findings highlight the potential of biosynthesized SeNPs as a specific and effective anti-biofilm agent for targeting bacterial biofilms and combating antibiotic resistance.

1 INTRODUCTION

Medicinal plants are valuable natural sources of bioactive compounds, which can be utilized to create pharmaceutical drug [1]. Natural compounds, including chemical compounds, have been found to have potential health benefits and are often found in medical plants[2], [3]. Selenium (Se) is an essential trace element in the human body. Approximately 40–300 micrograms of selenium are required daily as dietary supplements for a typical adult [4]. Lower concentrations of Selenium are necessary to maintain the healthy operation of the human and animal bodies [5]. Researchers found that the evolution of the bacteria was inhibited by the organoselenium [6]. Selenium nanoparticles (SeNPs) have demonstrated exceptional antibacterial effectiveness against the harmful bacteria Staphylococcus aureus in recent times. It has been discovered that elemental Se at the Nano scale has low toxicity and high biological activity [7]. Recently, SeNPs have shown remarkable antibacterial efficacy against the dangerous pathogen *Staphylococcus aureus*. At the nanoscale, elemental Se has been found to have great biological activity and minimal toxicity [8]. These nanoparticles potentially replace the functions of commonly used medications [9], [10]. NP can target a pathogen's cell wall directly without entering the cell. As a result, the bulk of MDR bacteria's defense mechanisms against ineffective treatment drugs are rendered ineffective [11]. SeNPs' properties extend beyond biological applications; they are also widely used in catalysis and oxidation-reduction processes [12].

2 MATERIALS AND METHODS

Camellia Sinensis was dried; all chemicals involved in the experiments are reagent grad. The formula for sodium selenite, an inorganic substance, is Na_2SeO_3, 0.0189 M. In 100 milliliters of deionized water, sodium selenite was produced and dissolved... The anti-bacterial test includes *Staphylococcus aureus*.

2.1 Preparation of Plant Extract

To prepare the *Camellia Sinensis* Extract (green tea), the leaves were washed using deionized water to remove crusts and cut into small pieces. 10 g of it and liquefy it in 100 ml of deionized water and put it on the magnetic jacket for 10 min at a temperature of

60°C and then in the centrifuge at a speed of 4000 rpm for 15 min then filtered with eight filter paper and placed in Cool place. Figure 1.

2.2 Synthesis of Se/Seo NPs by Using Camellia Sinensis L Extract

When the green tea plant extract is heated to 80 degrees Celsius on a magnetic stirrer, the sodium selenite solution is regularly distilled into the extract. The temperature is gradually raised so that it doesn't reach 160 degrees, and after 60 minutes, we observe a change in the solution's color, which indicates the acquisition of nanoscale selenium. Figure 2.

2.3 Characterization of Se/SeO NPs

Nanoparticles can be thought of in a variety of ways. Changing the color of the solution is the first and simplest method. For phase identification and characterization of crystalline metallic nanoparticles, the optical characteristics of Se/Seo NPs were measured using a Spectrophotometer UV-VIS.

The Energy-dispersive X-ray (EDX) spectra show the components in the prepared samples, which the fabricated samples are SeO nanoparticle.

In order to study the morphology and particle size of very large magnifications, the MIRA3 TESCAN Mashhad (MUMS) model (Fill Emission Scanning Electron Microscope) was used to measure the surface morphology characteristics (shape and particle size) of drying solution droplets placed on slides using high-resolution transmission electron microscopy (FESEM). During FESEM study, the electron beam absorbs energy between 10 and 30 kV.



Figure 1: Synthesis of dried leaves extract Camellia Sinensis.



Figure 2: Synthesis of SeO NPs using Na_2SeO_3 salt as a sources of selenium metal by Camellia Sinensis dried leaves extract.

2.4 Antibacterial Activity Test of SeNPs

In this study two isolated used, there are many sample taken from patients and determined by Vitek-2 System which indicated this isolate was *Staphylococcus aureus*. *Invitro* antibacterial activity was determined by agar-well diffusion method [13]. To determine the impact and efficacy of the nanoparticles made in this investigation against Gram-positive bacteria, specifically *Staphylococcus aureus*. The culture media arrived and sterilized the feed for 15 minutes at 121° C and 15 lb/inch of pressure. After being activated in the center of the nutrient broth, the bacteria were cultured for 24 hours at 37° C and stored in the refrigerator until they were needed. Following preparation and sterilization, the nutritional agar was transferred onto sterile Petri dishes.

The zone of inhibition test was active to observe the inhibitory effects of Azithromycin (15μg), Levofloxacin (5μg). Logarithmic phase bacteria (OD600nm 0.5) were diluted to approximately 107 CFU/mL with nutrient broth. The sample disk containing the antimicrobial agent solution was gently placed at the center of the, Mueller-Hinton agar plates and cultured overnight at 37 C. The diameter of the zone of inhibition around the disk is the standard of measure of the antibacterial activity.

2.5 Antibiofilm Activity of SeNPs Against Staphylococcus Aureus

In this study used isolate of *Staphylococcus aureus* which a biofilm-producing and a non-biofilm-producing isolate were used as controls. *Staphylococcus aureus* was determined by the method of [13], Antibiofilm effects of SeNPs and levofloxacin antibiotic, used 96 wells Agar well diffusion methods. Each well of the 96-well microtiter plate filled with 200 μL of the diluted bacterial culture. Incubate the 96-well plate at 37°C for 24-48 hours to permit biofilm formation After incubation period, If the absorbance analysis is higher than the negative control, it shows biofilm formation. After the contents of the microtiter plates were removed, 200 μL of phosphate buffered saline (PBS, pH 7.2) was used to wash the wells. After being emptied, microtiterplates were allowed to air dry. For 15 to 30 minutes, the microtiter-plates were stained with 200 μL of 1% crystal violet per well, which is used for Gram staining. To get rid of extra stain, each well was washed with 200 μL of 95% ethanol solution. Using a microplate reader, measure each well's absorbance at 590 nm. Then, compare the results with both positive and negative controls to verify the establishment of biofilms.

3 RESULTS AND DISCUSSION

3.1 Physical Properties

3.1.1 UV–Visible Spectroscopy and Color Change

A reduction of selenium ions to Se/SeO NPs using green tea extract is indicated by the solution's visual color changing from pale-pink to deep-yellow due to surface vibration of plasmas in Se/SeO NPs. In the UV–vis spectrum, SeNPs displayed surface plasma resonance of a peak centered at roughly 272 nm, which is equivalent to the absorption of Se/SeO NPs. (Figure 3).

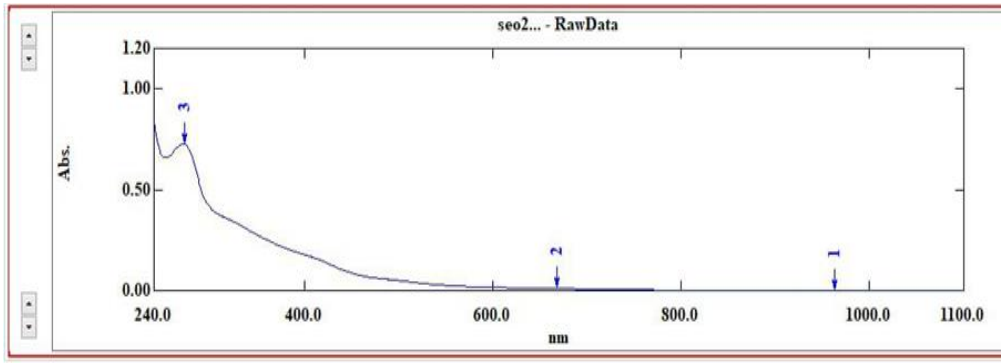
3.1.2 EDX Analysis

The Energy-dispersive X-ray (EDX) spectra show the components in the prepared samples, in which the fabricated samples are SeO nanoparticles. The EDS result in Figure 4, shows the presence of Se and O (Se: 70% and O: 30%).

3.1.3 Field-Emission Scanning Electron Microscope Analysis (FESEM)

The surface morphology for the synthesized nanoparticles was observed using a scanning electron microscope. Figure 5 shows the surface morphology of the SeO (from Na_2SeO_3 salt) nanoparticle respectively, in which the nanoparticles are found to be cylindrical in shape.

With the average size of nanoparticles at 20.5 nm. The results confirm the effect of the anion associated with selenium metal is very clear on the size and shape of the SeO nanoparticles since the different anions gave agglomeration of the nanoparticles and could be related to van der Waals forces among.



No.	P/V	Wavelength nm.	Abs.	Description
1	↑	963.0	0.003	
2	↑	668.0	0.014	
3	↑	272.0	0.728	

Figure 3: Absorbance spectrum as a wavelength function of Se/SeO NPs.

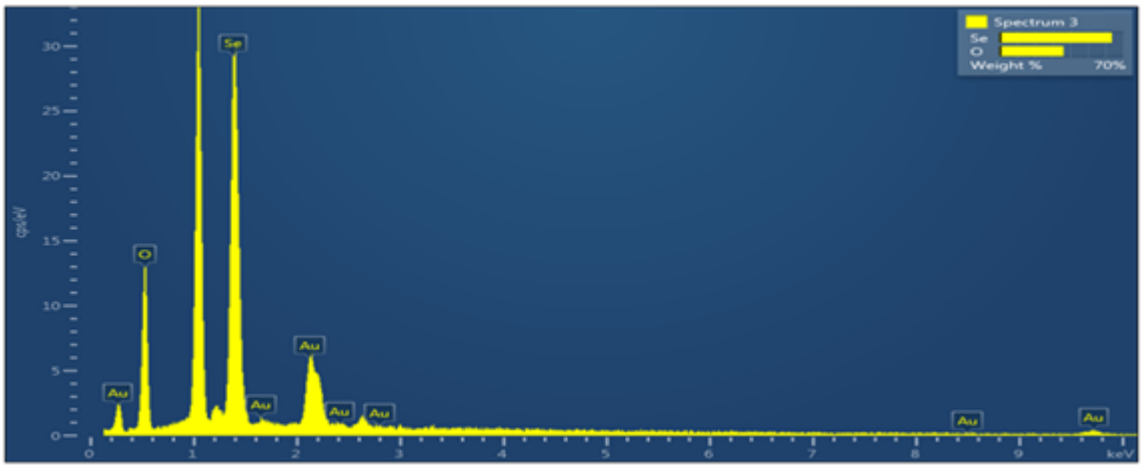


Figure 4: EDS image of SeO nanoparticles, from Na_2SeO_3 salt.

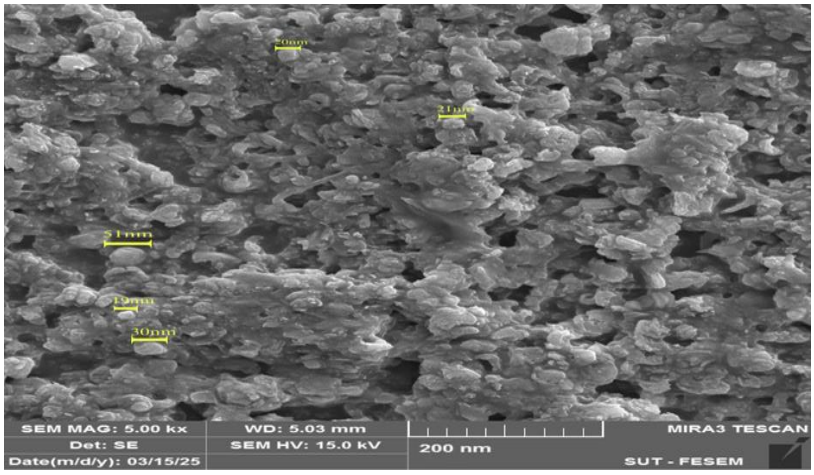


Figure 5: FESEM image of Se/SeO NPs by 200 nm.

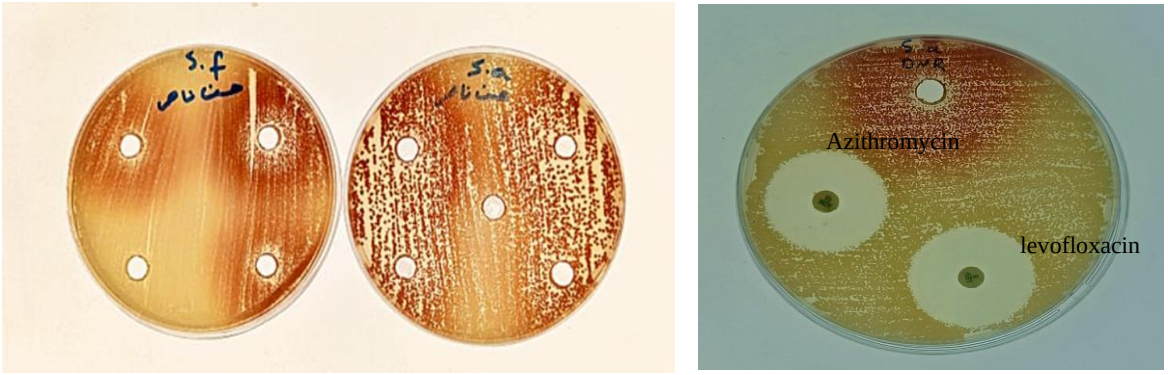


Figure 6: Antibacterial activity assessment of SeNPs against Staphylococcus aureus: a) Antibacterial activity of various concentrations of biosynthesized SeNPs; b) Comparative antibacterial activity of SeNPs, azithromycin, and levofloxacin (reference antibiotics).

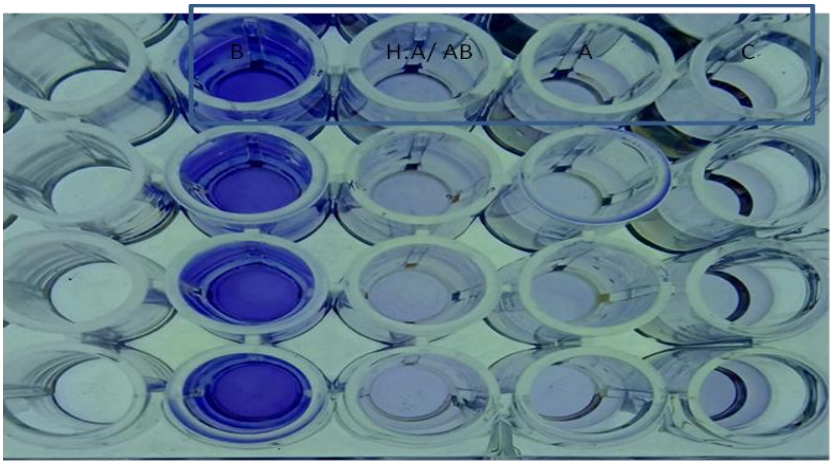


Figure 7: Biofilm activity of the biosynthesized SeNPs against Staphylococcus aureus. B=Biofilm test column, AB=Antibiofilm test line, A= antibiotics, C= Control.

3.2 Biological Properties

3.2.1 Antibacterial Activity of Selenium NPs Using Agar Well Diffusion Method

The effect of Se/SeO NPs has been studied in different concentrations (25, 50, 75, 100) %, Figure 6a Antibacterial inhibition zone.

Measurement is No activity, then compared with two types of antibiotics (Azithromycin and levofloxacin) on the inhibition of bacterial growth of Gram-positive (*Staphylococcus aureus)* as in Figure 6b, the inhibition zone measurement for both Antibiotics were 30 mm.

Since the characteristics of bacterial killing are comparable to those of nanoparticles in terms of volume, stability, and additional concentration to the growth medium, that gives the nanoparticles more time to interact with bacteria, there are a number of theories to explain how nanoparticles inhibit bacteria. A change in the overall structural and optical properties results from an increase in the surface-to-volume ratio, which can occur thousands of times [14].

Bacterial cell membranes of the highest quality have nanoscale stomata or pores. But in order to influence the growth of bacteria by interfering with the regular operation of cells, the nanostructures must penetrate or cross those membranes and settle sufficiently.

3.2.2 Ability of SeNPs to Remove the Established Biofilm

Impacts of SeNPs and levofloxacin antibiotic in eliminating the conventional biofilm (after 24 to 48 hours) were investigated in microtiter plates. According to the acquired results, the SeNPs generated under the investigated conditions exhibited antibiofilm effects against *Staphylococcus aureus*, compared with antibiotic levofloxacin both appeared a strong antibiofilm degrees 54.7, 55% sequentially. Figure 7.

The balance between pathogenic and helpful bacteria in the colon is one factor that distinguishes health from illness. When dysbiosis occurs, it is caused by an imbalance. Conditions like diabetes, obesity, colon cancer, and inflammatory bowel disease can be triggered when the host-microorganism interaction is altered. The formation and accrual of bacterial biofilms upsets this balance and is one of the major causes of chronic infections [15]. Over the past ten years, nanoparticles (NPs) have been the subject of much research as drug carriers in a variety of disciplines, such as neuroscience, immunotherapy, and oncology [16], [17], Additionally, preventing the formation of biofilms is crucial for managing microbial biofilms in a variety of industries, including food processing [18]. Research on selenium nanoparticles' antibacterial properties is rather rare in Iraq. Nevertheless, several studies have found similar results [19]. Selenium nanoparticles, for example, showed strong antibacterial activity against a range of harmful microorganisms in an Iraqi investigation. including *S. aureus* [20]. Even this result is promising but green nanoparticle Selenium need more experimental test to explore its action within others bacteria and tissue culture.

4 CONCLUSIONS

The study concluded that selenium oxide nanoparticles (SeO NPs), synthesized using green tea (Camellia sinensis) leaf extract and Na_2SeO_3 salt using a green, environmentally friendly method, exhibit unique physical properties in terms of size and shape. Their average size was 20.5 nm and they were cylindrical in shape. Although they did not demonstrate any direct antibacterial activity against Staphylococcus aureus when tested using the agar diffusion method, the results demonstrated a strong inhibition and disintegration of the biofilm produced by the same bacteria, reaching 54.7%. These results indicate that these nanoparticles hold promise for biomedical applications, particularly in combating chronic infections associated with biofilm formation. The study recommends further studies to evaluate their effectiveness against other types of bacteria and in more complex biological models.

ACKNOWLEDGMENTS

The authors appreciate the College of Education for Pure Sciences - University of Diyala – for using laboratories with the necessary equipment to carry out the experiment.

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Investigation of Indicators of Bacterial Pollution Using Environmental Genomics and Water Quality Index for the Purpose of Living Organisms in the Tigris River South of Mosul City

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Keywords: Tigris River, Bacteria, Pollution, Molecular, DNA.

Abstract: This study was conducted to investigate the presence of Total Plate Count (TPC) and Fecal Coliform (FC), and to isolate and identify some types of bacteria present in the Tigris River water, south of Mosul. The study area extended from Hammam Al-Alil to Al-Qayyarah (60 km). The presence of these bacteria is evidence of organic pollution in the river. The water quality index was used to assess the living organisms in the river, taking into account the physical and chemical factors specific to this model, represented by river temperatures, pH, and dissolved oxygen measurements. Traditional tests were also conducted to assess coliform bacteria by calculating the number of fecal coliform bacteria, which includes three stages: Presumptive Test, Confirmed Test, and Completed Test. The results of the physical and chemical tests for the living organisms in the river showed that the water temperature ranged between 10-24.5 °C and the concentrations of the indicator values. The acidity ranged between 6.4-7.9 and the dissolved oxygen results indicated a range of (6.4-10.3) mg/L. The range of phosphate and nitrate concentrations was between (0.01-0.14) and (0.001-0.99) mg/L, respectively. The results were within the Iraqi standard determinants and international specifications for surface water. The Tigris River water, south of Mosul, ranged between (moderate - good). The results of the bacteriological study also showed that the total number of bacteria ranged between (6-700) cells/100 ml, and colon bacteria ranged between 0-180 cells/100 ml, in addition to the isolation and identification of each of the bacteria (Acinetobacter radioresistens, Bacillus firmus, Pseudomonas aeruginosa

1 INTRODUCTION

Wisdom says that water is life. For years, water has been described as the womb that embraces life, as it constitutes a large percentage of the composition of living organisms. Although it is essential for life, when it is polluted, it can threaten it and destroy various organisms within it [1]. Water is a primary means of transmitting pathogens resulting from the discharge of human, animal, industrial and agricultural waste into it. Among the pathogens are typhoid, dysentery, polio, cholera, parasitic worms and others [2]. Reports from the World Health Organization indicate that 80% of diseases that affect humanity are caused by water pollution with pathogenic microorganisms, a problem that many countries in the world, including developed countries, still suffer from [3]. It indicates that there is a steady increase in the rate of infections with Rotavirus sp, which causes gastroenteritis, and infections with Salmonella bacteria, which causes typhoid, and Campylobacter bacteria [4]. Microbiological analysis of water is a safety valve for many epidemics and diseases that may result from contamination with human or animal waste. Bacteriological analysis of water is often relied upon to search for indicators of contamination, especially coliform bacteria, which are part of the intestinal bacteria family and live in various natural environments, in addition to living in the intestines of humans and animals. They can be found in waste, nutrient-rich water, soil, and rotting plant parts. Therefore, they may not be a sufficient indicator of water pollution. However, detecting Escherichia coli bacteria in potable water poses a significant risk, as it is one of the most important indicators of fecal pollution, and its presence in water confirms the recent contamination [5]. Clostridium perfringens bacteria is also one of the indicators of fecal pollution, the possession of this type of bacteria spores leads to its resistance to different

environmental conditions,. Fecal streptococcus is an important indicator of fecal contamination [6]. The aforementioned types of bacteria are indicators of the potential presence of pathogenic bacteria, due to the presence of these bacteria in the human intestine. Furthermore, these bacteria remain in water for a longer period and are present in greater numbers than pathogenic bacteria. They are also easier to isolate and purify [7].

2 MATERIALS AND METHODS

2.1 Water Sample Collection

The study was conducted on the Tigris River from its source in Mosul to the Qayyarah district, southeast of Mosul, within the administrative borders of Nineveh. The study spanned a total length of 66 km. Four main sites were identified along the river: Hammam al-Alil district, al-Safina village, al-Hood al-Fawqani village, and al-Qayyarah district, as shown in Figure 1 and Table 1. Samples were collected, one sample per site per month, for a period of twelve months, from February 2024 to January 2025. Clean polyethylene containers were used, and the containers were washed with the sample water in the field and laboratory.

2.2 Physical and Chemical Tests of Water Samples

Five factors were used to calculate the water quality index for aquaculture purposes: pH, dissolved oxygen, phosphate, nitrate, and temperature. Measurements were made according to standard and international methods [8]. Methods for measuring physical and chemical tests were followed in [9].

2.3 Bacteriological Tests of Water Samples

Water samples were collected in pre-sterilized glass bottles using an autoclave at a pressure of 1.5 pounds and a temperature of (121)°C for 15 minutes [10].

2.4 Culture Media

Prepared according to supplier's instructions (LABM) and sterilized in an autoclave at 121°C and 15 psi for 15 minutes. The media included Nutrient Agar ,MacConkey Broth, Peptone Water, and Eosin Methylene Blue Agar.

Table 1: Water sampling sites from the Tigris River within Nineveh Governorate.

Location	Location coordinates
43°16'06.5"E 36°10'38.7"N	**1**\ Hammam Al-Alil
43°21'01.2"E 35°58'48.1"N	**2**\ Al-Safina
43°18'46.1"E 35°51'27.4"N	**3**\ Al-Hood
43°17'12.7"E 35°46'31.1"N	**4**\ Al-Qayyarah

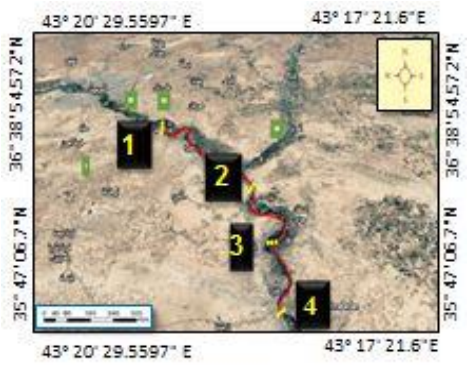


Figure 1: Main studied sites on the Tigris River south of Mosul (Google Earth).

2.5 Solutions, Reagents, and Dyes

They were prepared according to the instructions [9]. Such as normal saline, Kovac's reagent.

2.6 Detection of Cacteria in Water Samples

A series of tests was performed to detect the presence of bacteria. The following tests were performed.

2.6.1 Total Plate Count (TPC)

A dilution was made for each sample (up to 1-10 dilutions, 2-10 dilutions). 1 ml of the sample was taken and placed in a sterile Petri dish. The previously sterilized, cooled Nutrient Ager medium was poured over the sample. The plates were placed upside down in an incubator at 37°C for 24-48 hours. The number was then calculated using the following equation: TPC cell.ml-1 = Reciprocal of dilution × number of colonies growing on the medium

2.6.2 Counting the Number of Faecal Coliform Bacteria

The number of fecal coliform bacteria was calculated using the Multiple Tube Method and the Most Probable Number (MPN) mentioned by the American Association for Public Health, which includes three stages [10]**:**

2.6.2.1 Presumptive Test

Nine test tubes containing MacConkey Broth medium were inoculated with three concentrations (10, 1, and 0.1) ml of the sample, respectively. They were then incubated in a water bath at 44°C. After incubation, the tubes showed a gas accumulation and a yellow color, indicating the presence of fecal coliforms in the water sample. The results were recorded, and using the MPN table, the most probable number of coliforms present per 100 ml of the sample was determined [10].

2.6.2.2 Confirmed Test

The test was performed by taking a full loop from the positive tubes for the presumptive test and inoculating them onto previously prepared and sterilized Eosin Methylen Blue agar (EMB) using the schematic method. These tubes were then incubated upside down at 37°C for 24-48 hours to determine if the bacteria that formed the gas were negative. Whether the bacteria are Gram-positive (i.e., within the coliform group) or Gram-positive; this medium is capable of inhibiting the growth of Gram-positive bacteria, while the appearance of small, round colonies with dark centers surrounded by a dark green metallic sheen is evidence that the bacteria belong to the coliform group.

2.6.2.3 Completed Test

In this test, test tubes containing previously prepared and sterilized peptone water medium were inoculated by taking a full loop from the positive tubes in the hypothetical test. The tubes were incubated at 44°C for 24-48 hours. Drops of Kovacs reagent were then added. A red ring was observed on the surface of the medium, indicating the growth of E. coli and a number of different species and strains of Enterobacteriaceae. Referring to the MPN table, we can arrive at the results to obtain the most probable number of E. coli bacteria per 100 ml of the water sample.

2.7 Isolation and Identification

2.7.1 Isolation

Bacterial species were isolated from water samples using a full loop and cultured on Nutrient agar and MacConkey agar.

2.7.2 Cultural Identification

The morphological characteristics of the isolated colonies were studied after purification on selective culture media. These included the shape, texture, size, color, and borders of the isolated colonies [12].

2.7.3 Confirmation of Identification Using Molecular Methods

Polymerase Chain Reaction (PCR) was used to confirm the identification of bacterial isolates by following the following steps:

2.7.4 DNA Extraction

The Genomic DNA mini kit from Geneaid was used to extract DNA from Gram-positive and Gram-negative bacteria. The initial migration was followed by DNA detection, followed by Polymerase Chain Reaction (PCR), which included Primer sequence Table 2.

Table 2: Primer sequence.

Source	Result	Sequence Primer	Gene
NCBI	1300 pb	F GACCTCGTTTAGTTCACAGA	16Sr RNA
		R CACACGCTGACGCTGACCA	

Primers were prepared according to the manufacturer's instructions.

A PCR machine was used to amplify the 16S rRNA gene using specific forward and reverse primers, and the DNA concentration was adjusted for all study samples. DNA was then extracted from the agarose gel, and the resulting PCR bands were purified and sent for nucleotide sequencing using a Geneaid analysis kit. The nucleotides of the amplified fragments were then identified using DNA sequencing technology to identify and identify the species of the organism based on matching the gene sequences obtained with the gene sequences recorded for species and strains at the National Center for Biotechnology

Information (NCBI). The results were analyzed using the BLAST program.

3 RESULTS AND DISCUSSION

The results of the physical and chemical factors used to calculate water quality for aquatic habitats (CCME WQI) as shown in Table 3 and Figure 2 showed that temperatures ranged between 10-24.5 °C. High temperatures encourage the growth of microorganisms such as fungi, bacteria, and viruses, which can cause diseases for consumers, especially diarrhea in children and viral hepatitis in the hot months of the year [12]. The pH values ranged between in Table 3 (6.4-7.9).

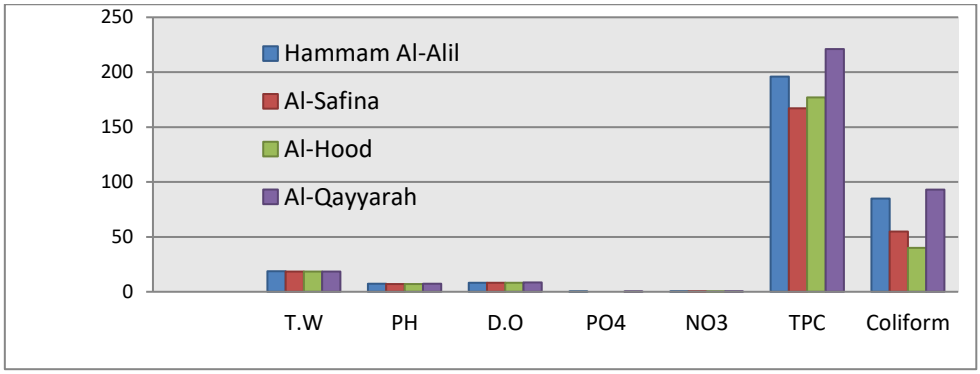


Figure 2: Physical and chemical factors guide water quality not for aquatic purposes.

Table 3: Averages, minimum and maximum concentrations of the physical and chemical factors under study.

Locations factors		Hammam Al-Alil	Al-Safina	Al-Hood	Al-Qayyarah
T. Water	Mean	18.6	18.3	18.4	18.5
	max	24.0	24.0	24.5	24.5
	min	10.0	10.1	10.4	10.4
PH	Mean	7.3	7.1	7.0	7.2
	max	7.9	7.7	7.6	7.8
	min	6.7	6.5	6.4	6.6
D.O mg/L	Mean	8.1	8.2	8.3	8.5
	max	10.2	10.2	10.3	10.2
	min	6.4	6.6	6.7	6.5
PO_4-3, mg/L	Mean	0.1	0.0	0.0	0.1
	max	0.14	0.12	0.11	0.13
	min	0.03	0.01	0.01	0.02
NO_3^-, mg/L	Mean	0.420	0.30	0.266	0.37
	max	0.990	0.69	0.650	0.88
	min	0.030	0.00	0.001	0.01
TPC ml/ 100	Mean	196	167	177	221
	max	650	580	600	700
	min	16	13	6	14
Coliform Bacteria ml/100	Mean	85	55	40	93
	max	165	130	90	180
	min	4	2	0	5

The results indicated that the river water tends towards alkalinity, as well as the lack of fluctuations in the pH values of the river water, which is attributed to the ability of Iraqi water and soil to neutralize the acidity (ANC) due to the high concentration of alkalinity in the water, as well as the high carbonate compounds CO3 in the bottom sediments [13], [14]. (Dissolved Oxygen (DO) and the results of Table 3 indicated that its value ranged between 6.4-8.5 mg/L, which indicates that the raw water is well-ventilated and free from causes of taste and odor resulting from anaerobic degradations [9], [15]. The results Table 3 of nitrate concentrations, which ranged between 0.001-0.990 mg/L, showed a variation in the extent of the decrease. The reason for the decrease in the nitrate value may be attributed to its consumption by floating algae and algae present in the area [16]. Phosphorus is one of the important nutrients for cell components, as it is an essential element for the growth, maintenance and repair of body tissues. It is necessary, along with calcium and magnesium, for healthy growth and bone formation in infants and children [17]. Its concentrations ranged between In our study, between 0.01-0.14, nitrates and phosphates were among the Iraqi standard determinants and international water specifications, as shown in Table 4, as the quality of the Tigris River water south of Mosul ranged between moderate - good and protected waters, rarely threatened, weak and deviating from the required level when compared with the rest of the rivers, especially in the southern part of Iraq, as it suffers from weakness.

3.1 Total Plate Count (TPC)

The total plate count included all types of aerobic, anaerobic, and facultative Gram-negative bacteria capable of fermenting lactose. Therefore, it is an important indicator of water safety and the extent of water contamination [18], [15]. The results in Table 3 showed that the total bacterial count ranged between 6-700 cells/100 ml. The reason for the variation in counts is due to the different factors affecting the presence of bacteria, the pH value, and the soil surrounding the river [19].

3.2 Fecal Coliform Bacteria

Intestinal bacteria are one of the most important causes of waterborne diseases. E. coli is the most prominent and widespread of these bacteria. They are naturally found in the intestines of humans and warm-blooded animals, and are therefore abundant in human and animal feces. This indicates water contamination if the number of coliform bacteria ranges, as shown. In Table 3 between 0-180 cells/100 ml, these pathogenic bacteria cause a group of infections inside and outside the intestine [20]. The Tigris River water is classified as category (A2) based on the classification provided by [21]. who classified surface water required for use into three categories, Table 5.

3.3 Molecular Diagnosis

The use of molecular diagnostic techniques is important and necessary due to their speed, particularly the use of Polymer Chain Reaction (PCR) technology. The study of the bacterial genome and comparison of its nitrogenous base sequences with those available in Gen Bank identified three bacterial species.

3.4 Cultural Diagnosis

Pseudomonas aeruginosa colonies on MacConkey agar were shown to be non-lactose fermenters. Bacillus bacteria were also isolated on nutrient agar and appeared white, chalky, dry, and rough to the touch.

Acinetobacter radioresistens was also isolated on nutrient agar and appeared as pink, lactose-fermenting colonies.

3.5 Molecular Diagnosis

This reaction was performed on purified genomic DNA samples extracted from the bacterial isolates using a primer specific for the 16srRNA gene.

Table 4: Iraqi and international standard specifications for water.

WHO 2006, 2003	Aquatic life	Physical and chemical characteristics
6.5-9.5	6.5-9	PH
–	15	Temp
50	13	mg/L NO_3
0.4	0.3	PO^{-4} mg/L
>5	5	DO mg/L

Table 5: Twort classification of surface water.

Maximum number of fecal colon cells allowed per 100 ml	Category
20	A1
2000	A2
20000	A3

3.6 Results of the Specialized PCR Reaction for Genomic DNA Samples

This reaction was performed on purified genomic DNA samples extracted from bacterial isolates. The strain was isolated using a primer specific for the 16s rRNA gene. After isolating the bacterial species from the samples under study and extracting their genomes [11], PCR was performed to confirm their molecular identification by detecting the 16s rRNA gene. The nitrogenous base sequences of each isolate were analyzed, and the results were compared at the National Center for Biotechnology Information (NBCI) using the Blast program. The results showed similarity between the three isolates, as shown in Table 6.

Table 6 shows the genera of isolated bacteria and the percentages of partial identification of the isolated bacterial species.

Table 6: Genera and identification percentages of isolated bacterial strains.

Reverse number	Molecular diagnosis and similarity ratio
MT367790.1	*Acinetobacter radioresiste* %100
MT457466.1	*Bacillus firmus* %100
ON032863.1	*Pseudomonas aeruginosa* %100

4 CONCLUSIONS

This study confirms the presence of moderate organic pollution in the Tigris River waters south of Mosul. The total bacterial count (TPC) ranged from 6–700 cells/100 ml, and fecal coliform counts (FC) ranged from 0–180 cells/100 ml. Species of health and environmental significance, such as Acinetobacter radioresistens, Bacillus firmus, and Pseudomonas aeruginosa, were isolated. Although the physical and chemical parameters (temperature, pH, dissolved oxygen, phosphate, and nitrate) were within the permissible limits according to Iraqi and international standards, the presence of bacteriological contaminants highlights the need for enhanced treatment procedures and continuous monitoring along the 60 km stretch from Hammam al-Alil to Qayyarah.

The relative distance from direct pollution sources and natural dilution processes have contributed to raising water quality levels to the "good" range in some locations. However, the continued discharge of untreated domestic and industrial waste threatens the sustainability of water resources and the health of local communities. Therefore, it is recommended to:

1) Install monitoring and biological filtration stations at critical points along the river.
2) Develop awareness and regulatory programs to reduce the discharge of sewage and industrial waste.
3) Conduct periodic studies that include analyzing additional environmental factors and monitoring long-term water quality developments.
4) These measures will ensure the preservation of the quality of the Tigris River and protect human health and aquatic ecosystems south of Mosul.

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Effect of Spraying Salicylic Acid and Seeding Rates on Wheat Plants under Water Stress

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Keywords: Water Stress, Averages of Seeding, Salicylic Acid, Wheat Plant.

Abstract: An experiment has been done in a flowerpot for the study of seeding effect and on salicylic acid on the production of wheat under moisture tension by using the system of split bars once, and tri-repeated Random Complete Block Design (RCBD).So, the whole or main plots has included irrigation treatments irrigation (every 7, 14, and 21 days), whereas the has included the averages of seeding (60, 80) kg. ha^{-1}),and the sub-sub-plots has included sprinkle plants with ordinary water (comparison treatment),and with the salicylic acid by concentration of (0, 100 and 200) ppm. Results have indicated that; the plants which were irrigated every 7 and 14 days are high-level average (32.31and 36.61) .cm, the area of a flag –leaf is (31.87and34.20) cm^2, the seeds amount is (28..31 and 29.84) seed.$pike^{-1}$), the protein rate is 21.96and 23.91)% but plants –which where irrigated every 14 days –have surpassed move spikes (4.43 $spike.m^2$) and thetotal wheat is (6.40 ton. ha^{-1}), the treatment has given (60kg. ha^{-1}),the higher value of seeds weigh is (0.44gm. $plants^{-1}$).results of the study have showed the surpassing irrigation treatment and besides seeding treatment (12 day and 60kg. ha^{-1}) at the production features and their components with the exception of the dry weigh which hasn't indicated differences .Additionally, the study results have showed the surpassing concerns traction of salicylic acid (100 ppm)at the studied production features with the exception of the length of a spike (200) and recorded (5.50cm). Finally, the results were revealed possibility using salicylic acid during fertilization stage, and it helps to increase seed yield and oil percent.

1 INTRODUCTION

Water is one of the most determining environmental factors for the growth and production of crop plants in the arid and semi-arid regions of the world in which Iraq is located, which are the areas most affected by drought and associated climate changes [1]. Iraq is facing severe droughts as a result of high temperatures, as it suffers from multiple types of drought, including climatic, agricultural and dry water bodies [2]. Drought has negative effects on the growth of plants, which it reduces their vegetative and reproductive growth through inhibition of photosynthesis processes and disturbance of nitrogen metabolism in them and its role in increasing the production of reactive oxygen species, which works to destroy proteins and cell membranes [3].

The wheat (Triticum aestivum L.) is the most important food crop in the world and this importance is between the balance between carbohydrates and proteins in its grains. Despite the importance of this crop, its production in Iraq is low, so it comes in sixth place among the importing countries of this crop, the seeding rate, which expresses plant density, is the most important agricultural practice, which greatly affects the determination of the ideal environment for the growth of the crop by achieving a balance to compete with plants for each other and the amount of seeds is a determining factor for the wheat crop in the use of available genetic resources [4].

To determine the appropriate seeding rate, a major role is played, especially in the wheat crop, as it directly affects the yield and its components [5], it has become common in recent years to use plant hormones as one of the agricultural techniques used to increase the tolerance of plants to various stresses, including drought. These regulators control the response of plants to various stresses by controlling many physiological processes, because they work to reveal and widen the carrier vessels in the bark and wood, contribute to facilitating the flow of water and food, and control the division and distinction of cells for some plant tissues and the development of flowering and organelles such as plastids and the orientation of photosynthetic products [6]. As well as controlling the aging of leaves [7], and increasing the

size of green plastids and grana discs in them and in the accumulation of nutrients and then dragging them to certain places of the plant such as leaves to form chlorophyll and prevent its loss [8], and maintain the normal level of endogenous hormones [9] and leads to the treatment of plants exposed to environmental stresses with salicylic acid to increase the tolerance of plants to those stresses and improve the growth qualities and yield of the plant [10], [11].

This occurs as a result of many physiological activities of this plant hormone, including its inhibition of ethylene synthesis and control of the movement of stomata and its opposite to the effectiveness of abscisic acid and its ability to bind to amino acids and give the plant systemic acquired resistance (SAR) and its non-enzymatic antioxidant and it is important to synchronize the spraying of growth regulators with the growth stages of plants directly related to the components of the yield.

The aims are to investigate the effect of spraying agents and the treatment of plant seedlings at different water stress to improve the performance and yield of wheat plants and the ability of using this technique to increase the plant's tolerance to water stress conditions.

2 MATERIALS AND METHODS

2.1 The Location of the Experiment and the Procedures for its Implementation

An experiment was carried out in a pot in an arboretum of the Directorate of Agriculture in Diyala Governorate during the winter season 2023-2024 with the aim of studying the effect of seeding and spraying rates with salicylic acid on the productivity of wheat crop under drought and humidity conditions.

2.2 Crop Management and Data Collection

Wheat grains of the Aba variety were planted in a pot of 2.5 kg. The experiment was applied by arranging the splinter panels and designing the whole randomized sectors with three replicates. The experiment included three factors, the main plots included three irrigation treatments (irrigation all 7, 14 and 21) days respectively, and the secondary factor seeding rates (60 and 80) $kg.ha^{-1}$ respectively and the sub-secondary factor spraying plants with tap water (comparison treatment), and salicylic at a concentration of (100 and 200) ppm respectively at the elongation stage. The seeds were planted on 25/11/2023 by 20 grains in the pot and an operation was performed Irrigation for all experimental units after the completion of planting until the completion of the emergence of seedlings, after which the irrigation treatments included in the experiment were initiated. The plant's requirements of nitrogen and phosphorus were added in the form of urea fertilizer (46% nitrogen) and phosphorus was added in the form of triple superphosphate 45% P_2O_5 and when the plants reached the stage of 100% flowering, the following characteristics were studied (plant height (cm), chlorophyll ($\mu g.cm^{-2}$) was estimated using the chlorophyll meter (Spad Plus Alrt 206-205), the area of the flag paper (m^2), the components and yield of the plant that included the number of spikes (m^2. $plant^{-1}$, spike length (cm), number of grains, $spike^{-1}$, grain weight (g. $plant^{-1}$), and grain yield ($ton.ha^{-1}$) and the weight of dry matter (g) and biological yield ($ton.ha^{-1}$). The data were analyzed following the method of analysis of variance according to the design used by the SPSS17 system, and the least significant difference test (L.S.D) at a significant level of 0.05 was used to compare the averages of the treatments (see Table 1).

Table 1: The chemical and physical properties of the soil.

The test	Unite	Value		
EC	D.S	2.45		
pH		7.3		
N	$mg.kg^{-1}$.soil	52		
P	$mg.kg^{-1}$.soil	12.12		
K	$mg.kg^{-1}$soil	176		
Ca	$mg.kg^{-1}$.soil	280		
Texture	$Gm.kg^{-1}$soil	Sand	Silt	Clay
		726 Mud	51	223
			Sandy Loam	

3 RESULTS AND DISCUSSION

3.1 Effect of Irrigation Treatments, Seeding Rates and Salicylic Acid on Growth Stages, Plant Height, Chlorophyll Content, Flag Leaf Area, Number of Grains, Grain Weight, Percentage of Protein

The results of Table 2 indicated the superiority of the plants that were irrigated every 7 and 14 days (in the characteristic of plant height and their value was (36.61 and 32.82), chlorophyll content 36.54 and 31.31 $\mu g.cm^{-2}$, the area of the flag leaf 34.20 and 31.87 cm^2 and the percentage of protein 23.91 and

21.36% with a non-significant difference between them. The plants irrigated every 7 days excelled in grain weight (0.59 gm.plant^{-1}) compared to all treatments.

The irrigation treatment, which provided appropriate amounts of moisture for the plant approaching its requirements, enabled it to form a large root system capable of absorbing water and nutrients with high efficiency, which reflected positively on a series of vital events directly or indirectly related to the accumulation of dry matter such as transpiration, gas exchange, respiration, photosynthesis, and others, increasing with it the number of stem buds below the soil surface [12] and increasing the growth rate of leaves and prolonging the duration of their effective stay.

Plants irrigated every 21 days gave the lowest values in plant height (22.56 cm), chlorophyll content (26.27 µg.cm^{-2}), flag leaf area (26.99 cm^2) and grain weight (0.18 gm. plant^{-1}). The reason for the decrease in growth stages may be attributed to the fact that the drought led to a reduction in the division and elongation of stem cells and their small size, which was reflected in the decrease in plant growth and negatively affected the process of carbon metabolism, transport and absorption of nutrients [13]. Their influence on other qualities is absent. As for the seeding rates, no significant differences appeared in the studied traits and the treatment exceeded 60 kg.ha^{-1} in the grain weight characteristic (0.44 gm.plant^{-1}). The reason is that the increase in plant density increases competition between plants for light, which prompted the stems to elongate to obtain the largest possible light needs [14]. Plants sprayed with salicylic acid at 100 ppm treatment outperformed all studied traits significantly except for protein (23.05%).

Salicylic acid has an effect on increasing the number and growth rates of cells due to the accumulation of metabolites in the developing peaks, an indicator of the increase in the effectiveness of DNA and RNA, the efficiency of carbon metabolism and the concentration of hormones such as auxins, kintien and gibberellins 9 and 16.

3.2 Effect of Irrigation Treatments, Seeding Rates and Salicylic Acid on Growth Parameters, Number of Spikes, Spike Length, Grain Yield, Dry Weight, Biological Yield Content

As for Table 3, the results show that the irrigation treatment is superior every 14 days in the characteristics of the yield and its components, as it significantly outperformed the number of spikes and reached a value of (4.43 spikes.m^{-2}), the length of the ears (5.94 cm), the yield of grains (6.40 tons.ha^{-1}) and the biological yield (23.29 tons.ha^{-1}).

The reason for this may be due to the irrigation treatment that provides the requirements of plants of moisture in converting the largest proportion of the formed seedlings into fertile seedlings through its role in increasing photosynthesis rates, and the high moisture deficiency leads to the failure to complete the grain contract or to abort it after holding it as a result of lack of processing with nutrients [15] while no significant difference appeared in the dry weight characteristic.

The reason is attributed to the increase in the vegetative total represented by the characteristics of plant height and the area of the flag leaf and its

Table 2: The effect of irrigation treatments, seeding rates and salicylic acid on some growth parameters.

Irrigation treatments (day)	Plant height (cm)	Chlorophyll (µg.cm^{-2})	Flag paper area (cm^2)	Number of grains (grain.spike^{-1})	Grain weight (gm.plant^{-1})	Protein %
7	32.82	31.31	31.87	28.31	0.59	21.96
14	36.61	36.54	34.20	29.84	0.52	23.91
21	22.56	26.27	26.99	21.32	0.18	19.16
L.S.D value p=0.05	5.98	2.02	1.53	6.05	0.12	1.91
Seeding rates kg. ha^{-1}						
60	31.75	33.23	32.52	28.81	0.44	22.40
80	29.58	29.52	29.52	24.18	0.42	20.95
L.S.D value P=0.05	3.19	2.49	3.30	3.39	0.18	2.79
Salicylic acid mg.L^{-1}						
0	27.03	28.72	26.86	22.75	0.25	20.05
100	33.50	33.39	34.17	29.51	0.83	23.05
200	31.48	32.00	32.03	27.21	0.21	21.92
L.S.D value P=0.05	3.77	1.80	2.39	4.08	0.13	2.30

competition for the root system on the products of photosynthesis of growing plants in conditions that provide moisture in addition to the lack of need for plant roots to expand to the soil layers may have contributed to their low dry weight.

As for the seeding rates, it appears from Table 3 that the sowing treatment (60 kg.ha^{-1}) is higher in the number of ears, and its value was (3.95 spike.m^{-2}), the length of the spike (5.71 cm), and the yield of grains (5.70 tons.he^{-1}) and significantly over the treatment of seeds (80 kg.ha^{-1}) respectively.

No significant differences were found in the dry weight and biological yield characteristic. The lower seeding rates increased the number of spikes and the length of the spike on the wheat plant in order to increase the number of branches due to the presence of sufficient distance [14].

The plants that were sprayed with salicylic acid at the time of treatment gave 100 ppm the highest values in the number of spikes and their value was (4.64 spikes.m^{-2}), the grain yield (6.10 tons.ha^{-1}) and the biological yield (21.95 tons.ha^{-1}) significantly respectively, and no significant differences were shown in the dry weight characteristic.

While the treatment exceeded 200 ppm in the length of the spike and significantly, as its value reached (5.50 cm), the reason may be that salicylic acid as a result of its role in the hormonal balance between cytokinin and auxins and increasing the level of cytokinin hormone metabolism within the plant and its contribution to increasing the proportion of the hormone florigen when the plant is exposed to drought stress and therefore it controls the emergence and differentiation and growth of flowers and thus in their number [16], [17]. Salicylic acid also contributes to inhibiting the metabolism of ethylene acid and maintains the water content of the leaves, raising the efficiency of water relations in the plant through its ability to conjugate with amino acids such as Glycine, Proline and Tryptophan, which works to regulate the osmotic leaves, withdraw water from neighboring cells, and maintain the fullness of cells when growing plants in drought conditions [18], [19].

In addition to its importance in increasing cell divisions and numbers and in the accumulation of amino acids and preventing their oxidation when growing plants in drought conditions or at high temperatures, it raises the level of antioxidants and inhibits the concentrations of Reactive Oxygen Species (ROS) leading to the degradation of proteins and oxidation of enzymes leading to reduced metabolism and accumulation of dry matter [20], [21].

3.3 Bilateral Interactions

The results of Tables 4 and 5 showed a significant effect of the interaction treatments between irrigation treatments and seeding rates, we note the superiority of irrigation treatment and seeding treatment (14 days and 60 kg.ha^{-1}) in the characteristic of plant height and its value was (38.39 cm), chlorophyll content (37.23 µg.cm^{-2}), the area of the flag leaf (36.16 cm^2) and the number of grains per spike (31.75 grains.spike^{-1}) and significantly.

As for the interaction of irrigation treatment and salicylic acid concentration, the results show significantly higher irrigation treatment and salicylic acid concentration (14 days and 100 ppm) in plant

Table 3: The effect of irrigation treatments, seeding rates and salicylic acid on yield, its components and other characteristics.

Irrigation treatments (day)	Number of spikes (spike.m^{-2})	Spike length (cm)	Grain yield (ton.ha^{-1})	Dry weight (gm.m^{-2})	Biological yield (tons.ha^{-1})
7	4.13	5.75	5.38	0.12	21.28
14	4.43	5.94	6.40	0.13	23.29
21	2.38	4.65	4.30	0.10	16.59
L.S.D value P=0.05	0.86	0.76	1.32	0.08	3.55
Seeding rates kg. ha^{-1}					
60	3.95	5.71	5.70	0.12	20.91
80	3.34	5.18	5.01	0.11	19.86
L.S.D value P=0.05	0.44	0.70	0.46	0.05	1.33
Salicylic acid mg.L^{-1}					
0	2.18	5.39	4.40	0.12	18.09
100	4.64	5.45	6.10	0.12	21.95
200	4.12	5.50	5.58	0.12	21.13
L.S.D value P=0.05	0.71	0.68	0.52	0.03	2.58

height (40.33 cm) and chlorophyll content (39.49 μg.cm^{-2}). While the irrigation treatment and the concentration of salicylic acid (7 days and 100 ppm) in the area of the flag leaf (38.21 cm^2) and grain weight (1.00 gm. plant^{-1}) and significantly superior to the rest of the treatments and the interaction treatment between the irrigation treatments and the comparison treatment (21 days and 0 ppm) gave the lowest values in the characteristic of plant height (19.53 cm), chlorophyll content (25.22 μg.cm^{-2}), flag leaf area (24.90 cm^2), number of grains (17.85 grains.spike^{-1}) and grain weight per plant (0.18 g. plant^{-1}).

The interaction of seeding rates with salicylic acid concentration exceeds the treatment (60 kg.ha^{-1} and 100 ppm) in the characteristic of plant height and its value was (35.16 cm), chlorophyll content (35.12 μg.cm^{-2}), the area of the flag leaf (35.08 cm^2), the number of grains per spike (33.05 grains. spike^{-1}) and the grain weight of the plant (0.84 gm. plant^{-1}).

The lowest values in plant height, chlorophyll content, leaf area and grain count were recorded by the interaction between seeding rates and the comparison treatment (80 kg.ha^{-1} and 0 ppm). The lowest grain weight value at the time of treatment (80 kg.ha^{-1} and 200 ppm).

The results of Table 5 also indicated that the bilateral interaction treatment between irrigation treatments and seeding rates (14 days and 60 kg.ha^{-1}) in the number of spikes and its value was (4.62 spike.m^{-2}), the length of the spike (6.49 cm), the grain yield (7.04 tons.ha^{-1}) and the biological yield (23.81 tons.ha^{-1}) significantly over the rest of the treatments.

There were no significant differences in the dry weight characteristic. As for the interference of irrigation treatments with salicylic acid concentration, the interference treatment (14 days and 100 ppm) outperformed the rest of the treatments significantly in the number of spikes and amounted to (5.55 spikes.m^{-2}), spike length (6.04 cm), grain yield (7.60 tons.ha^{-1}) and biological yield (25.38 tons.ha^{-1}), while no significant differences appeared in the dry weight characteristic.

Table 4: The effect of bilateral interactions between irrigation treatments, seeding rates and salicylic acid on some growth indicators is illustrated.

Irrigation treatments (Day)	Seeding rates	Plant height (cm)	Chlorophyll (μg.cm^{-2})	Flag paper area (cm^2)	Number of grains (grain spike^{-1})	Grain weight (gm.plant^{-1})
7	60	33.09	32.57	33.49	29.78	0.60
	80	32.56	30.05	30.25	26.84	0.58
14	60	38.39	37.23	36.16	31.75	0.52
	80	34.83	35.84	32.25	27.93	0.52
21	60	23.76	29.88	27.93	24.88	0.20
	80	21.36	22.65	26.05	17.76	0.16
L.S.D value p=0.05		5.53	4.31	5.71	5.88	0.31
Salicylic acid mg.L^{-1}						
7	0	30.81	29.70	26.48	24.15	0.26
	100	34.80	32.89	35.04	31.25	1.29
	200	32.87	31.36	34.09	29.55	0.22
14	0	30.74	31.25	29.20	26.26	0.31
	100	40.33	39.49	38.21	31.50	1.00
	200	38.77	38.87	35.21	31.76	0.25
21	0	19.53	25.22	24.90	17.85	0.18
	100	25.37	27.80	29.26	25.80	0.19
	200	22.79	25.78	26.18	20.33	0.17
L.S.D value P=0.05		6.54	3.12	4.14	7.08	0.23
Salicylic acid	Seeding rates					
60	0	27.66	30.72	28.56	24.21	0.27
	100	35.16	35.12	35.08	33.05	0.84
	200	31.98	33.83	33.93	29.16	0.23
80	0	26.40	26.72	25.16	21.30	0.24
	100	31.39	31.65	33.25	25.97	0.82
	200	30.97	30.18	30.14	25.26	0.20
L.S.D value P=0.05		5.34	5.55	3.38	5.78	0.19

Table 5: The effect of bilateral interactions between irrigation treatments, seeding rates and salicylic acid on yield and its components.

Irrigation treatments (Day)	Seeding rates	Number of spikes (spike.m^{-2})	Spike length (cm)	Grain yield (ton.ha^{-1})	Dry weight (gm.m^{-2})	Biological yield (tons.ha^{-1})
7	60	4.45	5.93	5.67	0.12	21.99
	80	3.81	5.57	5.08	0.12	20.57
14	60	4.62	6.49	7.04	0.12	23.81
	80	4.25	5.39	5.76	0.13	22.77
21	60	2.78	4.71	4.41	0.11	16.94
	80	1.97	4.58	4.19	0.09	16.24
L.S.D value (P=0.05)		0.77	1.22	0.81	0.09	2.30
	Salicylic acid					
7	0	2.50	5.82	4.46	0.11	18.57
	100	5.40	5.49	6.01	0.13	22.88
	200	4.49	5.95	5.66	0.12	22.38
14	0	2.65	5.91	5.02	0.11	19.86
	100	5.55	6.04	7.60	0.15	25.38
	200	5.11	5.86	6.56	0.13	24.65
21	0	1.41	4.43	3.71	0.09	15.83
	100	2.98	4.82	4.67	0.12	17.58
	200	2.75	4.69	4.51	0.10	16.36
L.S.D value (p=0.05)		1.23	1.17	0.91	0.05	4.48
Salicylic acid	Seeding rates					
60	0	2.38	5.57	4.82	0.11	18.21
	100	5.11	5.74	6.50	0.13	22.62
	200	4.36	5.82	5.79	0.12	21.91
80	0	1.98	5.21	3.97	0.10	17.96
	100	4.17	5.16	5.70	0.12	21.27
	200	3.87	5.18	5.36	0.11	20.35
L.S.D value (P=0.05)		1.00	0.96	0.91	0.04	3.66

As for the interaction of the seeding treatments with the concentrations of salicylic acid, the results show that the treatment exceeds (60 kg.he^{-1} and 100 ppm) in the number of spikes (5.11 spike m^{-2}), grain yield (6.50 tons.ha^{-1}) and biological yield (22.62 tons.he^{-1}) and significantly. No significant differences were shown in the dry weight characteristic.

While the interaction treatment between seeding rates and salicylic acid concentration (60 kg.ha^{-1} and 200 ppm) showed superiority in the description of spike length (5.82 cm), and the lowest value was recorded in the number of spikes (1.98 spike.m^{-2}), grain yield (3.97 ton.ha^{-1}), dry weight (0.10 gm.m^{-2}) and biological yield (17.96 tons.ha^{-1}), the interaction treatment between seeding rates and the comparison treatment (80 kg.he^{-1} and 0 ppm) respectively.

3.4 Triple Interactions

The results of Table 6 showed a significant effect of triple overlap between irrigation treatments, seeding rates and growth regulator in some yield characteristics. The interaction treatment between irrigation treatments, seeding rates and salicylic acid concentration (14 days and 60 kg.ha^{-1} and 100 ppm) in the number of spikes (5.93 cm^2), spike length (6.65 cm), number of grains per spike (34.66 grains.spike^{-1}), grain yield (8.29 tons.ha^{-1}), biological yield (26.10 tons.ha^{-1}) and protein percentage (26.60%) significantly compared to the interaction treatment between irrigation treatments, seeding rates and comparison treatment (7 days, 60 kg.ha^{-1} and 0 ppm).

The interaction treatment between irrigation treatments, seeding rates and salicylic acid concentration (7 days and 60 kg.ha^{-1} and 100 ppm) outperformed in the number of spikes (5.86 cm^2) and in the grain weight (1.30 gm. plant^{-1}) and significantly over the rest of the treatments.

The lowest values in the number of spikes (1.66 cm^2), spike length (4.36 cm), number of grains (15.43 grains.spike^{-1}), grain yield (3.45 tons.ha^{-1}), biological yield (15.16 tons.ha^{-1}) and protein percentage (17.50%) were recorded by the interaction treatment

Table 6: The effect of triple interaction between irrigation treatments, seeding rates and growth regulator on some traits.

Irrigation treatment (Day)	Seeding rates	Salicylic acid	Number of spikes (cm^2)	Spike length (cm)	Number of grains ($grain.spike^{-1}$)	Grain weight ($gm.plant^{-1}$)	Grain yield ($ton.he^{-1}$)	Biological yield ($tons.ha^{-1}$)	Protein %
7	60	0	2.83	5.88	25.20	0.28	4.77	18.77	20.76
		100	5.86	5.65	32.50	1.30	6.26	24.10	23.70
		200	4.66	6.27	31.66	0.23	5.99	23.10	22.33
	80	0	2.16	5.76	23.10	0.25	4.14	18.38	20.66
		100	4.93	5.33	30.00	0.28	5.77	21.66	22.83
		200	4.33	5.63	27.43	0.21	5.33	21.66	21.48
14	60	0	2.66	6.33	27.16	0.32	5.72	19.38	21.96
		100	5.93	6.65	34.66	1.00	8.29	26.10	26.60
		200	5.26	6.48	33.43	0.26	7.09	25.96	25.50
	80	0	2.63	5.50	25.36	0.31	4.32	20.33	20.73
		100	5.16	5.43	28.33	0.99	6.92	24.66	24.66
		200	4.96	5.25	30.10	0.24	6.03	23.33	23.89
21	60	0	1.66	4.50	20.26	0.20	3.98	16.50	19.43
		100	3.53	4.93	32.00	0.21	4.94	17.50	21.33
		200	3.16	4.72	22.40	0.20	4.30	16.05	20.00
	80	0	1.66	4.36	15.43	0.16	3.45	15.16	17.50
		100	2.43	4.71	19.60	0.18	4.41	17.50	19.10
		200	2.33	4.66	18.26	0.15	4.71	16.05	18.33
L.S.D value P=0.05			1.74	1.66	10.01	0.33	1.29	6.34	5.64

between irrigation treatments, seeding rates and comparison treatment (21 days and 80 kg.ha-1 and 0 ppm) respectively, and the interaction treatment (21 days, 80 kg.he-1 and 200 ppm) recorded the lowest value in grain weight and was (0.15 g.plant-1).

The significant overlap between irrigation treatments and seeding rates and growth regulator for a particular trait is evidence of the response of that trait to the three variables in a homogeneous manner, while the non-significant interaction indicates a difference in the response of the trait to those variables.

4 CONCLUSIONS

The results of this study highlight the effectiveness of using salicylic acid and optimal seeding rates to improve wheat growth and yield under water stress conditions. Irrigation every 14 days combined with a seeding rate of 60 $kg.ha^{-1}$ and foliar application of salicylic acid at 100 ppm showed the most positive impact on growth traits, grain yield, and protein content. This combination significantly improved plant height, chlorophyll content, spike number, and overall productivity. Salicylic acid proved beneficial in enhancing the plant's tolerance to drought by promoting physiological activities and improving water-use efficiency. Additionally, lower seeding density reduced competition among plants, contributing to better yield components. The application of salicylic acid at 100 ppm during the elongation stage, along with moderate seeding rates and scheduled irrigation, can be an effective strategy to enhance wheat performance under limited water availability.

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Evaluation of Salicylic Acid and Proline Treatment on Tomato Plant Growth under Biotic Stress by Alternaria Alternata Fungus

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Keywords: Proline, Alternaria Alternata, Salicylic Acid, Early Blight, Tomato.

Abstract: An experiment was conducted in 2024-2025 to examine the effects of treatment with different concentrations of salicylic acid SA and proline on pathogenic fungus *Alternaria alternata* on tomato plants. Seven fungal isolates of A. alternata fungi were obtained from tomato fruits showing symptoms of late blight infection, the pathogenicity of these isolates showed that two isolates (Ala2) and (Ala4) gave the highest percentage of pathogenicity, as the seedling death rate, and Ala4 isolate was chosen for the laboratory experiment and the greenhouse experiment. The results of the tested experiment also showed the inhibitory effect of SA on the growth of fungal mycelium on SDA medium, as the inhibition rate was 100% at all concentrations, As for proline, the concentration 0.5 recorded highest inhibition rate of 91.27%, Then the effect of spraying with five concentrations of resistance inducing agents (SA and proline) was tested, which are (0.5, 1, 1.5, 2, 2.5) ml. L-1 were sprayed on 40 day old tomato seedlings after treatment with the pathogenic fungus ,The results showed that superiority of spraying with SA in reducing the infection rate with early blight disease, the infection rate with the disease decreased with increasing the concentrations of spraying with salicylic acid. Accordingly, the lowest infection rate was in the spraying treatment with 2.5 ml. L-1 and was 1.96%. The results also show that spraying with proline had different results than spraying with SA, as the infection rate did not decrease with increasing concentration, and increasing the concentration of the treatment did not affect the pathogen. The results show that the treatment with SA and proline did not affect the average number of leaves or the leaf area of the plant compared to the control treatment without the pathogenic fungus, but it outperformed the control treatment with the pathogenic fungus.

1 INTRODUCTION

Tomato (Solanum lycopersicon L.) is one of the most important and popular vegetables in the world. It is a strategic crop due to its high nutritional value [1]. It is also an economically important plant, as it is the second most important crop after potatoes [2]. Tomato is an annual staple crop, produced in more than 170 countries worldwide, including Iraq, where tomato cultivation has spread in covered and open fields in arid and temperate regions [3]. The cultivated area in 2018 amounted to 18,186 hectares, with a production capacity of 266,294 tons. As for tomato production in 2020, according to statistics from the Ministry of Planning, the Central Statistical Organization (CSO), and the Food and Agriculture Organization of the United Nations, Iraq's tomato production amounted to 744 million tons [4]. Tomato crop is exposed to many diseases, which are the main limiting factor in its production, causing significant losses in the crop. Infection is often very rapid when the environmental conditions are suitable, especially in contaminated soil. Tomato productivity is affected by many fungal and non-fungal infections worldwide, as these diseases can spread from one plant to another in the field [5]. One of the most widespread fungal pathogens is the fungus *Alternaria alternata*, which is responsible for late blight of tomato plants [6]. A. alternata is a common pathogenic fungus of tomato plants. This fungus invades the fruits due to the high nutritional content. The high moisture content of the fruit and the availability of a thin outer skin make tomato fruits susceptible to infection and colonization through infected or weakened tissues, causing fruit rot disease [7]. Ripe fruits are more susceptible to infection than fruits in the early stages of development [8] with infection occurring either in the field or during harvest or storage [9]. The fungus infects plant parts such as leaves, stems, fruits, tubers, and roots.

The disease manifests as necrotic disease causing black spots on the surface of ripe fruits, causing fruit spoilage [10]. Recent studies have shown that the residual effects of chemical pesticides on plants exceed the permissible limit. This, in turn, poses a significant risk to consumers and the environment. Therefore, studies and research have focused on finding alternative and safer methods for combating plant diseases. Among these methods is the use of plant resistance-inducing substances, which work to control the pathogen [11]. Plants have developed a defense system to survive, where plants resort to several methods and mechanisms to defend themselves against pathogens, such as physical defenses such as thick cell walls, which are the main site and target of infection by pathogens, or through the formation of a waxy surface on leaves or the presence of thorns [12].The other type of defense is chemical defenses, which are represented by the production of chemical compounds that kill or inhibit the pathogen, such as the production of phytoalexins [13], or through systemic acquired resistance (SAR) that occurs after infection with a non-fatal disease, which leads to the stimulation of defense mechanisms in all parts of the plant, which gives the plant the ability to resist subsequent diseases. Therefore, the current study aimed to study the effect of treatment with different concentrations of salicylic acid and proline on the pathogenicity of the fungus *Alternaria alternata* that caused late blight on tomato plants in the laboratory and on the symptoms of the disease on the tomato plant and the growth of the tomato plant under biotic stress by the pathogenic fungus.

2 MATERIALS AND METHODS

2.1 Isolation of the Pathogenic Fungus Alternaria Alternata

Several samples of tomato fruits showing symptoms of the disease as shown in Figure 1 were collected from the local market of Baqubah, the capital of Diyala Governorate, during February 2024-2025. The samples were transferred to the Mycology Laboratory in the Department of Life Sciences, College of Education for Pure Sciences, University of Diyala. Afterwards, the samples were washed well with running water and dried on blotting paper. The fruits were then superficially sterilized with 1% sodium hypochlorite solution for 2 minutes, after which the fruits were washed with sterile water three times, then the samples were dried using sterile filter papers. After that, the infected parts of the fruit were removed and cut into 1 cm long pieces and transferred to 9 cm diameter plastic dishes containing the Sabouraud Dextrose Agar (SDA) culture medium, sterilized by an autoclave at 121°C and a pressure of 15 psi for 15 minutes. After that, the dishes were placed in an incubator at 25 ± 2°C until the appearance of fungal growths [14]. The fungus *Alternaria alternata* was diagnosed according to the morphological and microscopic characteristics of the fungus mentioned in [15]. To prepare a suspension of pathogenic fungal spores, ten ml of sterile distilled water was added to a plate containing a seven-day-old fungal colony. The harvested spores were then filtered through two layers of cheesecloth, and the spore concentration in the resulting suspension was adjusted to 1×105 spores.ml-1 by using a hemocytometer slide.



Figure 1: Some samples of Tomato fruits collected from local markets in Baqubah city show symptoms of early blight.

2.2 Pathogenicity Test

To test the pathogenicity Tomato seeds Solanum lycopersicum L. variety Superrogena were used for this. These seeds were obtained from the agricultural offices in Diyala Governorate and their variety was confirmed by testing them in the laboratories of the Ministry of Science and Technology, Baghdad. The seeds were sterilized in sodium hypochlorite solution 1% for 10 minutes, then washed three times with distilled water, and dried on paper towels, then placed in suspension of pathogenic fungal spores for 10 minutes. Then the seeds soaked in the pathogenic fungus were transferred to plastic penny dishes containing filter papers to maintain the moisture of the growth medium. The dishes were left in a dark place and were watered when the filter paper dried. As for the control treatment, seeds not soaked in the pathogenic fungus were used. After 15 days of planting, the Percentage of seedling death were calculated as in the following equations [16]:

Germination rate (%) = (N. of germinated seeds / Total N. of seeds) × 100.

Death rates (%) = (N. of dead seeds / Total N. of tested seeds) × 100.

2.3 Effect of Salicylic Acid and Proline on Alternaria Alternatalinear Growth

According to the poisoned food technique [17], the effect of salicylic acid and proline on the most virulent fungal isolates was evaluated in a pathogenicity experiment for the growth of A. alternata (Ala4), where 6 concentrations of each were tested, namely (0.0, 0.5, 1.0, 1.5, 2, 2.5) ml. L-1. The pathogenicity test was performed using the culture intoxication technique, and sterile SDA medium was used for this purpose. Plates were inoculated with a 5 mm diameter disc taken from a freshly grown fungal colony, and each plate was inoculated with one disc. Each treatment was repeated 3 times, after which the plates were incubated in an incubator at 25 ± 1 °C for 10 days. Then, the readings were taken by measuring the diameters of the fungal cultures (mm) and the inhibition percentage was calculated according to the equation [18] as follows: Percent inhibition%= (Radial growth in control- Radial growth of treatment / Radial growth in control) × 100.

2.4 Greenhouse Experiment

2.4.1 Preparing Tomato Seedlings

Tomato seeds variety Super Rogina were used, which were planted in plastic cork plates after filling them with peat moss sterilized by an autoclave at a temperature of 121°C and a pressure of 15 pounds for 20 minutes. The seeds then planting in it and watered as needed. The seedlings were used after they had grown and reached the age of 40 days in the greenhouse experiment.

2.4.2 Experiment Implementation

A factorial experiment was conducted in the greenhouse of the Nursery Department of the Agricultural Research Department in Baqubah - Diyala Governorate - Iraq during the agricultural season 2023-2024 on tomato plant Solanum lycopersicum L. Super Rogina variety, which was previously grown in special dishes. The seedlings were transferred at the age of 40 days to plastic pots filled with sandy mixture soil mixed with peat moss at a ratio of 3:1 and sterilized by solar pasteurization according to the method of [19]. The pots were planted at a rate of one plant per pot. The experiment was carried out with the aim of studying the effect of spraying with salicylic acid and proline on the resistance of tomato plants to infection with the pathogenic fungus *Alternaria alternata*.

The treatment was carried out by artificial infection with the pathogenic fungus and at 6 concentrations (0.0, 0.5, 1.0, 1.5, 2, 2.5) ml. L-1 of salicylic acid and proline spraying after two days of artificial infection with the pathogenic fungus. Spraying was repeated for both methods three times after planting at equal intervals. As for the control treatment, it was sprayed with distilled water only. As for the pathogenic fungus, the treatment was carried out by spraying the pathogenic fungus suspension on the entire plant at a rate of 10 ml per plant. After 45 days of planting in plastic pots, the following measurements were taken:

- Disease incidence was computed according to [20]: Disease incidence % = (N. of Diseased Plants /Total N. of Plant Inspected) ×100;
- Average of leaves number ;
- Leaf area calculation (cm2): was calculated for tomato leaves at all concentrations using Image J [21].

2.5 Statistical Analysis

A factorial experiment was conducted using a Randomized Complete Block Design (R.C.B.D.). The results were statistically analyzed using SPSS. The differences between the means were compared using Duncan's multiple range test at a probability level of 0.05 and P value.

3 RESULTS AND DISCUSSION

3.1 Isolation of the Fungus Causing Early Blight Disease

The results of isolating the fungus causing early blight on tomato fruits showed that the causative agent was one, namely the fungus *Alternaria alternata*, as 7 pure isolates were obtained, namely Ala1, Ala2, Ala3, Ala4, Ala5, Ala6, and Ala7. Figure 2 shows a colony of the fungus *Alternaria alternata* on SDA medium, Colonies were olive-black to black or gray, resembling tanned to tangled leather, and the fungus grew quickly on SDA media. Under a microscope, simple, occasionally branched, short, or oblong sporangia were used to create branching spore chains of multicellular spores simultaneously. The spores had smooth or warty walls, were dull brown in color,

spherical or spheroidal, occasionally oval or ellipsoidal, and frequently had a short, conical or cylindrical beak, and this agreement with [22].

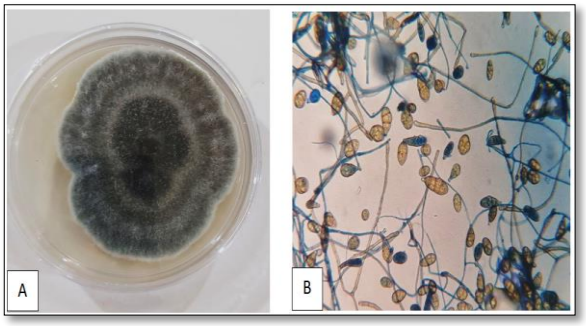


Figure 2: Colony of *Alternaria alternata* fungus on SDA medium a), *Alternaria alternata* fungus under a light microscope at ×40 magnification b).

3.2 Pathogenicity Test

The results in Table 1 show the pathogenicity test results for 7 fungal isolates of *Alternaria alternata* on tomato seeds after 15 days of germination. All isolates were pathogenic to varying degrees. The two isolates Ala6 and Ala7 recorded the highest percentage of seedling death, which was 100%, followed by the two isolates Ala3 and Ala5, with a seedling death percentage of 97%. As for the isolate Ala1, it recorded a percentage of 90%, while the two isolates Ala4 and Ala2 recorded the lowest percentage of seedling death, which was 66% and 50%, respectively, compared to the control treatment, in which the percentage of seed germination was 100% and the percentage of seedling death was 0%.

Table 1: Testing the effect of different fungal isolates of Alternaria alternata on the germination rate and seedling death of tomato after 15 days of growth in plastic dishes.

Fungi isolates	Germination rate, % after 15 day	Seedling death rate, % after 15 day
Ala1	1.00 d	90 a
Ala2	5.00 b	50 c
Ala3	0.33 d	97 a
Ala4	3.33 c	66 b
Ala5	0.33 d	97 a
Ala6	0.00 d	100 a
Ala7	0.00 d	100 a
Control	100 a	0 d

Note: Similar letters indicate that there are no significant differences between the means, at a probability level of 0.05. Note that the probability value p-values ≤ 0.00.

The decrease in germination rate and the increase in seedling death rate resulting from seed infection by pathogenic fungi are attributed to the ability of the fungus to produce decomposing enzymes as well as to secrete mycotoxins, all of which can reduce seed germination rate or cause seedling death after germination [23], [24]. Also the difference between fungal isolates in their pathogenicity can also be attributed to the difference in environmental conditions between one region and another or the difference in the genetic composition of the fungus, which allows its development, spread, and high adaptability [25].

3.3 Effect of Salicylic Acid and Proline on Alternaria Alternata Linear Growth

Table 2 shows and Figure 3 that treatment with different concentrations of salicylic acid led to inhibition of the growth of the fungus *Alternaria alternata*, where the average diameter in all treatments was 0.00 mm compared to the control treatment, in which the diameter of the fungal colony was 75.0 mm, with an inhibition rate of fungal growth of 100%. The treatment with proline shows that the average colony diameter decreased with an increase in the concentration of proline, and the treatment with a concentration of 2.5 ml. L-1 achieved the lowest rate of diameter growth, which was 19.12 mm, with an inhibition rate of 74.51%, followed by treatments (2, 1.5, 1, 0.5) ml.L-1 in which the average diameters were (28.00, 46.22, 59.56, 68.45) mm, respectively, with inhibition rates of (62.67, 38.37, 20.59, 8.73)%, respectively. It is known that salicylic acid plays an important role in stimulating plant defenses against pathogens and also has the ability to directly affect pathogens, especially fungi, by inhibiting fungal growth. This is what the results of the current study confirmed and is consistent with the results of previous studies, including [26], [27].

3.4 Greenhouse Experiment

The results in Table 3 show the effect of spraying with salicylic acid and proline on the infection rate of tomato plants with early blight disease on leaves. The table shows the superiority of spraying with salicylic acid in reducing the infection rate with early blight disease. As we note from the results in the table, the infection rate with the disease decreased with increasing the concentrations of spraying with salicylic acid. Accordingly, the lowest infection rate was in the spraying treatment with 2.5 ml.L-1 and was

1.96%. As for the spraying treatment with a concentration of 0.5 ml.L-1 it recorded the highest infection rate with the disease and was 6.66%, while the control treatment infected with the pathogenic fungus and without spraying recorded an infection rate of 44.11%. The results of the table also show that spraying with proline had different results than spraying with silicic acid, as the infection rate did not decrease with increasing concentration, and increasing the concentration of the treatment did not affect the pathogen. We find that the 0.5 ml.L-1 concentration treatment achieved the lowest infection rate of the disease, which was 16.66%, while the 2.5 ml.L-1 treatment recorded a rate of 23.43%.

The results in Table 4 show the effect of spraying with salicylic acid and proline on the average number of leaves of tomato plants growing under biological stress with the pathogenic fungus *Alternaria alternata*. The spraying treatments with salicylic acid show that the average number of leaves decreased compared to the control treatment without pathogen, which was 15.5 leaves, and the concentration treatment of 0 ml.L-1 (pathogen without spraying), which was 13.6 leaves. The results show that the average number of leaves decreased with increasing salicylic acid spray concentration, which was (12, 10.2, 10.8, 11, 10.2) leaves, respectively, for concentrations (0.5, 1, 1.5, 2, 2.5) ml.L-1, respectively. As for the proline spray treatments, the results show that the number of leaves increased with increasing concentrations, which were (0.5, 1, 1.5, 2) ml.L-1, respectively, and the average number of leaves was (13.2, 13.4, 15.5, 15.6) leaves, respectively, while the 2.5 ml.L-1 concentration treatment recorded a decrease in the average number of leaves to become 12.8 leaves.

Table 5 and Figure 4 shows the effect of spraying with salicylic acid and proline on the average leaf area of tomato leaves under biological stress by the pathogenic fungus *Alternaria alternata*. The results show the varying effect of spraying with different concentrations of salicylic acid on the leaf area, as the largest leaf area was in the 0.5 ml/L concentration treatment, which was 10.85 cm, and the smallest leaf area was in the 1.5 ml/L concentration treatment, which was 7.66 cm. As for the proline spraying treatment, the results show that the 0.5 ml/L concentration treatment achieved the highest average leaf area, which was 14.43 cm, while the 0 ml/L concentration treatment, which was without spraying, recorded the lowest average leaf area, which was 8.03 cm, while the leaf area in healthy plants in the control treatment was 11.25 cm.

The above results confirm the positive role of salicylic acid and proline in reducing the incidence of tomato plants with early blight disease caused by the pathogenic fungus *Alternaria alternata*, through their direct effect on the growth of the fungus, as shown in the results in Table 2 and Figure 3. The decrease in the incidence of the disease is also explained by the ability of both salicylic acid and proline to induce resistance in the plant by increasing the concentration of secondary metabolites or active compounds, in addition to increasing the concentration of antioxidant enzymes and proteins associated with pathogenesis. Consequently, the symptoms of the disease decreased in the treated plants [28] , [29] , [30] , [31], [32]

Table 2: The effect of treatment with salicylic acid and proline in inhibiting the growth of pathogenic fungi Alternaria alternata.

Concentrations	Salicylic acid		Proline	
	Radial growth (mm)	Percent of inhibition (%)	Radial growth (mm)	Percent of inhibition (%)
0	75.00	-------	75.00	-------
0.5	0.0	100	68.45	8.73
1	0.0	100	59.56	20.59
1.5	0.0	100	46.22	38.37
2	0.0	100	28.00	62.67
2.5	0.0	100	19.12	74.51

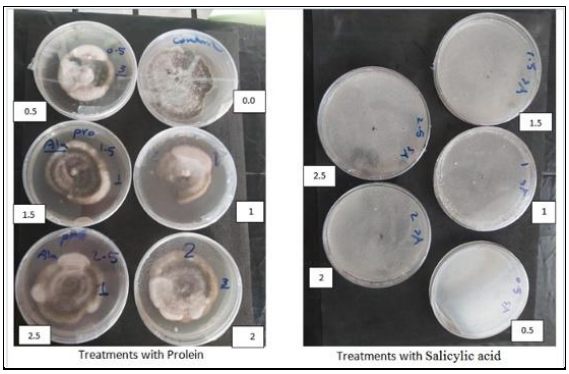


Figure 3: Effect of treatment with Salicylic acid (A) and Proline (B) in the growth of pathogenic fungi Alternaria alternata on SDA media.

Table 3: Effect of treatment with Salicylic acid and Proline on the incidence rate (%) of early blight disease on tomato plants caused by the fungus Alternaria alternata.

Concentrations	Incidence rate %	
	Salicylic acid	Proline
0	44.11 a	44.11 a
0.5	6.66 c	16.66 b
1	3.92 c	20.89 b
1.5	3.70 c	19.35 b
2	1.81 c	17.94 b
2.5	1.96 c	23.43 b

Note: Similar letters indicate that there are no significant differences between the means , at a probability level of 0.05. Note that the probability value p-values ≤ 0.00.

Table 4 : Effect of treatment with salicylic acid and proline on the number of leaves of tomato plants growing under biotic stress by the pathogenic fungus *Alternaria alternata* .

Concentrations	Leaves number	
	Salicylic acid	Proline
0	13.6 b	13.6 b
0.5	12 cd	13.2 bc
1	10.2 d	13.4 b
1.5	10.8 cd	15.5 a
2	11 cd	15.6 a
2.5	10.2 d	12.8
Control	15.5 a	

Note: Similar letters indicate that there are no significant differences between the means , at a probability level of 0.05. Note that the probability value p-values ≤ 0.00.

Table 5: Effect of treatment with salicylic acid and proline on leaf area (cm2) of tomato plants growing under biotic stress by the pathogenic fungus *Alternaria alternata* .

Concentrations	leaf area (cm^2)	
	Salicylic acid	Proline
0	8.03 e	8.03 e
0.5	10.85 bc	14.43 a
1	8.07 e	11.75 b
1.5	7.66 e	10.76 bc
2	9.76 cd	10.06 bcd
2.5	8.84 de	11.10 bc
Control	11.25 bc	

Note: Similar letters indicate that there are no significant differences between the means, at a probability level of 0.05. Note that the probability value p-values ≤ 0.00.

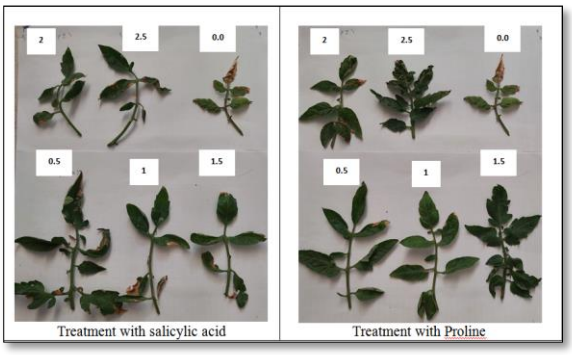


Figure 4: The effect of treatment with salicylic acid and proline on disease symptoms in tomato plants infected with the fungus *Alternaria alternata*.

4 CONCLUSIONS

The results of the current study show that the isolates of the pathogenic fungus causing early blight on tomato plants were highly virulent, as the seven isolated strains had a strong effect in inhibiting the growth of tomato seedlings. The results of the induction factors used in the study, including salicylic acid and proline, also indicated that they had an inhibitory effect on the growth of the pathogenic fungus in the laboratory and also led to the inhibition of the disease infection on tomato plants grown in greenhouse conditions. The results showed that the effect of salicylic acid on disease inhibition increased with increasing concentration. Therefore, the results of this study are considered promising results for combating plant diseases without resorting to the use of chemical pesticides that have harmful environmental and biological effects, which supports the goals of sustainable development and environmental conservation.

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Green-Synthesized Calcium Oxide Nanoparticles for Adsorption, Antibacterial Applications, and Alleviating Water Stress in Fenugreek

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Keywords: Green Synthesis, Caonps, Antibacterial, Adsorbent, Fenugreek.

Abstract: In this research, a novel synthesis of CaONPs has been developed via an environmentally friendly, green method. Garlic extract (Allium sativum) was used as a green-reducing and stabilizing agent for CaONPs. The average particle size of CaONPs was approximately 24.42 nm. The synthesized CaONPs were identified by using Fourier transform infrared (FT-IR) spectroscopy, U.V.-vis spectrum, X-ray diffraction (XRD), Field Emission-Scanning Electron Microscopy (FE-SEM), Transmission Electron Microscopy, transmission electron microscopy (TEM), Energy Dispersive X-ray spectroscopy (EDX), Atomic Force Microscopy (AFM), and zeta potential (Zp) analysis. The current study highlights the notable applications for CaONPs. First, an antimicrobial assay revealed a high antibacterial and antifungal activity, with the maximum zone of inhibition observed at different concentrations of CaONPs. Secondly, adsorbent for CaONPs in an aqueous solution containing M^{+2} ions (Co, Ni, Cu). The removal percentages (R%) were Co (II) 93.47%, Ni (II) 87.58%, and Cu (II) 88.53%. Thirdly, the study of fenugreek under water stress revealed that CaO-NPs positively enhance water stress tolerance.

1 INTRODUCTION

Green synthesis protocols used for synthesizing (MONPs) have become recently drawn the attention of researchers due to their lower cost, greater credibility, and simplicity [1], [2]. CaONPs are safe, non-toxic, environmentally friendly, and have a high reputation across all elements because of their stability [3]. Different sizes of CaONPs have been synthesized using plant materials such as the leaves of Trigon sp., which yielded nanoparticles with an average size of 51.64 nm [4]. Piper nigrum with 47.08 nm [5], Moringa oleifera with 32.08 nm [6], and Pistacia Atlantica with sizes ranging from 40-130 nm [7].

Nanotechnology has demonstrated the potent antibacterial capabilities of metal oxide nanoparticles (MONPs) [8]. Similarity investigations have illustrated the antibacterial activities of metal oxide nanoparticles [9], [10], [11]. Nanoparticles are commonly employed in wastewater treatment due to their large surface area and small size, which confer strong adsorption reactivity and capacity [12], [13]. Moreover, MONPs have been extensively utilized in agriculture to mitigate water stress, playing crucial roles in signaling pathways, defense metabolism, and regulatory activities [14], [15]. In this study, we established protocols for the green synthesis of CaONPs using garlic extract. The synthesized CaONPs were evaluated for their applications, including their efficiency as adsorbents for removing Co (II), Ni (II), and Cu (II) from aqueous solutions, and their effect on antibacterial activity. Additionally, we investigated the impact of CaONPs on enhancing fenugreek's (Trigonella foenum-graecum L.) resistance to water stress.

The use of plant-based extracts for the synthesis of nanoparticles is an emerging area of interest due to its sustainable and eco-friendly approach. Garlic extract, in particular, has been recognized for its rich bioactive compounds, which can act as reducing and stabilizing agents in nanoparticle synthesis [16]. This method not only reduces the need for hazardous chemicals but also aligns with the principles of green chemistry. Furthermore, the adsorption properties of CaONPs were analyzed to determine their effectiveness in removing heavy metal ions from wastewater.

This application is particularly relative given the growing concerns about water pollution and the need for efficient and cost-effective treatment methods. The antimicrobial properties of CaONPs were also examined, providing insights into their potential use in medical and agricultural settings. Finally, the study explored the role of CaONPs in enhancing the water stress tolerance of fenugreek plants. This aspect is crucial for developing strategies to improve crop resilience under drought conditions, which is a significant challenge in agriculture today. By integrating nanotechnology with botany, this research contributes to the development of innovative solutions for sustainable agriculture and environmental management.

2 MATERIALS TECHNIQUES AND METHODS

2.1 Sample Collection

Deionized water (DI) was obtained from the Chemical Lab, College of Education for Pure Science / Ibn Al-Haitham - University of Baghdad. Cobalt sulphate $CoSO_4$, copper sulphate pentahydrate $CuSO_4 \cdot 5H_2O$, nickel sulphate heptahydrate $NiSO_4 \cdot 7H_2O$, calcium sulphate $CaSO_4$, and sodium hydroxide NaOH were purchased from Merck, Germany. All chemicals were used without further purification. Garlic was sourced from the local market in Baghdad, Iraq. Fresh garlic bulbs were cleaned with water to get rid of dust, peels, and other contaminants, then dried at 37 ^oC and grated to facilitate extraction. A 500 mL beaker was filled with 20 g of garlic and 200 mL of deionized water. The mixture was heated to 80 ^oC with continuous stirring for 30 min. After cooling to room temperature, the yellowish mixture was filtered to remove impurities. The filtrate was then centrifuged at 4000 rpm for 15 min to eliminate any remaining fine suspended materials.

2.2 CaONPs Preparation by Garlic Extract with CaSO4

Calcium Sulphate $CaSO_4$ 4g was dissolved in 100 mL of deionized water at a concentration of 0.1M in a beaker with a volume of 500 mL, to which 100 mL of garlic fruit extract that had previously been prepared gradually at a temperature of 90 ^oC was added with continuous stirring, gradually add drop by drop a solution of sodium hydroxide with a concentration of 1M through the dissolution of 2 g of NaOH in 50 mL of deionized water until the pH reaches 12. The solution was left for 48 h, to form a light brown precipitate. The precipitate was separated using a centrifuge (4000 – 4500 rpm), then filtered and washed with deionized water and hot ethanol. after that it was dried by using an electric oven at a temperature of 300 ^oC for 15h, leaving a white powder as shown in Figure 1.

2.3 Instrumentation Techniques

An electronic balance (A 220/C/1 model), PLC centrifuge (4000 – 4500 rpm, FAITHFUL), electric oven, water bath with shaking (SCL F), pH tape, and a UV-Vis spectrophotometer (160/UV, Shimadzu) were utilized in this study. The FT-IR studies were conducted using a Shimadzu 8500S spectrometer in the range of 400 – 4000 cm-1 at Baghdad University. (XRD) analysis was performed using a Holland/Phillips system at the laboratory center of Baghdad. (FE-SEM) was carried out using a 300 Hv system (Germany-Z. S). Energy (EDX) and (TEM) (EMIOC-100Kv, Germany) were conducted at Kashan University, Iran. (AFM) (Nano surf AG. Liestal, Switzerland) was performed at the College of Science/ University of Baghdad, and Zeta potential measurements (14011201, Mahamax – 45184- Zeta-03 nzt) were also conducted at Kashan University, Iran. Two reference bacteria, Staphylococcus aureus (G+) and Escherichia coli (G-), were used to examine the synthesized CaONPs, as well as the fungal species Candida albicans, using the diffusion method and nutrient media, including Mueller Hinton Agar (MHA) and Potato Dextrose Agar (PDA).

2.4 Types of Bacteria and Fungi

Two strains of bacteria and one fungal species were used in the studies: Gram-negative Escherichia coli, Gram-positive Staphylococcus aureus, and the fungal species Candida albicans. The disc diffusion method was performed using nutrient media, specifically, Mueller Hinton Agar (MHA) for bacterial studies, and the same method was applied

for antifungal activity. The CaONPs were prepared using garlic extract and tested at different concentrations, ranging from 2 to 0.25 mg/mL.

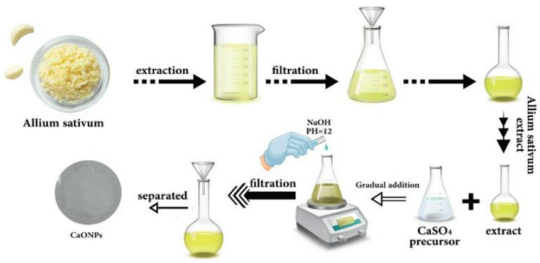


Figure 1: Preparation steps of CaONPs using garlic extract.

2.5 Adsorption Studies

In this study, the effect of metal ions (II) such as Co, Ni, and Cu on CaONPs showed promising results. $CoSO_4$, $NiSO_4 \cdot 7H2O$, and $CuSO_4 \cdot 5H_2O$ were used as the sources of these ions, each at a concentration of 12.5 mg/L. In each experiment, 0.1 g of CaONPs was mixed with 12.5 mg/L of each metal ion in a tube and agitated using a shaker water bath at 35 ºC with a rate of 120 rpm and pH 6. The initial concentrations of ($M^{(II)}$: Co, Ni, and Cu) were taken from synthetic metal ion solutions. After adsorption, the samples were filtered by centrifugation, and the liquid was analyzed for these metal ions using a UV-visible spectrophotometer.

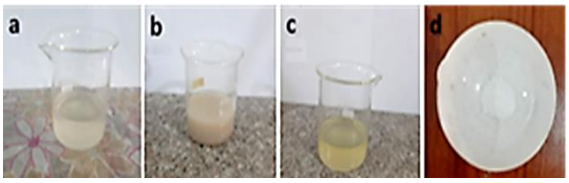


Figure 2: Color change solutions of CaSO4 and bio-redactor of Garlic (Allium Sativum) extract a) Turbid, b) Milky, c) Pale yellow, and d) CaONPs powder.

The experimental design layout was applied in the execution of each experiment. The formula was used to calculate the adsorbent's removal efficiency, or

$$R\%. [17, 18]. R\% = \frac{100 (Co - Ct)}{Co}. \quad (1)$$

Where Co and Ct (mg /L) are the initial concentration and concentration at time 10-80 min. R: represents the percentage of adsorption of nickel, copper, and cobalt ions.

2.6 Pots Experiment

The experiment was performed as a factorial experiment in a plastic house belonging to the Biology Department, College of Science, Al-Mustansiriyah University. The study aimed to reduce the adverse effects of water stress, represented by three irrigation periods, on the fenugreek crop using different concentrations of calcium oxide nanoparticles (CaONPs). Ten seeds were germinated in plastic pots containing 5 kg of soil, and the harvest was taken 70 days after planting to study vegetative growth, yield, and some antioxidant concentrations. The treatments included T1 (5-day irrigation period), T2 (10-day irrigation period), T3 (15-day irrigation period), T4 (T1 + 50 mg/L CaONPs), T5 (T1 + 100 mg/L CaONPs), T6 (T2 + 50 mg/L CaONPs), T7 (T2 + 100 mg/L CaONPs), T8 (T3 + 50 mg/L CaO NPs), and T9 (T3 + 100 mg/L CaONPs).

The parameters studied were plant weight (cm), dry weight (g), number of pods, seed weight (g), proline concentration (µg/g) [19] salicylic acid (SA) concentration (µg/mL) [20], ascorbic acid (ASA) concentration (mg/100g) [21], and α-tocopherol (α-TOC) concentration (µg/g) [22]. Three replicates were used in a completely randomized design (CRD), and the least significant difference (LSD) test was utilized to statistically evaluate the data at a 5% probability level [23].

3 RESULT AND DISCUSSION

An aqueous extract of garlic was used with CaSO4. The changing color of the solutions is shown in Figure 2. Initially, the solution was turbid Figure 2a, which then turned milky Figure 2b, and subsequently became pale yellow Figure 2c. The final product was a white powder of CaONPs Figure 2d.

3.1 FT-IR Spectroscopy

FT-IR was used to identify CaONPs as shown in Figure 3. Peaks located at 540.07, 497.64, and 466.78 cm^{-1}, confirming the formation of CaONPs [6]. Peaks were observed at 659.66 and 567.08 cm-1 for A. sativum crude extract [24].

3.2 UV-Visible Absorption Spectroscopy Analysis

UV-vis spectrum confirmed the presence of CaONps as shown in Figure 4 at 248nm. The following

equation was used to determine the energy gap band. Eg = 1239.83 / λ. Lambadi peak absorption is represented λ, and the bulk band, Eg, is given in eV as follows: Eg = 1239.83 / 248 = 4.99 eV [25], [26].

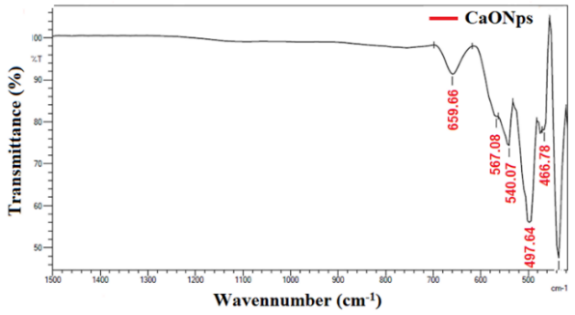


Figure 3: FT – IR spectrum of CaONPs.

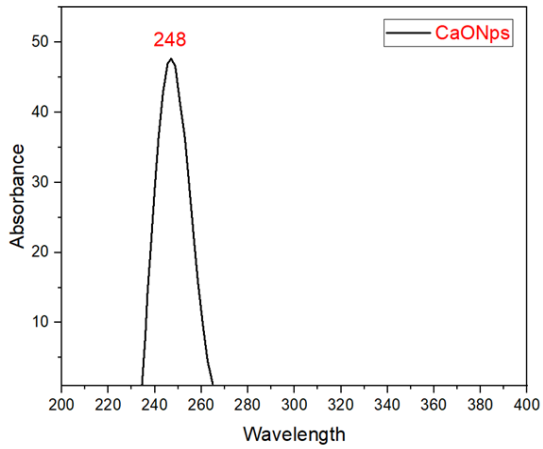


Figure 4: UV-vis spectrum of CaONPs.

3.3 X-Ray Diffraction

Further proof of the product CaONPs over the diffraction angle (2θ) range of 20° - 80°. Figure 5 shows the peaks observed at 2θ = 38.74° (111), 42.99° (200), 63.12° (220), 75.32° (311), and 80° (222). The average size (D) of the synthesized CaONPs was determined to be 24.42 nm by applying the Scherrer equation to measure the crystallite size. The polycrystalline and monophasic cubic structure of CaONPs, as character by the XRD pattern, is revealed by the sharp peaks. The crystal structures and the provided JCPDS data (JCPDS powder diffraction data card No. 77-2376) are in good agreement. Miller indices are shown in Table 1. The diffraction peaks' line broadening suggests that the CaONps are in the nanometer range. The products had good crystallinity, as evidenced by the distinctive peaks' lower spectral width and higher intensity. The final results of synthesized products are high-quality CaONps, as indicated by the absence of peaks related to contaminants [27].

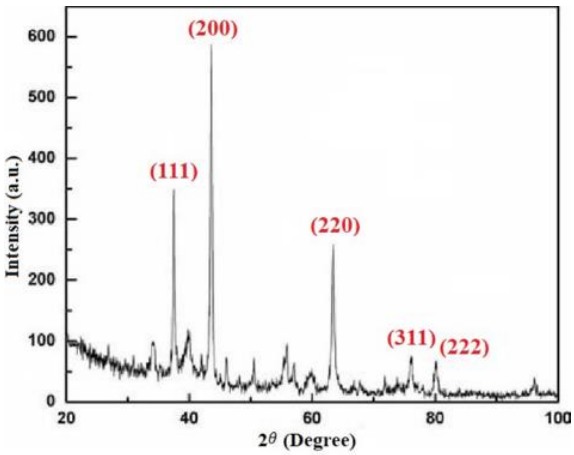


Figure 5: XRD pattern of CaONPs.

Table 1: Summary of XRD data analysis for CaONPs.

Pos. [°2θ]	Height [cts]	FWH-M [°2θ]	d-spacing [Å]	Tip width [°2θ]	Matched by
38.74	644.9	0.29	2.32	0.35	29.81
42.49	712.7	0.34	2.12	0.41	25.86
63.12	210.4	0.39	1.47	0.47	24.75
75.32	250.5	0.39	1.26	0.47	26.65
79.94	208.4	0.72	1.23	0.86	15.05

3.4 Morphological Study

FE-SEM and TEM images are shown in Figure 6, and Figure 7, respectively. In the FE-SEM images of CaONps, a variety of shapes such as tubes, cubes, and flowers, with several spaces between nanoparticles were observed. The mean size of the particles is 24.20 nm. This represents the size and morphology of the synthesized CaONPs [28].

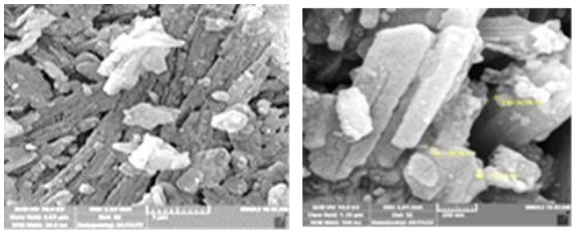


Figure 6: FE-SEM image of CaONPs.

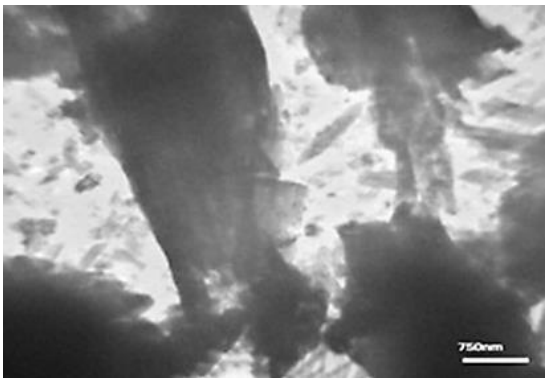


Figure 7: TEM image of CaONPs.

3.5 Energy Dispersive X-Ray Spectroscopy

In general, EDX is used to identify the chemical composition, as shown in Figure 8. The EDX spectrum of CaONPs showed a strong peak for calcium and a medium peak for oxygen, proving that there are no observable impurities in the calcium, which indicates it is chemically pure. The presence of Au is also confirmed by the EDX, which may have resulted from the usage of the standard and sample preparation for SEM-EDX analysis. [29].

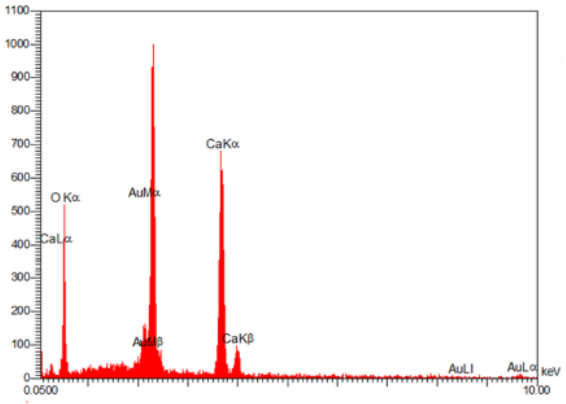


Figure 8: EDX composition spectrum of CaONPs.

3.6 Atomic Force Microscopy

The AFM offers visualization and analysis in three dimensions, revealing that the nanoparticles' size is in good identical to the data obtained from FE-SEM and TEM. The AFM histogram and statistical particle analysis of CaONPs powder with topography parameters, and 3D AFM images of the nanoparticles indicating the pyramid-like grains are presented in (Figure 9, and Figure10) [30] .

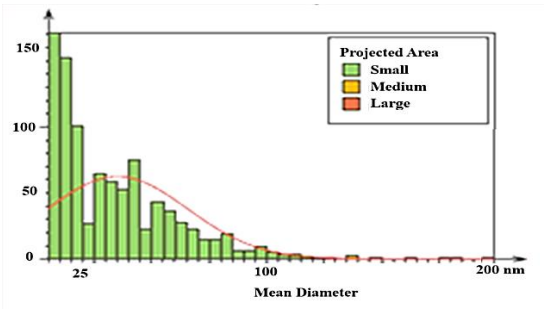


Figure 9: AFM histogram and statistical particle analysis of CaONPs powder.

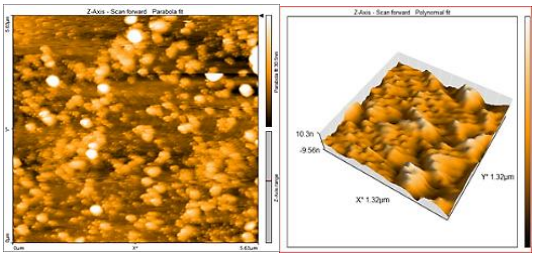


Figure 10: 3D AFM surface structure images of CaONPs.

3.7 Zeta Potential of CaONPs

Figure 11, depicts, the Zeta potential of CaONPs, with a value of -20.5 mV ± 2.3 mV. The high level of stability values of the CaONPs produced are highly charged particles, indicated by the high zeta potential values, which in turn prevents aggregation and agglomeration due to their large repulsion force [31].

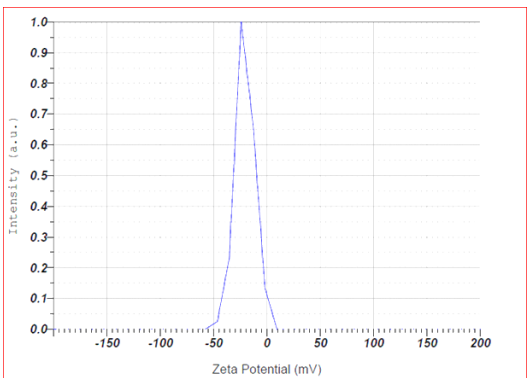


Figure 11: Zeta potential values of CaONPs.

3.8 Antibacterial Study

Using the agar well diffusion method, the antibacterial effectiveness of CaONPs was assessed against Escherichia coli, Staphylococcus aureus, and the fungal species Candida albicans. The outcomes

show that the HCl dilution negative control did not exhibit an inhibition zone, indicating that the control in its natural state, devoid of nanoparticles, exhibited no antibacterial activity. When the concentrations of the CaONPs sample (A) were 2, 1, 0.5, and 0.25 mg/mL, it was found out that at all these concentrations, the CaO nanoparticles caused a growth delay in all microorganisms. Higher antibacterial activity was observed in the range of 35 – 39 mm at different concentrations from 2 to 0.25 mg/ml [32]. All these results and the diameters of the inhibition zones are represented in Table 2.

Table 2: Shows the diameters of the inhibition zones in mm of CaONPs sample in different concentrations.

CaONPs Sample	D1	D2	D3	D4
Concentration	2mg/mL	1mg/mL	0.5mg/mL	0.25mg/mL
Micro organisms' type	Diameter of inhibition zone (mm)			
S.auras	39	37	38	35
E. Coli	35	32	31	28
C. albicans	25	22	19	17

3.9 Adsorption Study

According to the results, CaONPs demonstrated effective removal of various divalent metal ions, specifically cobalt, nickel, and copper, from a solution of distilled water, as shown in Figure 12. The percentage of ions removed by CaONPs decreased from 95.90%, 87.33%, and 89.02% after 10 min to 91.86%, 86.92%, and 88.40%, respectively, indicating the number of active sites available on CaONPs. During the first 80 min of the adsorption process, the sites on CaONPs gradually became saturated. The removal efficiencies (R%) for the divalent metal ions of cobalt, nickel, and copper were higher, with values of 93.47%, 87.58%, and 88.53%, respectively [33].

3.10 Study of The Impact of Water Stress on The Growth of Fenugreek at Current CaONPs Concentrations

The results in Table 3, shows a significant effect of (irrigation periods) and water stress on reduced plant height, dry weight, number of pods, and seed weight. When spacing irrigation periods from 5 days (T1) to 15 days (T3), these parameters decreased by 27.95%, 55.61%, 59.26%, and 37.67%, respectively. Furthermore, CaONP spraying mitigated the harmful effects of water stress by increasing the vegetative growth parameters and yield contents mentioned above. Specifically, in the T3 treatment, the usage of (50 mg/L of CaONPs) (T8) resulted in significant increases of 15.60%, 62.63%, 63.64%, and 28.76%, respectively. Additionally, the usage of (100 mg/L of CaONPs) (T9) rendered in significant increases of 23.93%, 68.69%, 54.55%, and 37.73%, respectively.

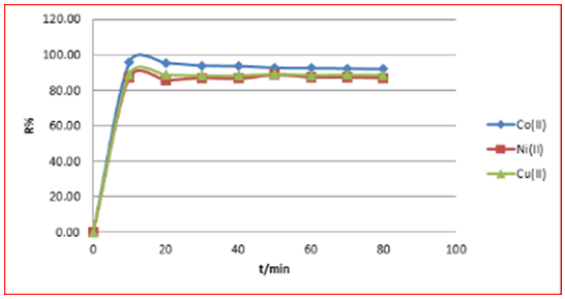


Figure 12: Represented of adsorption of cobalt, nickel, and copper metal ions with CaONPs, garlic extract, and calcium sulphate.

Table 3: Effect of water stress and CaONPs on vegetative growth and yield of fenugreek.

Treat.	Plant height (cm)	Dry weight (g)	Pods No.(pod)	Seeds weight (g)
T1	38.97	2.23	13.50	6.08
T2	30.62	1.47	7.50	4.28
T3	28.08	0.99	5.50	3.79
T4	41.56	2.45	15.50	6.40
T5	43.94	2.68	16.50	6.79
T6	36.98	1.84	9.50	5.79
T7	37.26	2.15	12.00	5.92
T8	32.46	1.51	9.00	4.88
T9	34.80	1.67	8.50	5.22
LSD (0.05)	1.70	0.27	1.63	0.18

The results in Table 4, showed an increase in antioxidant concentration with increasing water stress. The T3 treatment yielded high values of proline, salicylic acid, ascorbic acid, and α-tocopherol, with rates of 172.47%, 161.75%, 62.44%, and 64.85%, respectively, compared to the T1 treatment (control). When CaONPs were sprayed, there was a significant decrease in the average antioxidant concentrations mentioned above, especially in the T3 treatment. The usage of (50 mg/L of CaO NPs) (T8) resulted in significant

decreases of 35.87%, 44.14%, 16.69%, and 17.87%, respectively. Similarly, the usage of (100 mg/L of CaONPs) (T9) resulted in significant decreases of 50.97%, 53.88%, 22.34%, and 35.59%, respectively.

Table 4: Effect of water stress and CaONPs on antioxidants.

Treat.	Proline (µg.g-1)	Salicylic acid (µg.ml-1)	Ascorbic acid (mg.100g-1)	α-tocopherol (µg.g-1)
T1	3.96	2.51	55.75	42.10
T2	8.41	4.35	80.28	60.50
T3	10.79	6.57	90.56	69.40
T4	2.19	1.47	50.29	37.90
T5	1.98	1.20	42.52	34.20
T6	4.98	2.75	64.53	49.70
T7	3.19	2.52	62.47	44.40
T8	6.92	3.67	75.45	57.00
T9	5.29	3.03	70.33	44.70
LSD (0.05)	0.77	0.66	3.45	9.92

Drought is an environmental factor that decreases plant growth and yield. Many studies have shown that drought negatively affects morphological growth (plant height, leaf area, root development, branch number, and biomass accumulation), metabolic processes (photosynthesis and respiration), and productivity [34]. The reduction in morphological and physiological characteristics and yield may be due to a decrease in water content in plant tissues, reduced leaf area and chlorophyll concentration, and ultimately a decreased photosynthetic rate and protein synthesis [35]. Furthermore, drought can alter the plant's CO_2 metabolism by decreasing Rubisco inactivation, RuBP regeneration, and stomata open system [36]. Recent studies have shown that water deficits in plant tissues reduce yield by affecting flowering time and causing pollen grain sterility [37]. In addition, water stress increases reactive oxygen species (ROS) and accumulates proline in cells [38].

Plants respond by producing secondary compounds like phenols and stimulating enzymatic and non-enzymatic antioxidants to scavenge ROS [39]. Nanoparticles (NPs) enhance the effectiveness of the antioxidant system in different plants by reducing ROS in plant tissues or increasing the concentration of photosynthetic pigments (anthocyanins and β-carotene) to improve growth and yield. Moreover, NPs can alter metabolism beyond the physiological and biochemical levels, potentially inducing the gene expression of antioxidant biosynthesis [40].

In this research, CaONPs played a positive role in water stress tolerance by enhancing morphological growth, resulting in greater plant height, dry weight, and improved yield content. Foliar application of CaO ameliorated the harmful effects of drought by enhancing proline accumulation and reducing antioxidant concentrations (SA, ASA, and α-TOC). Additionally, CaONPs positively affected plant growth at morphological, physiological, biochemical, and molecular levels, thereby increasing drought stress tolerance [41].

4 CONCLUSIONS

Allium sativum (garlic extract) has been successfully used to synthesize CaONps via the biosynthesis method. FT-IR and UV-visible spectra confirmed the presence of CaO bonds, with a peak centered at 245 nm and an energy gap (Eg) of 4.99 eV. XRD analysis of CaONPs shows a monophasic cubic structure with an average crystallite size of 24.42 nm. FE-SEM and TEM images reveal a variety of shapes, such as tubes, cubes, and flowers. EDX peaks indicate the presence of Ca and O peaks. AFM provides 3D images of nanoparticles, showing pyramid-like grains indicative of the presence of CaONPs. The Zeta potential value of -20.5 mV indicates highly charged particles.

CaONPs demonstrated antimicrobial activity, with inhibition zones ranging from 25 to 39 mm, indicating strong inhibition against the growth of *S. aureus*, *E. coli*, and the fungal species *Candida albicans.* CaONPs effectively removed Co (II), Ni (II), and Cu (II) from aqueous solutions, with yields of 95.90%, 87.33%, and 89.02% after 10 min, and 91.86%, 86.92%, and 88.40% after 80 min, respectively. The adsorption efficiencies (R%) were 93.47%, 87.58%, and 88.53%, respectively. The study of water stress in fenugreek (Trigonella foenum-graecum L.) shows that CaONPs have positive growth-promoting effects on the growth of fenugreek.

ACKNOWLEDGMENTS

To University of Ashur / College of Health and Medical Technology, and College of Education of Pure Science, Ibn- Al Haitham / Department of Chemistry- University of Baghdad / Iraq. For support.

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Study Effect of Biochare, Earthworms and Mycorrhizal Fungi on Cauliflower Plant Growth and Some Soil Characters

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Keywords: Mycorrhizal, Biochare, Earthworms, Cauliflower, Growth.

Abstract: The study aimed to determine the effect of different concentrations of biochare and there interaction with the mycorrhizal fungus Funneliformis mosseae and Earthworms Eisenia Fetida on growth of Cauliflower plant Brassica oleracea var. botrytis L. plant cultivated in plastic pots. The results of the study showed that there were changes in the properties of the studied soil, including organic matter, electrical conductivity, and the soil content of available phosphorus and potassium. However, the soil texture did not undergo any change, in addition to a decrease in the soil acidity compared to the control treatment. As for the effect of the presence of earthworms and biochare on mycorrhizal fungi, the results showed that the percentage of infection of cauliflower roots with mycorrhizal fungi increased, ranging from 60% to 100% in the interaction treatments compared to the mycorrhizal fungi only treatment, which was 20%. As for the severity of root infection with mycorrhizal fungi, it decreased in the interaction treatments of mycorrhizal fungi with earthworms and biochare , where it ranged from 23.33% to 42%, with the exception of the interaction treatment of mycorrhizal fungi with 2 gm of biochare, which was 56.66%, while the mycorrhizal fungi treatment was 50%. The results also showed that all treatments increased vegetative growth characteristics of cauliflower, including plant height, stem diameter, number of leaves, leaf area, and fresh and dry vegetative weight, as well as root growth characteristics, including root length and fresh and dry weight of the root system, compared to the control treatment without fertilizer. The results of this study contribute to environmental sustainability and soil conservation by reducing the use of chemicals as fertilizers.

1 INTRODUCTION

Cauliflower plant Brassica oleracea var. botrytis L., is an important winter vegetable crop it belongs to the Brassicaceae (cruciferae) family, which includes other crops such as cabbage and broccoli, The name cauliflower comes from the Latin word "cauliss," meaning stem, and "floris," meaning flower [1]. Cauliflower is an important food source rich in sulfur, which occurs in the form of compounds known as glucosinolates (GLS), GLS is one of the plant's defensive metabolic compounds and is responsible for its taste and flavor [2]. Eating vegetables rich in glucosinolates reduces the risk of cancer [3]. Several studies have indicated that eating cauliflower contributes to the prevention of metabolic disorders, asthma, and Alzheimer's disease, reduces the incidence of type 2 diabetes [4], and also reduces the risk of heart disease [5].

The extensive use of fertilizers over the past decades has significantly increased global food production capacity. However, in recent years, numerous studies have highlighted the inefficiency and unevenness of fertilizer use across countries, leading to environmental problems, soil nutrient imbalances, and suboptimal food production [6]. One alternative to chemical fertilization is the use of biofertilizers such as fungi, especially arbuscular mycorrhizal fungi represent a sustainable solution for enhancing plant growth because they form symbiotic relationships with many plants, They also contribute to plant growth by increasing the availability of nutrients in the soil and making plants more tolerant to abiotic stresses such as drought, salinity, heavy metals in the soil, and temperature changes and increase the plant's ability to withstand biotic stresses such as inducing plant resistance to

pathogens and competing with pathogens in the rhizosphere [7], [8].

Biochare is one of the sustainable development goals because it contributes to the removal of carbon dioxide from the atmosphere by stabilizing atmospheric carbon. Therefore, it plays a role in reducing greenhouse gas emissions [9]. Biochare production also plays an important role in reducing waste. Many farmers have begun producing biochare from agricultural waste, which studies have shown contributes to improving soil properties, increasing organic matter, and increasing nutrient availability. It also enhances the growth of the soil's biological community, thus improving plant growth [10], [11], [12]. Earthworms are among the largest organisms that inhabit agricultural soils. They are among the largest animal groups in the soil and play a fundamental role as bioindicators for intensive agriculture [13]. They are of great importance in improving the physical, chemical, and biological properties of soil, as their presence accelerates the decomposition of soil pollutants, reduces heavy metal activity, and enhances crop growth [14], [15].
Due to the paucity of studies on the cauliflower crop Brassica oleracea var. Botrytis and the fertilization factors mentioned above. An experiment was carried out in plastic pots under greenhouse conditions with the aim of studying the effect of biofertilization with the mycorrhizal fungus Funneliformis mosseae and adding biochare and earthworms Eisenia fetida and the interaction between them in Some physical and chemical soil characteristics before and after planting, Mycorrhizal characteristics of cauliflower, and Vegetative and root growth characteristics of cauliflower plant.

2 MATERIALS AND METHODS

In order to evaluate the effect of treatment with mycorrhizal fungus Funneliformis mosseae, adding biochare, earthworms Eisenia fetida and their interaction on the growth of cauliflower (Brassica Oleracea var. botrytis), an experiment was carried out in plastic pots during the autumn of the 2024-2025 agricultural season in the greenhouse of the government nursery of the Directorate of Agriculture in Baqubah - Diyala Governorate. The area of the greenhouse was 50 m2 (length 10 m and width 5 m), and the land was leveled and weeds were removed to prepare it for the implementation of the experiment. The experiment was carried out according to the following steps:

2.1 Preparing Cauliflower Seedlings

Cauliflower seeds, Hybrid variety, were obtained from the local market in Baqubah, Diyala Governorate, For the purpose of growing cauliflower seedlings, plastic dishes were used, filled with peat moss sterilized by an autoclave at a temperature of 121°C and a pressure of 15 pounds. Inch2 for one hour for two consecutive days.The seeds were planted in them after being superficially sterilized with a 1% sodium hypochlorite solution, at a rate of one seed per hole. Planting took place on 9/10/2024 until they were transferred to plastic pots on 10/10/2024, with the seedlings being 30 days old.

2.2 Mycorrhizal Fungus Inoculum

Funneliformis mosseae mycorrhizal fungus was obtained from the Agricultural Research Department in Zafaraniya, affiliated with the Ministry of Science and Technology. The fungal inoculation consists of soil containing fungal spores and pieces of white corn roots infected with the mycorrhizal fungus. The mycorrhizal fungus was added to the soil at a rate.

2.3 Earthworms Inoculum

Earthworms Eisenia Fetida, they were obtained from the Khalis cow station of the National Company, northwest of Baqubah - Diyala Governorate, which was an organic soil containing immature worms, noting that every 100 grams of soil contains 100 worms of small and medium sizes. Earthworms were added at a rate of 20 worm to each plastic pot. Figure 1 shows the earthworms added to the soil.



Figure 1: Organic soil containing earthworms Eisenia fetida indicated in yellow.

2.4 Biochare

The biochare was obtained from the Mycology Laboratory at the College of Education for Pure Sciences, University of Diyala, which was prepared by one of the former graduate students from the

woody branches of the eucalyptus plant. Biochare was added at three concentrations 0, 1, and 2 gm.

2.5 Experiment Implementation

The experiment was conducted in a greenhouse at the government nursery affiliated with the Diyala Agriculture Directorate in Baqubah, Diyala Governorate. Plastic pots were filled with a mixture of soil and peat moss, 15 kg per pot, after placing a filter paper at the bottom of the pot. For the addition of earthworms, a piece of plastic clip was placed at the bottom of the pot to prevent earthworms from escaping from the pot holes. Cauliflower seedlings were then planted in plastic pots on October 10, 2024, when they reached 3 or 4 leaves, at a rate of 2 plants per pot. These were subsequently thinned to one plant per pot. The experiment included 12 treatments with three replicates, bringing the number of treatments and their replicates to 36 experimental units as shown in Table 1 shows the treatments included in the experiment. The experiment was completed on 25 December, 2024, and several measurements were taken, including.

Table 1: Experimental treatments.

Treatment code	Treatment details
Con	Control
1BCH	1 gm of Biochare
2BCH	2 gm of Biochare
MF	Mycorrhizal fungus
MF+1BCH	Mycorrhizal fungus+1 gm of Biochare
MF+2BCH	Mycorrhizal fungus +2 gm of Biochare
EW	Earthworms
EW+1BCH	Earthworms+1 gm of Biochare
EW+2BCH	Earthworms + 2gm of Biochare
MF+EW	Mycorrhizal fungus+ Earthworms
MF+EW+ 1BCH	Mycorrhizal fungus + Earthworms+1gm of Biochare
MF+EW+ 2BCH	Mycorrhizal fungus+Earthworms +2gm of Biochare

2.5.1 Physical and Chemical Properties of Soil

Two months after planting, soil samples were taken from each treatment from a depth of 20 cm. They were air-dried and placed in plastic bags, three samples for each treatment. The samples were sent to the soil analysis laboratory of the Soil and Water Division of the Agriculture Directorate in Baqubah, Diyala Governorate, to determine the physical and chemical properties of the soil after planting.

2.5.2 Mycorrhizal Characteristics

Mycorrhizal traits, including the percentage and severity of mycorrhizal fungal infection, were measured in the Mycorrhizal Laboratory at the Agricultural Research Department in Zafaraniya, affiliated with the Ministry of Science and Technology , The method of Phillips and Hayman [16] was followed to calculate the percentage of mycorrhizal fungal infection of roots. and the infection rate was calculated according to the equation mentioned in [17] . The severity of mycorrhizal infection was calculated according to the equation mentioned in [18].

2.5.3 Plant Growth Characteristics

Shoot growth parameters includes Plant height (cm), stem diameters, fresh and dry weight for shoot part, leaves number , leaf area [19]. The characteristics of root system growth included root length (cm) , The fresh and dry weight of root group.

2.6 Statistical Analysis

A factorial experiment was conducted using a Randomized Complete Block Design (R.C.B.D.). The results were statistically analyzed using SPSS. The differences between the means were compared using Duncan's multiple range test at a probability level of 0.05 also the percentage increase between treatments compared to the control treatment was calculated.

3 RESULTS

3.1 Physical and Chemical Properties of the Soil after Planting

The results in Table 2 showed that there was a change in the concentration of organic matter in the soil, and the highest concentration was in the treatment of interaction between mycorrhizal fungi, earthworms and 1 gm of biochare, which was 3.5% compared to the control treatment, which was 2.4%. The table also shows that the soil pH decreased in all treatments and ranged between 7.3 to 7.8, while the soil pH in the control treatment was 8.1. The results in the table also show a change in the degree of conductivity in the soil in all treatments, which ranged between 0.7 to 1.3 dsm^{-1} compared to the control treatment, which was 1.0 dsm^{-1}. As we find

Table 2: Physical and chemical properties of soil after and before planting.

Treatments	pH	Organic Matter (%)	Available phosphorus ($mg.kg^{-1}$)	Available potassium ($mg.kg^{-1}$)
Control	8.1	26	35.1	2.4
MF	7.6	27	29.9	2.8
EW	7.4	32	40.3	2.9
MF+EW	7.3	30	39.7	2.6
1BCH	7.8	32	22.8	3.2
2BCH	7.8	33	14.1	1.6
MF+1BCH	7.6	29	45.6	1.6
MF+2BCH	7.5	26	35.1	3.0
EW+1BCH	7.5	27	22.4	3.1
EW+2BCH	7.3	34	38.2	2.6
MF+EW+1BCH	7.5	38	40.6	3.5
MF+EW+2BCH	7.3	29	48.6	2.4

Table 3: Effect of Earthworms and Biochare on percentage and severity of Cauliflower roots infection with the mycorrhizal fungus.

Treatments	Percentage of infection, %	Severity of infection, %
Control	0	0
MF	20	50
MF+EW	100	33.33
MF+1BCH	60	42.5
MF+2BCH	71.4	56.66
MF+EW+1BCH	100	23.33
MF+EW+2BCH	100	25

from the results shown in Table 1, there was a change in the soil content of available phosphorus, and the interaction treatment between mycorrhizal fungi and earthworms and the addition of 2 grams of biochare achieved 48.6 $mg.kg^{-1}$ compared to the control treatment, which was 35.1 $mg.kg^{-1}$. As for the available potassium in the soil, the results show that the interaction treatment between mycorrhizal fungi and earthworms and the addition of 1 gram of biochare achieved the highest content of available potassium in the soil, which was 38 $mg.kg^{-1}$ compared to the control treatment, which was 26 $mg.kg^{-1}$.

3.2 Mycorrhizal Characteristics

The results of Table 3 indicate that there are differences between the treatments for the infection rate and infection severity trait, as the treatment of mycorrhizae and metabolite worms, the treatment of mycorrhizae and metabolite worms and biochare at a concentration of 1%, and the treatment of mycorrhizae and metabolite worms and biochare at a concentration of 2% were superior, giving the highest value of 100% in the infection rate compared to the treatment of adding mycorrhizae, which was 20%. Table 2 also indicates the superiority of the treatment of interaction between mycorrhizae and biochare at a concentration of 2%, as it achieved the highest value of 56.66% in infection severity compared to the treatment of adding mycorrhizae, which was 50%.

3.3 Shoot Growth Parameters of Cauliflower Plant

The results in Table 4 and Table 5 indicate an increase in the height of the cauliflower plant if the interaction treatment between mycorrhizal fungus and biochare at a concentration of 1% was superior, as it achieved the highest increase of 47 with an increase rate of 65.90% compared to the control treatment, which was 28.33. The table shows an increase in the fresh weight of the urban group without the flower, as the biochare treatment at a concentration of 2% was superior, as it achieved the highest increase of 33 - 523 with an increase rate of 223.72% compared to the control treatment, which was 161.66. The table also shows an increase in the dry weight of the vegetative group without the flower, as the interaction treatment between mycorrhizal fungus and biochare at a concentration of 1% was superior, as it recorded the highest increase of 43.33 with an increase rate of 282.43%

compared to the control treatment, which achieved 11.33. The table indicates an increase in the diameter of the stem when the interaction treatment between mycorrhizal diameter and biochare at a concentration of 1% was superior. 1% compared to the control treatment, which was 4.00%1, where it achieved the highest increase, which was 5.66, with an increase rate of 41.5. Table 5 shows an increase in the number of cauliflower leaves if the treatment with mycorrhizal fungi was superior, as it achieved the highest increase, which was 15.66, with an increase rate of 42.36% compared to the control treatment, which was 11. The table indicates an increase in the leaf area of the cauliflower plant when treated with metabolite worms and biochare at a concentration of 2%, as it recorded the highest increase, which was 259.06, with an increase rate of 193.98% compared to the control treatment, which recorded 88.12. The table shows an increase in the leaf area of the cauliflower plant if the interaction treatment between metabolite worms and biocarbon at a concentration of 2% was superior, as it achieved the highest increase, which was 3737.4, with an increase rate of 283.42% compared to the control treatment, which was 974.73. The table shows an increase in the content of Chlorophyll in cauliflower leaves, as the interaction treatment between worms and biochare at a concentration of 1% and the interaction treatment between worms and biochare at a concentration of 2% were superior, as they recorded 50.7, an increase of 31.92% compared to the control treatment, which was 38.43 .

Table 4: Effect of inoculation with Earthworms and mycorrhizal fungus and their interaction with biochare on Root growth for Cauliflower plant.

Treatments	Plant height, (cm)	Stem diameter, (mm)	Leaves number
Control	28.33 ^{d}	4.00 ^{c}	11.00 ^{e}
MF	44.00 ^{ab}	4.66 ^{bc}	15.66 ^{a}
EW	32.00 ^{cd}	4.00 ^{c}	13.00 ^{cd}
MF+EW	35.66 ^{cd}	3.83 ^{c}	13.33 ^{c}
1BCH	35.66 ^{cd}	4.66 ^{bc}	15.33 ^{ab}
2BCH	44.33 ^{ab}	4.66 ^{bc}	15.33 ^{ab}
MF+1BCH	47.00 ^{a}	5.66 ^{a}	15.33 ^{ab}
MF+2BCH	45.00 ^{ab}	5.00 ^{ab}	14.33 ^{abc}
EW+1BCH	35.00 ^{cd}	4.16 ^{bc}	14.00 ^{bc}
EW+2BCH	38.66 ^{bc}	4.33 ^{bc}	14.33 ^{abc}
MF+EW+1BCH	33.33 ^{cd}	3.83 ^{c}	12.00 ^{de}
MF+EW+2BCH	36.33 ^{cd}	4.00 ^{c}	12.00 ^{de}

Note: Similar letters indicate that there are no significant differences between the means , at a probability level of 0.05. Note that the probability value p-values ≤ 0.00.

Table 5: Effect of inoculation with Earthworms and mycorrhizal fungus and their interaction with biochare on Root growth for Cauliflower plant.

Treatments	Leaf area (cm^2)	Shoot fresh weight (gm)	Shoot dry weight (gm)
Control	88.12 ^{g}	133.33 ^{e}	11.33 ^{b}
MF	166.62 ^{cdef}	431.67 ^{a}	36.33 ^{a}
EW	175.37 ^{cde}	190.00 ^{cd}	17.67 ^{b}
MF+EW	214.49 ^{abc}	170.67 ^{de}	17.00 ^{b}
1BCH	119.24 ^{f}	256.67 ^{b}	18.67 ^{b}
2BCH	194.75^{bcde}	443.33 ^{a}	38.00 ^{a}
MF+1BCH	230.00 ^{ab}	425.00 ^{a}	43.33 ^{a}
MF+2BCH	226.68 ^{ab}	418.33 ^{a}	42.33 ^{a}
EW+1BCH	162.50 ^{def}	157.33 ^{de}	16.67 ^{b}
EW+2BCH	259.06 ^{a}	237.00 ^{bc}	16.67 ^{b}
MF+EW+1BCH	157.37 ^{ef}	139.33 ^{de}	14.00 ^{b}
MF+EW+2BCH	209.37^{abcd}	178.00 ^{de}	17.00 ^{b}

Note: Similar letters indicate that there are no significant differences between the means , at a probability level of 0.05. Note that the probability value p-values ≤ 0.00.

Table 6: Effect of inoculation with Earthworms and mycorrhizal fungus and their interaction with biochare on Root growth for Cauliflower plant.

Treatments	Root length (cm)	Root fresh weight (gm)	Root dry weight (gm)
Control	32.00 ^{f}	50.00 ^{f}	6.33 ^{e}
MF	41.00 ^{bcde}	116.66 ^{b}	15.00 ^{ab}
EW	33.66 ^{ef}	98.33 ^{bc}	10.66 ^{bcd}
MF+EW	36.00 ^{def}	45.33 ^{def}	11.66 ^{abc}
1BCH	45.33 ^{bc}	73.33 ^{cd}	11.33 ^{abc}
2BCH	43.33 ^{bcd}	115.00 ^{b}	15.66 ^{a}
MF+1BCH	48.00 ^{b}	106.66 ^{b}	12.66 ^{ab}
MF+2BCH	57.66 ^{a}	158.33 ^{a}	13.66 ^{ab}
EW+1BCH	32.00 ^{ef}	53.00 ^{def}	14.33 ^{ab}
EW+2BCH	33.66 ^{ef}	59.00 ^{de}	12.33 ^{abc}
MF+EW+1BCH	40.00 ^{cde}	26.66 ^{f}	7.00 ^{de}
MF+EW+2BCH	33.66 ^{ef}	35.00 ^{ef}	8.33 ^{cde}

Note: Similar letters indicate that there are no significant differences between the means , at a probability level of 0.05. Note that the probability value p-values ≤ 0.00

3.4 Root Growth Parameters of Cauliflower Plant

Table 6 shows an increase in root length in cauliflower, as the treatment that combined mycorrhizal fungi with 2% biochare achieved the highest increase of 57.66, representing an increase of 80.18% compared to the control treatment, which recorded 32.00. The table also indicates an increase in fresh root weight, as the treatment with mycorrhizal fungi outperformed, recording the highest increase of 116.66, representing an increase of 133.32% compared to the control treatment, which recorded 50.

4 DISCUSSION

The changes in soil properties two months after planting cauliflower, treatment with mycorrhizal fungi, earthworms, two concentrations of biochare, and their interaction confirm the direct impact of these factors on soil properties. These changes are expected to increase with the duration of their presence in the soil, particularly in the percentage of organic matter, which increased in the earthworm treatment as a result of the earthworms' biological activity in the soil [20]. Biochare also contributes to the incorporation of organic carbon into the soil [21], improving soil structure, promoting the formation of soil aggregates [22], and providing an ideal environment for soil microorganisms [23]. Mycorrhizal fungi (AM) play an important role in the formation and stabilization of soil organic matter (SOM) [24]. Soil additives also contribute to increasing the availability of phosphorus and potassium in the soil, as mycorrhizal fungi contribute to reducing nutrient loss in the soil and increasing its availability [25]. The results also show that soil texture did not change. This is attributed to the short duration of the experiment, as changing soil texture takes a long time.

The results also showed that the percentage of root infection by mycorrhizal fungi was positively affected by the presence of earthworms and biochare. Earthworms and arbuscular mycorrhizal fungi (AMF) belong to the soil community and are beneficial soil organisms at different trophic levels. Both improve soil fertility and structural development, which enhances plant growth and nutrient uptake. Earthworm activities redistribute mycorrhizal spores and exert diverse effects on mycorrhizal colonization. Co-inoculation with both earthworms and AMF significantly enhances plant growth response by increasing soil enzyme activities and altering nutrient availability [26], also the application of biochar generally increased both arbuscular mycorrhizal fungi (AMF) root colonization and relative amount of neutral lipid fatty acid [27].

The current study also confirmed a significant increase in the vegetative and root growth characteristics in all treatments, compared to the control treatment without adding. the inoculation with AMF enhances plant growth, increases yield and improves quality, especially under stressful conditions, especially elements and nutrients [28]. It enhances the plant's ability to tolerate drought,

salinity, temperature changes and heavy metal toxicity, thus contributing to reducing damage caused by climate change [29]. It increases water absorption through fungal hyphae outside the root or by changing the root structure, enhances water and nutrient use efficiency, regulates hormone balance (auxin, abscisic acid and cytokinin), regulates stomata function, and increases the rate of photosynthesis [30].

Earthworms contribute to improving plant growth by enhancing soil fertility and increasing the mixing of organic matter in the soil, which facilitates the access of plant roots to it, in addition to improving soil aeration and decomposition of organic matter, thus increasing the availability of nutrients in the soil, in addition to enhancing the growth of microscopic soil organisms [31].

Biochare has demonstrated numerous positive environmental effects, such as carbon sequestration, reduced greenhouse gas emissions, and soil improvement. Biochare application has also shown tremendous benefits when applied to agricultural systems, including improved plant growth, either under optimal conditions or under biotic or abiotic stress. Several mechanisms have been described, such as enhancing soil microbial diversity and thus increasing soil nutrient cycling functions, improving soil physical and chemical properties, stimulating microbial colonization, or increasing soil phosphorus, potassium, and nitrogen content, to exert these positive effects on plant growth [32], [33].

5 CONCLUSIONS

The results of the current study confirm the importance of protecting natural resources in achieving sustainable development goals by avoiding the use of chemical fertilizers in agriculture. The results showed the positive effect of adding biocarbon, earthworms, and endophytic mycorrhizal fungi, either individually or in combination with each other. These treatments achieved a significant increase in the vegetative and root growth characteristics of cauliflower, which is considered one of the important economic plants around the world. The results obtained from the GC-MS analysis identified several phytochemical constituents with known biological activities, including 2,3-butanediol, oxirane derivatives, and 6-oxa-bicyclo [3.1.0] hexan-3-one. These compounds are known to exert antimicrobial, antioxidant, and anti-inflammatory effects, which may act through mechanisms such as membrane disruption, protein synthesis inhibition, or interference with bacterial metabolic pathways. The results also showed that the above treatments had an impact on some physical and chemical soil properties, which are likely to show greater variation with increasing time. Therefore, the study recommends adopting environmentally friendly methods to improve plant growth.

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Effect of Clove Extract on the Histological Structure of the Kidneys in Swiss Mice

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Keywords: Plant, Syzygium Aromaticum, Mus Musculus, Kidney.

Abstract: Syzygium aromaticum is a medicinal plant known for its antioxidant and anti-inflammatory properties. It is used in traditional medicine to treat a variety of conditions, but it can cause toxic effects at high doses. The study aims to effect the extract of the clove plant (Syzygium aromaticum) about the histological architecture of the kidneys in Swiss mice (Mus musculus), which took 30 days. 24 mice were used and divided into four groups according to the dose of clove extract, each group includes 6 mice. The first group (the first control experiment group), The second group received an injection of 100 mg/kg per day of the extract, the third group received 200 mg/kg per day, and the fourth group received 300 mg/kg per day of the extract. The results of the examination of the histological sections of the kidneys of animals in the injected experimental group demonstrated the presence of several abnormal histopathological alterations, including the amplation of the Bauman space, glomerular contraction, degeneration and necrosis of the cells forming the urinary tubules, degeneration of glomerular cells and cells of the parietal layer of the capsule, and the presence of congestion in the blood vessels and the formation of a blood clot with an increase in the thickness of the vascular wall, and some cellular changes such as enlargement of nuclei, cell swelling, the occurrence of vascular degeneration and cytoplasmic degeneration with infiltration of inflammatory cells, in addition to the occurrence of vascular centered fibrosis, increased thickness with density of the glomerular basement membrane.

1 INTRODUCTION

Since ages, Medicinal plants have been utilized as spices, dyes, and flavorings, in addition to their therapeutic applications by a significant portion of the global population to address various health issues, including cancers, diabetics, obesity, liver and kidney diseases, and heart diseases [1]. One of these plants is the clove plant Eugenia or LM Perry, the scientific name of which is Syzygium aromaticum (L.) is one of the types of plants that are used as a spice with common fragrances that is widely used globally In food as well as alternative medicine, as well as in traditional and folklore medicine, the oil is utilized in dentistry due to its potent analgesic and antiseptic characteristics [2]. Clove extracts and essential oil have extensive medical applications as anticancer, antimicrobial, anti-breath, antidiabetic, anti-obesity, anti-inflammation, antioxidants , antiviral and aphrodisiac, including several applications [3]. The carnation is an unopened flower bud of the clove tree, belonging to the Myrtaceae family, and is indigenous to Indonesia. It is utilized as a culinary spice worldwide and is originally from the Kings Islands (Indonesia), though it is now extensively cultivated in various tropical regions, including Zanzibar, Madagascar, Pakistan, India, and Sri Lanka for commercial purposes. Since 300 BC, Indonesia has served as a hub for Arab, Chinese, Indian, and European traders of the principal Indonesian spices, particularly the clove plant (Syzygium aromaticum L) [4]. Numerous investigations suggest that S. aromaticum L. has roughly 15-20% essential oils, while clove essential oil (CEO) is rich in phenolic compounds exhibiting different biological functions, particularly anti-fungal, anti-bacterial, insecticidal, and antioxidants capabilities. [5], [6]. The kidneys are one of the basic organs that the body needs to carry out many important functions, including maintaining balance, regulating the environment outside the cell, and emptying metabolites and toxic metabolites and drugs [7]. The kidneys are an organ that is rapidly affected by some types of drugs, i.e. the main function of the kidneys is

to maintain the state of balance and the amount of fluid in the body, and to excrete both excess water, electrolytes and waste in the blood [8]. In addition, it is a major gland that secretes some hormones, such as Erythropoietin. It facilitates the synthesis of red blood cells by activating the bone marrow, which in turn does so, and the resonance hormone Renin regulates blood pressure [9]. Due to the extreme importance of the kidneys in the sustainability and quality of life, due to their locations and functions and the lack of most previous research of the histological effects caused by extracts on them. The objective of this study was to ascertain the impact of the aqueous extract of the clove plant on the histological architecture of the kidneys in the white mouse.

2 MANUSCRIPT PREPARATION

2.1 Clove Plant Collection

The clove plant was obtained from the local markets and then cleaned by hand to get rid of impurities and then ground with a household electric grinder to obtain a fine powder and then kept in an airtight box until the powder is used.

2.2 Preparation of Aqueous Clove Extract

Prepare the extract by taking 50 grams of clove powder that has been ground and then dissolved in 500 ml of non-ionic water and then placed on a Hote plate for 72 hours to stir the mixture to dissolve the powder in water and then drain with filter paper to get rid of impurities is not solvent and then placed in the refrigerator to precipitate the precipitate and take the filtrate and put it in the center view device at a speed of 3000 cycles / minute to get rid of the remaining precipitate and then take the aqueous extract placed at a temperature chamber to evaporate the water and obtain the aqueous extract to be dissolved with distilled water and ready for injection of laboratory animals [10] .

2.3 Experimental Animals

Twenty four females were obtained from the Swiss musculus pregnant egg mice from the Institute of Embryology Research and Infertility Treatment / Al-Nahrain University, whose weight ranged from 32 to 54 g. The therapies administered were tailored to the specific requirements of the experiment:

- **Group 1:** The healthy control group.
- **Group 2:** This group received an injection of 100 mg/kg per day of the extract.
- **Group 3:** This group received an injection of 200 mg/kg per day of the extract.
- **Group 4:** This group received an injection of 300 mg/kg per day of the extract.

2.4 Clove Extract Dosage Preparation

The dose for clove extract was prepared based on the lethal half-dose (LD50) whose value in mice is 565.7 mg/kg for clove extract [11]. Where three concentrations of clove extract were selected to test the extent of its effect on mice, namely 100, 200 and 300 mg of extract / kg of the weight of the mice used in the experiment between (32-54) g. The required amount of extract was then dosed depending on the concentration (dose) by injection once a day for one month per concentration. The dosage for the specific clove extract was calculated based on the following (1) [12].

$$\frac{x}{D} = \frac{w}{1000}. \quad (1)$$

Where,

- w - the weight of the mouse used in the experiment (32–54 g);
- D - the specific dose of clove extract administered (100, 200, or 300 mg/kg of body weight);
- x - the amount of extract to be injected into each mouse, measured in milligrams.

3 RESULTS AND DISCUSSION

The kidneys work to excrete waste, acids, minerals, medicines, or metabolites of them, and because of this function may lead to histological changes in blood vessels, tubules and interstitial tissues [13], [14]. The results of the current study of the histological sections of the kidneys in the control group showed that the kidney consists of two regions, namely the cortex and the medulla. The renal cortex contains renal glomeruli with a normal shape and tufts of glomerular capillaries surrounded by the capsular space and Bowman's capsule and the proximal convoluted tubules, which have a narrow lumen and pyramidal cells with spherical nuclei and a narrow lumen as shown in Figure 1, the experimental group injected with clove plant extract at a concentration of 100 mg had histological changes in the kidneys, including

severe congestion in the renal tubules and suppuration in them, in addition to strong bleeding between the renal tubules and necrosis in some epithelial cells of the tubules and the separation of part of the cells from the basement membrane, in addition to that, it was noted in the current results that inflammatory cells combine the tubules as in Figures 2, 3 and 4.

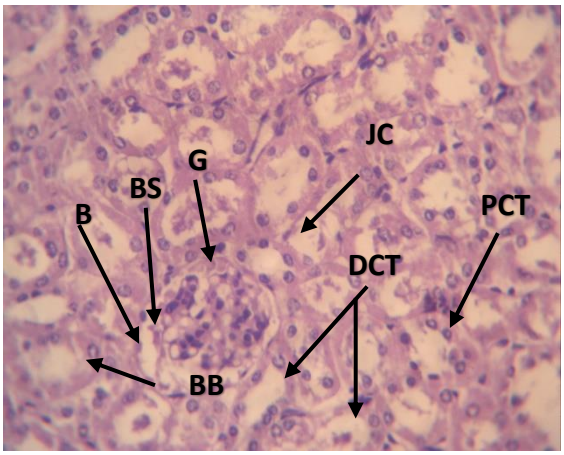


Figure 1: A transverse section of the kidney of pregnant female mice from the control group showing the cortex area. Note G glomerulus, PCT proximal convoluted tubule, JC paraglomerular cells, B Bowman's capsule, BS Bowman's space, DCT distal convoluted tubule, BB brush border stained (H&E 40X).

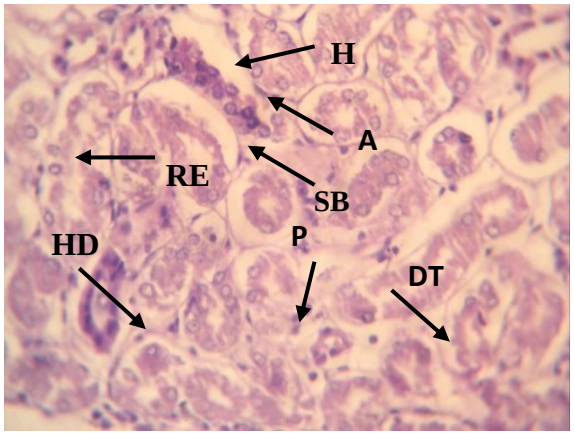


Figure 2: Section in the kidneys of pregnant female white mice experimental group injected with clove plant extract at a concentration of 100 mg / kg shows changes in the area of the cortex illustrates. H hemorrhage Re dilated tubules, SB, cell separation from basal membranes, HD alpine degeneration, P nucleolithrosis, DT destruction of some cells, inflammatory cell aggregation A, (H&E 40X).

The microscopic examination results indicated alterations characterized by the invasion of inflammatory cells. Infiltration may result from drug buildup or inflammation, as Bartoli [15] demonstrated that medications can induce systemic immune responses, resulting in inflammation and renal damage. Additionally, pre-existing renal injuries, such as fibrosis, may draw immune cells to the kidneys [16].

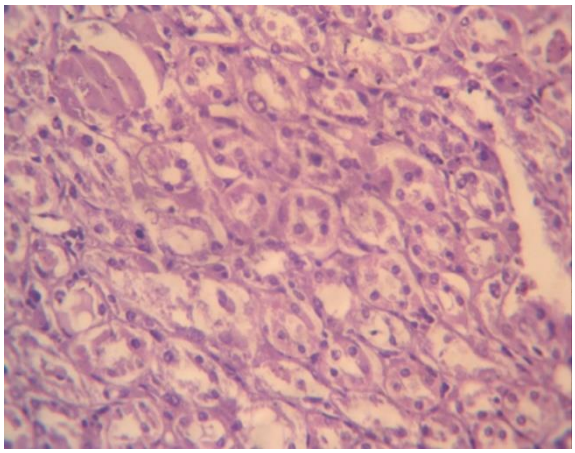


Figure 3: Section in the kidneys of pregnant female white mice experimental group injected with clove plant extract at a concentration of 100 mg/kg shows changes in the cortex area illustrates. A inflammatory cell infiltrate, V cytoplasm of the collector ducts cells, N tubule cell necrosis, P nucleololi (H&E 40X).

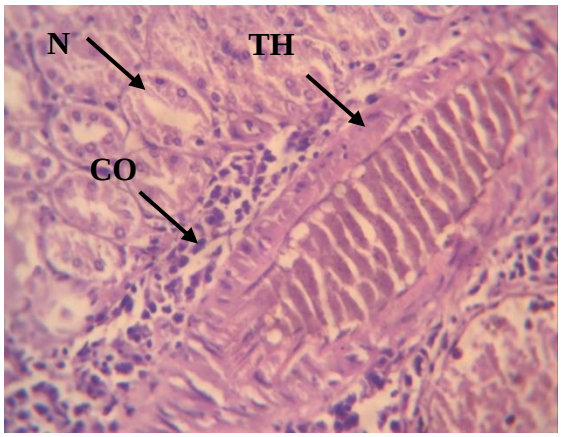


Figure 4: Section in the kidneys of pregnant female white mice experimental group injected with clove extract at a concentration of 100 mg / kg for 30 days shows vascular Vascular wall congestion and hypertrophy in the renal cortical region. CO noted acute vascular congestion, TH vascular wall hypertrophy, and N cellular necrosis (H&E 40X).

Vascular congestion in the cortical blood vessels was noted, along with hypertrophy of the vessel walls, maybe attributable to the toxic effects of clove extract or a response to inflammation resulting in augmented blood flow to the damaged region [17]-[19]. Histological sections have shown necrosis and degeneration due to the toxic effect of the extract or hypoxia, necrosis can be linked to ATP depletion, ultimately resulting in death of cells [20], [21].

Lara-Prado [22] also noted that the lack of blood flow to the kidneys may cause ischemia which increases ROS levels, modifies mitochondrial pathways, resulting in ATP depletion, cellular calcium releasing, caspase activation, and oxidative damage to lipids, DNA, and protein, eventually resulting in death of cells, as well as inhibiting antioxidant mechanisms (e.g. catalase, superoxide dismutase and glutathione peroxidase).

The examination outcomes indicated the detachment of renal tubule cells from their basal membranes, accompanied by hypertrophy and necrosis. This may stem from the influence of clove extract on the arteries, which diminishes blood flow necessary for cellular sustenance, resulting in cell death. Additionally, the extract may impact the enzymes produced by the kidney that govern arterial relaxation and contraction, such as prostaglandins [23].

The results of the current study showed that the injection of clove plant extract in pregnant female white mice at a concentration of 200 mg / kg led to pathological changes in the kidneys more severely than the previous concentration and for a period of 30 days, and these degenerative changes, which makes their cavities narrow as we did not notice the separation of some of the tubule cells from the basement membrane, and the occurrence of bloody bleeding between the renal tubules and observed enlargement and lobulation of the glomeruli so that they almost fill the capsular space, which led to the small area of the bowman space bleeding in addition to the death of some cells inside, either in some histological sections has been observed atrophy or contraction glomerulus, which led to the accumulation of cells inside it in the form of mass and the large space Bowman in it or its death and the capsule remains empty as in Figures 5, 6 and 7.

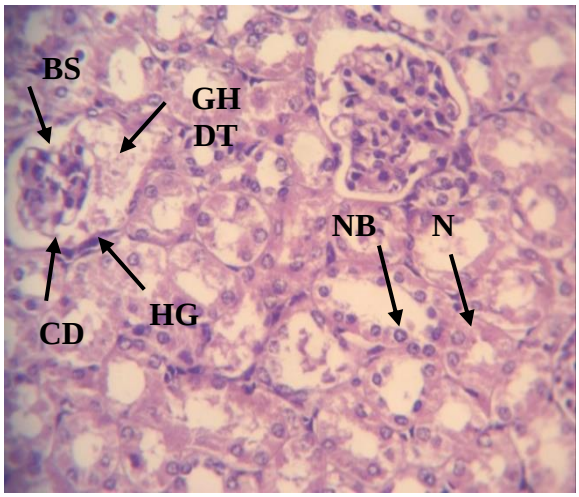


Figure 5: Section in the kidneys of pregnant female white mice Experimental group injected with clove extract at a concentration of 200 mg / kg for a period of 30 days, showing changes in the kidney cortex DT Cells are collected in the middle of the tubule N Urinary tubule cell necrosis, enlarged glomeruli HG, capsular space BS, CD cell death in the glomerulus, GH intraglomerular hemorrhage, NB nucleolary hyperplasia. Colorful (H&E 40X) .

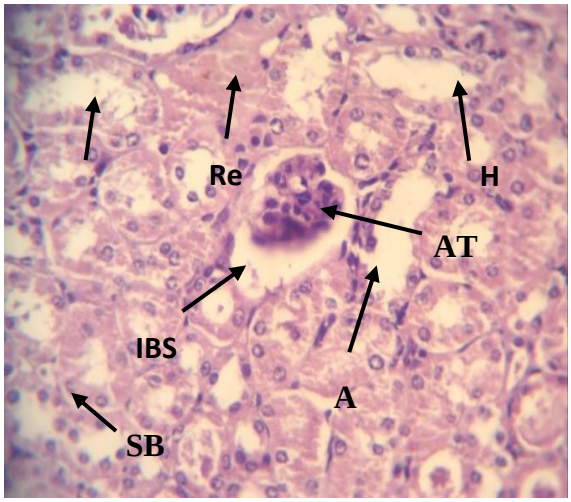


Figure 6: Section in the kidneys of pregnant female white mice experimental group injected with clove extract at a concentration of 200 mg/kg to period of one month, showing glomerular contraction (AT), inflammatory cell infiltration (A), intertubule hemorrhage (H), SB separation of cells from basal membranes, amplitude of Bowman space (IBS), dilated tubules (Re). Coloured (H&E 40X).

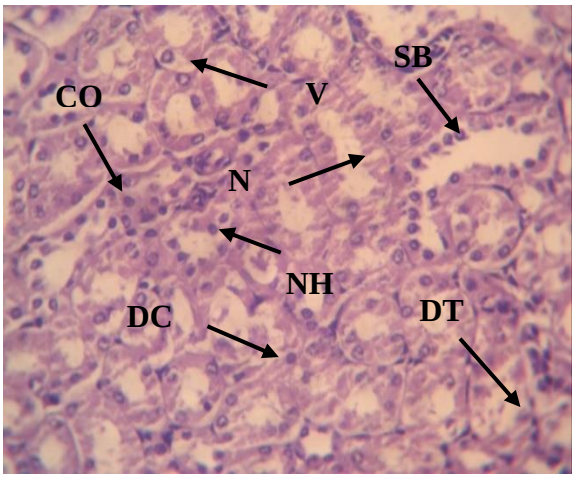


Figure 7: Cross section in the kidneys of pregnant female white mice experimental group injected with clove plant extract at a concentration of 200 mg / kg for 30 days, showing the occurrence of high degeneration of the pulposal region and renal rosids within the kidney. SB separation of cells from basal membranes, HN thickening of cell nuclei, Vtfgic cells, DT crash of some cells, N cell necrosis, CO vascular congestion, DC small size of cells, colored (H&E 40X).

The expansion of the Bowman space and the contraction of the glomeruli may result from the degeneration and demise of glomerular cells and their atrophy, or alternatively, from the influence of the extract on the hormone Angiotensin I, which stimulates mesangial cells to contract, leading to the constriction of glomerular capillary blood vessels. This cells support the capillary blood vessels, and their shrinking contributes to the narrowing of the glomerular capillary blood vessels, thereby increasing the area of the Bowman space [24]. The results of the current study showed that the injection of clove extract in pregnant female egg mice at a concentration of 300 mg / kg kidneys more severely when compared with the previous two concentrations and for a period of 30 days and these changes were kidney damage represented by severe bleeding inside the glomerulus and death of some cells in them and the occurrence of bleeding between the renal tubules and the detachment of specific cells from the basement membrane and the onset of defloration and the separation of cells and their collection inside the tubule in addition to thickening.

The death of some nuclei inside the tubules as well as the enlargement of some nuclei in the epithelial cells lining the tubules as shown in Figure 8. Uetrecht *et al*., [25] has shown that some types of drugs may affect angiotensin II, which elevates intraglomerular pressure and causes kidney damage, and that the mechanisms that contribute to injury include reduced renal blood flow, vasoconstriction of incoming glomerular arteries, or vasodilation of the outgoing glomerular arteries. The lack of blood flow works on ischemia and causes various damages such as necrosis, degeneration or cell death, and this supports what the researchers said [26], [27].

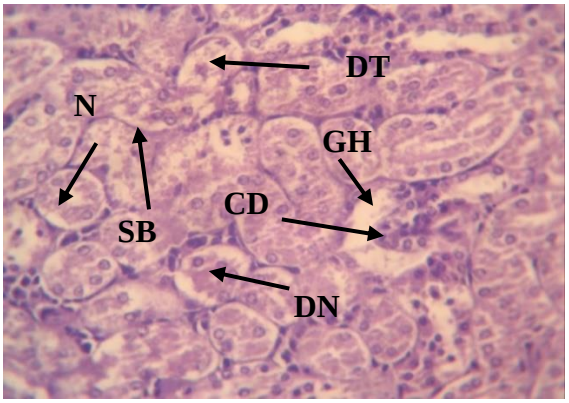


Figure 8: Cross section of kidney tissue Pregnant female white mice Experimental group injected with clove extract at a concentration of 300 mg / kg for a period of 30 days shows the occurrence of severe degenerative changes within the kidney. Intraglomerular hemorrhage GH, cell death in the glomeruli CD, SB separation of cells from basal membranes, DT Destruction of some cells, N necrosis, DN Death or decomposition of colored nuclei (H&E 40X).

The histological sections showed degenerative changes in the pulposal area in the kidney, in which there are tubules of different diameters, which include both the slim and thick Henley buttonhole, in addition to the presence of the university tubules, and these changes include bleeding between these tubules and the separation of their cells from the basement membranes and the emergence of degenerative between the cells lining the tubules and also occurs the crash of some of these cells Enlarged nuclei in most tubules and necrosis occurs between cells as we also note a small size of the tubules as shown in Figure 9.

The results from our current investigation demonstrated a rise in both the thickness and density of the glomerular basement membrane with some urine tubules, as well as an expansion for Enlargement of capsular space as a result of shrinking glomeruli and damage as glomeruli appeared accumulated cells, and atrophy glomeruli atrophy in some sections, and severe bleeding was seen inside the glomeruli and between the tubules and necrosis in the glomerulus as well as the occurrence of cell degeneration in the parietal region of the glomeruli as in the figure 10.

Acute medication-induced kidney injury is a serious problem and accounts for about 20% of cases of acute renal insufficiency among hospitalized patients [28].

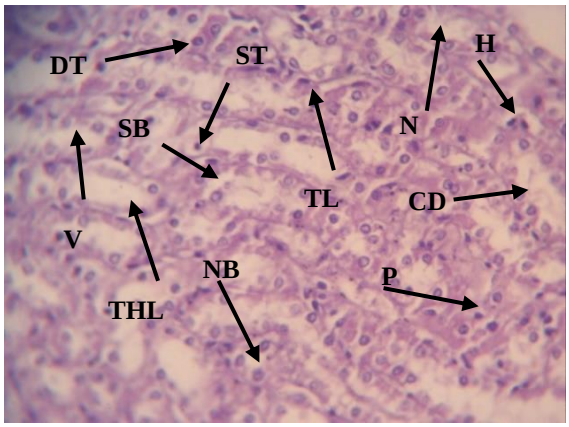


Figure 9: Clip in the kidney of pregnant white mice An experimental group injected with clove extract at a concentration of 300 mg/kg for a period of 30 days shows the pulp area of the kidney. Note THL thick Henley buttonhole, TL slim Henley buttonhole, CD combined canal, SB separation of cells from basal membranes, cell breakdown and aggregation in the center of the DT tubule, NB nucleolal hyperplasia, H intertubule hemorrhage, thickening of some P nuclei, N-cell necrosis, V-ductile tones, small size of tubules ST colored (H&E 40X).

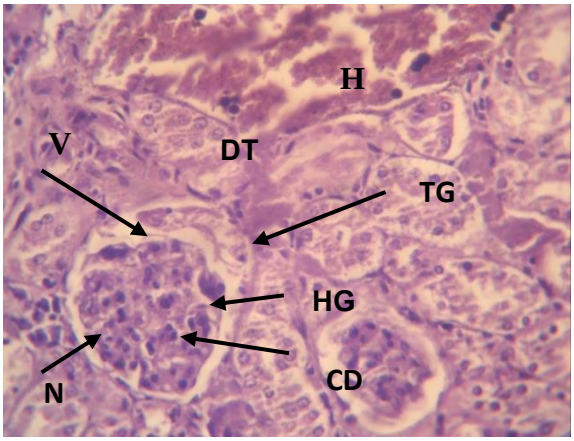


Figure 10: Section in the kidneys of pregnant female white mice experimental group injected with clove plant extract at a concentration of 300 mg / kg for a period of 30 days, shows the occurrence of TG increased thickness and density of the basement membrane of the glomeruli, H hemorrhage between tubules, DT breakdown and aggregation of cells inside the tubule, Vtfji cytoplasm cells in the glomerulus, HG intraglomerular hemorrhage, glomerular necrosis N death of some CD cells. Colored (H&E 40X).

While the results of the current study do not agree with the study Negm et al., [29], which indicated that after injection of clove extract at 300 mg/kg/day, there was a significant improvement in kidney indicators such as increased serum creatinine, blood urea nitrogen (BUN), and hyperphosphatemia resulting from adenine. Another study showed protective effects of all concentrations used, 150 mg/kg, 350 mg/kg, and 450mg/kg, of clove extract on the kidneys of white rats, due to the clove extract containing secondary metabolites in the form of saponins, tannins, flavonoids, and steroids that help repair damaged kidney cells [30].

4 CONCLUSIONS

The results of the examination of the histological sections of the kidneys of animals in the injected experimental group demonstrated the presence of several abnormal histopathological alterations, including the amplation of the Bauman space, glomerular contraction, degeneration and necrosis of the cells forming the urinary tubules, degeneration of glomerular cells and cells of the parietal layer of the capsule. The results indicate that clove (S. aromaticum) extract affects the histological structure of Swiss rats (Mus musculus), with significant dose-dependent histological changes observed. At low doses, the extract may exhibit protective effects due to its antioxidant and anti-inflammatory properties, helping to reduce oxidative damage to renal tissue. At high doses, it may lead to pathological changes such as dilatation of renal tubules, infiltration of inflammatory cells, and changes in the glomeruli, suggesting potential toxic effects at high doses. These findings underscore the importance of dose control when using clove extract to avoid negative effects on kidney health.

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The Effect of Rosemary Plant Treated with Fructose on Insulin Sensitivity and Inflammatory Mediators in Male Albino Rats

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Keywords: Fructose Consumption, Insulin Sensitivity, TNF-α, IL-6, STAT3, Rosemary.

Abstract: The current study aimed to understand the effects of fructose sugar on experimental animals and its impact on blood sugar levels, insulin hormone, insulin sensitivity, as well as its effect on inflammatory proteins including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and signal transducer and activator of transcription 3 (STAT3), Additionally, the study sought to investigate the role of rosemary plant extract, This study was conducted on 15 male rats with an average weight of 180-230 grams and ages of 4-5 months, which were divided into three groups of 5 animals each This study was conducted on 15 male rats with an average weight of 180-230 grams and aged 4-5 months. They were divided into three groups, with 5 rats in each Group 1 Considered the control group and given water and regular feed, Group 2 given with 2 g/kg of fructose Group 3 given with 2 g/kg of fructose and 50 ml of aqueous rosemary extract All groups were orally administered their respective treatments for 30 days, The results showed a significant increase in sugar levels, insulin, insulin resistance, and inflammatory proteins (TNF-α, IL-6, STAT3) in the fructose-treated group compared to the control group, In contrast , the group treated with fructose and rosemary extract exhibited a significant reduction in sugar levels, insulin, insulin resistance, and inflammatory proteins, This decrease is attributed to the role of rosemary in mitigating the effects of fructose , Rosemary might be beneficial in reducing blood sugar levels, enhancing insulin secretion , protecting pancreatic beta cells, and demonstrating inhibitory effects on IL-6, TNF-α, and STAT3 Alkaloids, phenolic acids, saponins, diterpenes, flavonoids, and essential oils are all found in rosemary, which also has anti-inflammatory.

1 INTRODUCTION

High fructose sucrose (HFCS) contains the simple sugar fructose as a free monosaccharide. It is a typical sweetener that is present in a lot of produced and processed foods and drinks that people consume on a regular basis. The consumption of fructose-sweetened drinks has risen significantly and is strongly linked to metabolic diseases. These diseases, along with systemic inflammation, infections, and harmful effects across generations, are widely associated with the increased intake of fructose [1]. Excessive intake of free sugars, such as fructose, is a major contributor to obesity and metabolic syndrome globally [2]. It is well-established that eating habits significantly influence the body's metabolism [3].

In recent decades, dietary patterns have shifted globally, leading to a significant increase in fructose consumption, particularly through sugar-sweetened beverages [4]. Clinical, experimental, and epidemiological research has shown that excessive consumption of fructose, beyond the recommended levels, can result in several negative health effects [5]. Higher fructose intake diminishes the sensation of fullness, promoting a positive energy balance, and enhances fat storage, which contributes to the buildup of visceral fat [6]. Increased arterial blood pressure and blood vessel damage are caused by fructose, which also encourages the accumulation of ectopic fat, especially in the liver and skeletal muscle, resulting in insulin resistance, inflammation, and abnormal fat metabolism [7]. Therefore, through a number of different processes, increased fructose consumption is linked to the beginning of a number of heart and metabolic problems, such as cardiovascular diseases, non-alcoholic fatty liver disease (NAFLD), type 2 diabetes, obesity, and insulin resistance [8]. A diet rich in fructose can cause inflammation in the colon and pancreas, enhance intestinal permeability, lead to fat accumulation in liver tissue, and increase levels of pro-inflammatory

cytokines [9]. It is important to note that fructose plays a crucial role in non-alcoholic fatty liver disease, with both preclinical and clinical research demonstrating a robust correlation between fructose intake and the degree of fibrosis and inflammation. Fructose is also regarded as a risk factor for liver cancer, The detrimental effects of fructose on the body, especially in the liver, can be explained by the activation of several pro-inflammatory, pro-fibrosis, and pro-tumor signalling pathways [10]. Obesity and overweight are becoming more common worldwide. According to data from the World Health Organisation (WHO), 39% of adults over the age of 18 were overweight in 2016 and 13% were obese, Furthermore, the proportion of overweight children and adolescents rose sharply from 4% in 1975 to 18.5% in 2016 alterations in eating patterns, such as the intake of beverages with added sugar, processed foods high in sugar, fat, and refined carbohydrates, along with the adoption of the Western diet and low physical activity, are contributing factors to the growing obesity rates worldwid [11].

Excessive fructose intake has been linked in numerous studies to adipose tissue buildup, systemic inflammation, adipokine production, elevated oxidative stress, and, ultimately, insulin resistance in a variety of tissues [12]. The body's immune reaction to damage or infection is inflammation, which is essential to innate immunity. The usual clinical signs of pain, swelling, heat, and redness are the outcome of a complex cascade of molecular and cellular signals that alter physiological responses [13].

The pancreas secretes the hormone insulin, which helps move glucose from the bloodstream into the cells of muscles, fat, and the liver, where it is used for energy [14]. The pancreas secretes insulin into the bloodstream to help reduce glucose levels and maintain them within normal ranges, When muscle, fat, and liver cells lose their sensitivity to insulin, it becomes harder for them to absorb glucose, leading to the development of insulin resistance, In order to facilitate glucose access into the cells, the pancreas is compelled to create more insulin [15]. If the pancreas can produce enough insulin to compensate for the cells' reduced responsiveness, glucose levels will stay within a healthy range. However, persistently high blood glucose levels can lead to prediabetes in individuals with insulin resistance or those whose pancreatic beta cells fail to produce sufficient insulin. People with genetic predispositions or unhealthy lifestyles are at a higher risk of developing insulin resistance or prediabetes [16]. Obesity is a major risk factor for insulin resistance, type 2 diabetes, and metabolic syndrome because chronic inflammation plays a major role in decreased insulin sensitivity. Increased inflammatory cytokine production sets off a number of signalling pathways that encourage fat cell growth and lead to the development of insulin resistance [17].

The use of plant sources to treat human and animal diseases has gained significant attention in several countries, as the compounds found in plants offer a diverse array of biologically active components that can help address various complications arising from diseases [18]. In the modern era, medicinal plants have gained significant interest due to their use in treating chronic diseases and their recognition as a preventive health approach in many developed countries [19]. The rosemary plant, which belongs to the Lamiaceae family, is one of the most important of these plants. Rosemary grows in the Mediterranean basin and is cultivated in many countries around the world due to its multiple uses, including as a cooking spice and food preservative, thanks to its antioxidant properties. It has been used as a medicinal herb for centuries, possessing the ability to resist many diseases and is considered an anti-inflammatory [20]. Natural and chemical toxins lead to significant negative effects on human health in various ways. In this context, the use of herbal medicines is considered a safe alternative to combat these toxins, the plant rosemary, scientifically known as Rosmarinus officinalis, Rosemary and its components, such as carnosic acid, rosmarinic acid, and carnosol, have a number of health benefits, including anti-inflammatory, antioxidant, antimutagenic, antibacterial, and antiviral properties, as well as analgesic and neuroprotective effects [21]. Alkaloids, phenolic acids, saponins, diterpenes, flavonoids, and essential oils are all found in rosemary, which also has anti-inflammatory, anti-cancer, neuroprotective, cardioprotective, and hepatoprotective properties. Although most people believe that rosemary is safe to eat and apply topically, some people have experienced rashes and allergic responses [22].

Objective: The purpose of the current study is to investigate how fructose affects blood serum levels of several biochemical variables, such as glucose, insulin, insulin resistance, interleukin-6, and tumour necrosis factor-alpha (TNFα) and signal transducer and activator of transcription 3 (STAT3), The study also assesses rosemary extract's defensive properties.

2 MANUSCRIPT PREPARATION

2.1 Experimental Animals and Group Organization

The study was conducted on 15 male white mice, weighing between 180–230 grams and aged between 4–5 months. The animals were randomly divided into three equal groups (5 mice per group) as follows:

- Group 1 (Control – CTRL): Received food and distilled water only orally for 30 days.
- Group two (fructose – FRC): Each mouse received 1 ml of a fructose solution at a concentration of 2 grams/kg of body weight, administered orally daily for 30 days.
- The third group (fructose + rosemary – FRC + ROSY): Each mouse received: 1 ml of fructose solution with a concentration of 2 grams/kg of body weight 1 ml of rosemary water extract at a concentration of 50 mg/kg body weight, both administered orally daily for 30 days.

2.2 Drugs Used in the Experiment

In this study, the following drugs and extracts were used:

- Fructose sugar, obtained as a white crystalline powder from the Spanish company Charlie, was dissolved in distilled water. The rats were then administered a dose of 1 ml per rat, at a concentration of 2 g/kg of body weight [23].
- The rosemary aqueous extract was prepared in the laboratory Using a lyphollizer and dissolved in distilled water. It was then given to the mice at a dosage of 1 ml per mouse, with a concentration of 50 mg/kg of body weight [24].

2.3 Collection of Blood Samples

Following the trial, the animals were given no food for a full day. The jugular vein was then severed in order to get blood samples. (Jugular vein) and left in test tubes for half an hour at ambient temperature , The serum was then separated from other components using a centrifuge set to 3000 rpm for 15 minutes, Micropipettes were used to separate the serum, which was then put in Eppendorf tubes and kept in a deep freezer at -80°C until biochemical testing was completed.

2.4 Biochemical Tests

Glucose concentration was estimated. Glucose using (kit) Produced by Biolabo-France Insulin and insulin resistance using a ready-made research kit produced by Sunlong-China and evaluation of IL-6 protein concentration and TNF-α protein concentration and the concentration of the STAT3 protein using a ready-made research kit produced by Sunlong-China using Elliza technology

2.5 Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) version 20 was used to statistically analyse the study's findings, and The ANOVA test (which is a one-way variance and means test) was used to compare the groups, and the significant differences between the means were tested using the Duncan multiple range test to compare three groups at a significance level of ($P \leq 0.05$) [25].

3 RESULTS

The results presented in Table 1 indicate a significant increase ($P<0.05$) in blood sugar levels, insulin, insulin resistance, and inflammatory proteins, including tumor necrosis factor alpha (TNF-α) and interleukin-6 and Signal Transducer and Activator of Transcription 3, as a result of fructose consumption in the second group (FRC) of mice that were dosed with fructose, compared to the control group (CTRL). The FRC+ROSY group showed a significant decrease ($P<0.05$) in blood sugar, insulin, insulin resistance, and inflammatory proteins, including TNF-α and IL-6 and STAT3, compared to the fructose-only group (FRC). This decrease is attributed to the protective role of rosemary in mitigating the harmful effects of fructose.

4 DISCUSSION

Our findings align with previous research [26]. which demonstrated that increased fructose intake leads to elevated glucose and insulin levels, potentially raising the risk of developing type 2 diabetes, A study [27]. also indicated that excessive fructose consumption induces an acute insulin response . though fructose itself doesn't directly trigger insulin secretion.

Table 1: Effect Fructose (FRC) and fructose + rosemary extract (FRC+ROSY) on the studied indicators in the serum of male mice.

Transactions	Groups	Mean±SD
Sugar mg/dl	CTRL	85.0±2.0 c
	FRC	137.75±1.71a
	FRC+ROSY	96.5±3.87 b
Insulin (ng/ml)	CTRL	11.04±0.3 c
	FRC	30.82±0.94 a
	FRC+ROSY	23.44±3.48 b
Resistant (Pg/ml)	CTRL	41.71±2.12c
	FRC	188.7±7.75 a
	FRC+ROSY	94±2.57 b
TNF-α (Pg/ml)	CTRL	144.33±5.57 c
	FRC	557.64±31.72a
	FRC+ROSY	280.79±20.8 b
IL-6 (Pg/ml)	CTRL	89.38±6.43 c
	FRC	297.11±34.61a
	FRC+ROSY	199.49±17.63b
Stat3(Pg /ml)	CTRL	8.79±0.67
	FRC	31.91±1.54
	FRC+ROSY	23.39±2.66

Instead, it increases insulin content in beta cells [28]. Obese adolescents exhibit a significant insulin response to fructose, suggesting a possible link between fructose intake and hyperinsulinemia [29]. High fructose consumption promotes fat accumulation in the liver, increasing the risk of non-alcoholic fatty liver disease (NAFLD), which impairs liver sensitivity to insulin and leads to insulin resistance [30] . Consuming large amounts of fructose disrupts leptin signaling, which promotes overeating and weight gain. Obesity exacerbates insulin resistance [31]. TNF-α is one of the inflammatory cytokines that play a role in the immune response and inflammation. Studies indicate that excessive consumption of fructose, especially from industrial sources like high-fructose corn syrup (HFCS), may lead to increased production of TNF-α in the body, This rise in TNF-α may contribute to chronic inflammation and oxidative stress, which are associated with diseases such as obesity, type 2 diabetes, and cardiovascular diseases [32]. Excessive consumption of fructose may lead to insulin resistance, a condition associated with increased levels of TNF-α. Insulin resistance can exacerbate chronic inflammation, leading to increased production of inflammatory cytokines such as TNF-α and IL-6 [33]. Insulin resistance and type 2 diabetes are linked to inflammatory cytokines including IL-6, TNF-α, and hs-CRP because they suppress the transcriptional activity and protein expression of numerous molecules involved in insulin signalling and its processes, including GLUT-4, Insulin's capacity to attach to its receptors and start the last stage of cellular signalling is weakened by this inhibition., leading to insulin resistance [34]. A study found that exposing mice to a high-fructose diet stimulates the production of pro-inflammatory cytokines from immune cells, such as IL-6 and TNF-α, which activate STAT3 [35]. Fructose increases insulin secretion at high concentrations, which may stimulate pathways such as PI3K/Akt that interact with STAT3 and enhance its activity [36]. Rosemary contains alkaloids, flavonoids, and phenols with biological activity that lower blood sugar levels and have many roles [37]. Rosemary is considered one of the herbaceous plants rich in bioactive compounds, which may contribute to lowering blood sugar levels. Thus, rosemary plays a supportive role in blood sugar management through multiple mechanisms [38]. Rosemary is rich in phenolic compounds, which have been proven to possess numerous health benefits, including anti-hyperglycemic properties. Research indicates that rosemary and its components may enhance insulin secretion and improve glucose metabolism [39]. Rosemary compounds activate insulin signaling pathways in the body, such as the AMPK pathway, which increases cell sensitivity to insulin and reduces resistance, especially in patients with type 2 diabetes [40]. Results showed that ferulic acid and the flavonoid quercetin affect pancreatic cells, enhancing the proliferation of beta cells and leading to increased insulin secretion. [41]. The results showed a decrease in TNF-α levels in the groups treated with rosemary plant extract, due to the plant's ability to reduce reactive oxygen species (ROS) either through direct interaction with free radicals or indirectly. Additionally, rosemary contains the compounds carnosic acid and carnosol, which act as antioxidants [42]. The results of the current study are consistent with [43]. They indicated that rosemary contributes to lowering interleukin-6 levels (IL-6). This is due to the presence of biologically active components in rosemary

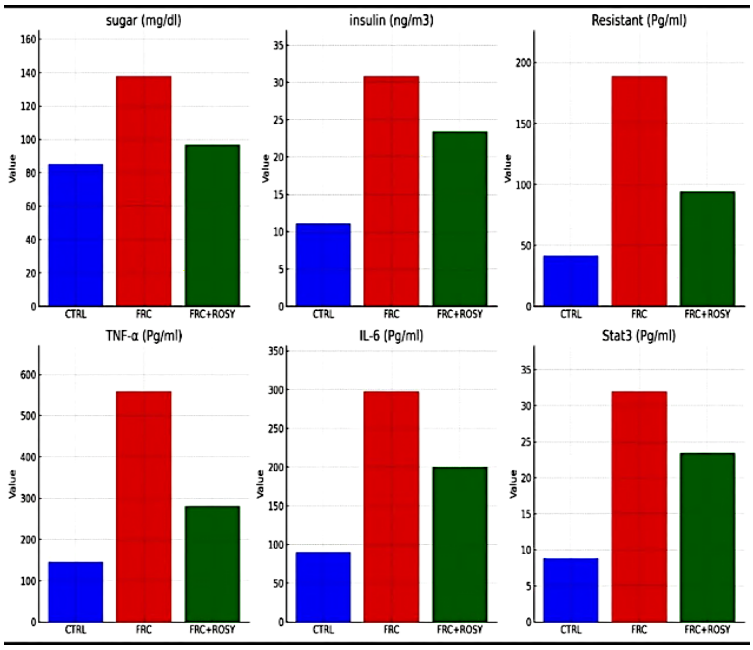


Figure 1: Graph to visually illustrate statistical differences.

that possess anti-inflammatory, antibacterial, and antitumor properties, in addition to its antioxidant properties and its ability to control and neutralize oxidizing agents [44] (Fig. 1). Therefore, this plant exhibits a capacity similar to that of other drugs [45]. The protective role of rosemary and its main compounds against natural and chemical toxins in laboratory and biological studies. The protective effects of rosemary and its components are mainly mediated through various mechanisms such as inhibiting oxidative stress, reducing inflammatory mediators, including tumor necrosis factor-alpha (TNF-α), interleukin-6, interleukin-17, cyclooxygenase-2 (COX-2), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), as well as modulating apoptotic signaling pathways and mitogen-activated protein kinase (MAPK) [45]. Scientific research indicates that rosemary extracts contain chemical compounds such as carbohydrates, coumarins, glycosides, and phenols, which may contribute to this effect in reducing inflammation, thereby lowering STAT3 protein levels [45].

Based on a group of five mice, the data are shown as the mean ± standard deviation. Significant differences are indicated by the vertical arrangement of the numbers and letters; statistical significance is established at a probability level of less than 0.05 ($P<0.05$).

5 CONCLUSIONS

The results of this study indicate that excessive consumption of fructose may be one of the main causes behind the development of insulin resistance, metabolic syndrome, and type 2 diabetes. It also contributes to elevated oxidative stress and increased levels of inflammatory proteins in the body, thereby exacerbating many chronic diseases. The data showed that fructose led to a significant rise in glucose levels, insulin, and inflammatory proteins such as TNF-α, IL-6, and STAT3, reflecting an acute inflammatory response and disruptions in glucose metabolism.

In contrast, the group treated with rosemary extract showed a notable improvement in these indicators, with a reduction in blood sugar, insulin, insulin resistance, and inflammatory protein levels. This improvement is attributed to the active compounds in the rosemary plant, such as carnosic acid, carnosol, and rosmarinic acid, which possess antioxidant and anti-inflammatory properties and positively influence the regulation of metabolic pathways related to glucose utilization.

Rosemary extract may enhance insulin secretion, protect pancreatic beta cells, and reduce the harmful effects of fructose, making it a promising natural option for alleviating the consequences of certain metabolic and inflammatory diseases. Based on these findings, the study recommends further clinical trials in humans to assess the efficacy and safety of

rosemary extract as a complementary therapy for preventing or minimizing the health risks associated with excessive fructose consumption.

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Quantitative Genetic Diagnosis of Medical and Forensic Insect Species in Diyala Governorate Using Geometric Wing Morphometrics

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Keywords: Chrysomya Megacephala, Sarcophaga Carnaria, Musca Domestica, Geometric Morphometric.

Abstract: A morphometric analysis was conducted on the right wing of adult males of some forensic insects, such as *Chrysomya megacephala*, and medical insects such as *Sarcophaga carnaria* and *Musca domestica*, collected from different areas in Diyala Governorate. The aim of this research was to differentiate between male medical and forensic insects based on the shape and size of the right wing central centroid. The analysis of the right wings in this work was carried out from adult males of the species *S. carnaria*, M. domestica, and *C. megacephal*. Samples were collected using light traps, and 19 marks were identified on each wing and placed at the ends of the longitudinal veins and between the intersections of the veins with the longitudinal veins. Significant differences were found in the mean right wing centroid and wing shape between adult males of medical and forensic insects. The results showed that the mean right wing centroid was 1576.52, 1633.33, and 1126.15 for *C. megacephala* and *S. carnaria* and *M. domestica*, respectively, when comparing *C. megacephala* with *S. carnaria* (f = 3.49, p = 0.03 and t = 1.13, p = 0.27 and AD = 56.81), comparing *C. megacephala* with *M. domestica* (f = 2.19, t = 0.15 and t = 15.8, p = 1.77 and AD = 450.35), and comparing *S. carnaria* with *M. domestica* (f = 7.65, p = 0.00 and t = 10.82, p = 1.61 and AD = 507.17). When comparing the right wing morphology between males of *C. megacephala*, *S. carnaria* and *M. domestica*, a significant difference was observed (f = 0.00, p = 0.99). A significant difference in wing size (f = 0.18, p = 1.00) was also observed between species.

1 INTRODUCTION

All flies part of the order Diptera are of criminal, medical and veterinary medicine Significance, as they are good mechanical vectors of many diseases that threaten human life throughout the world, especially in developing countries[1, 2]. Flies feed on numerous plant species and animal remains and are known disease vectors throughout history [3]. Diptera insects are distinguished from other insects by possessing a well-developed pair of forewings used for flight, while the second pair of wings is modified into structures known as balance pins (halters) [4]. Some of these insects are considered medically important pests as they transmit dangerous diseases affecting human and animal health. The impact of medically significant pests, such as *M. domestica* (housefly), is exacerbated in developing countries due to neglect in public health measures [5]. Additionally, insects play a crucial role in forensic veterinary entomology, as they are often the first living organisms to colonize a corpse immediately after death. This can help estimate the post-mortem interval by identifying the age of larvae from the first batch of eggs laid on the victim's body [6]. Insects contribute significantly to crime scene investigations, and forensic entomology has evolved into a specialized field within entomology, highlighting their role in forensic investigations [7]. Some insect species feed directly on corpses, such as the metallic blue blowflies from the Calliphoridae family and flesh flies from the Sarcophagidae family, both belonging to the order Diptera [8]. Therefore, an effective, efficient, and prompt method for identifying that insects is essential. This brings up an important subject: the taxonomic identification of fly species through wing geometry, which serves as a valuable tool for entomologists and forensic entomologists [9]. The geometric morphometric system for determining wing shape and size is an advanced technique used in quantitative genetics to measure significant variations in wing morphology. This method

enhances the accurate identification of forensically important species [10].

Extensive studies on medically important insects have shown that the analysis of geometric morphometric of wings of insect has a high capacity for detecting variations among geographically isolated populations within the same species or different species with great accuracy [11]. The primary goal of employing this quantitative genetic technique is to determine whether the analysis of geometric morphometric of insect wings be able to serve as an alternative or complementary tool to molecular markers in studying insect populations with medical and forensic significance. Consequently, this technique plays a vital role in accurately identifying the origin of any collected specimens. Furthermore, landmark-based geometric morphometric analysis is widely used for species identification due to its ease of application and low cost [12]. The geometric morphometric (GM) technique is a new tool in the field of biological research that is used to distinguish species with phenotypic affinity and to study the extent of morphological variations of organisms in different environments, is a new approach [11], and is frequently was accustomed to detect species in beings with analogous morphology and to study morphological differences in dissimilar surroundings particularly, with homologous insect species [13]. Recently, GM's landmark-based approach has been Serves to distinguish three genera of mosquitoes Aedes albopictus, Aedes Aegypti, and Aedes Scutellaris in Thailand and he discovered that this technique is very accurate in classifying species [14].

The current research aims to use a landmark-based genetic engineering approach to efficiently discriminate on three types of flies *C. megacephala*, *S. carnaria*, *M. domestica* that are criminally and medically important.

2 MATERIALS AND METHODS

2.1 Specimen Collection and Identification

Samples of the species *C. megacephala*, *S. carnaria* and *M. domestica* were gathered from different farms and in several areas of Diyala Governorate (muqdadiya) by installing fly traps and putting poison on animal waste, then the samples were kept in plastic bottles with the addition of naphthalene to prevent rotting of the samples [15], and then the sample was sent to the Natural History Museum at the University of Baghdad for the purpose of identifying and classifying the sample using classification keys.

2.2 Specimen Preparation and Landmark Collection

In this study, a total of 45 right wings (15 per species) were used to investigate the quantitative genetic diversity in wing size and shape among three medical and forensic insect species from Diyala Governorate: *C. megacephala*, S. *carnaria*, and M. *domestica*. The research employed the geometric morphometric (GM) technique.

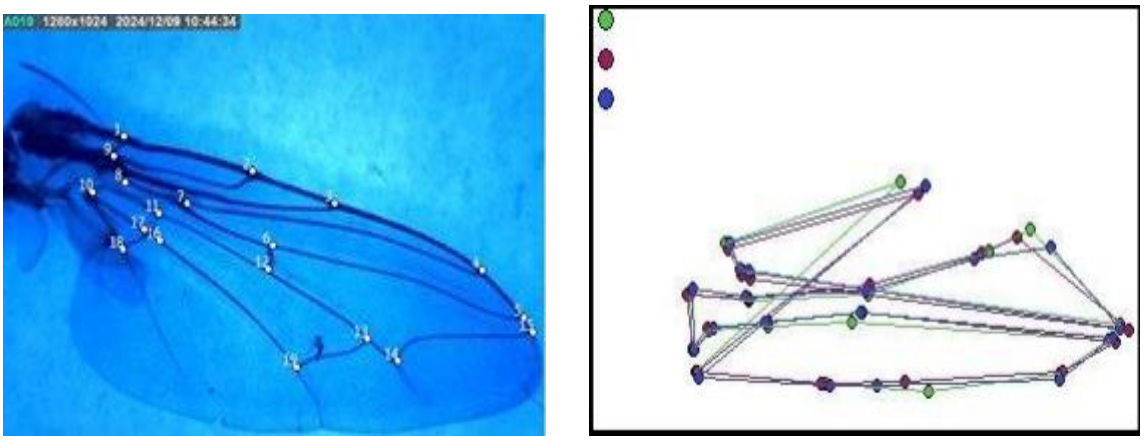
For the preparation, 15 male samples were isolated for each species. A technique followed [16] was used to prepare slide-mounted specimens. The right wing of each insect was removed and placed between two glass slides. The edges of the slides were securely sealed using G2100 adhesive paper tape. Each slide was labeled with the species, sex, and sample number.

After preparation, the slides were visualized using a digital microscope at a magnification power of x50. The intersections of the longitudinal wing veins and cross-veins were learned (Fig. 1). Specialized statistical analyses were then employed to assess differences between the species, including:

- Centroid size analysis.
- Discriminant analysis.
- Shape and size analysis.
- Mahalanobis distance analysis.

2.3 The Analysis of Geometric Morphometric

In wing size examination, the mean central size (MCS) wsas calculated as a means of estimating right wing size in medical and forensic insects, which is calculated via the square root of the sum of squares between the center of the landmark formation and every individual Anatomical point [17]. Then, statistical tests for wing size among males in each Non-parametric permutation tests (1000 cycles) with Bonferroni correction at a significance level of $P < 0.05$ were used to analyze the fly species. Similarly, wing shape analysis for *C. megacephala*, *S. carnaria* and M. *domestica*, where shape variables were evaluated by principal component analysis (PCA) of partial warp points (also known as relative warp), which were calculated after performing a generalized regression analysis of coordinates to assess variation in wing shape among species [18].



(a) (b)

Figure 1: Landmark configuration and morphological variation in wing venation among fly species: a) The locations of numbered landmarks on the ends of longitudinal and transverse veins. b) Histogram of the distribution of mean landmark configuration in each fly species. Green dot: *C. megacephala*, Red dot : S. *carnaria*, Blue dot: M. *domestica*.

2.4 The Software of Geometric Morphometric

The Collection of Landmarks for Identification and Characterization package (CLIC), release 97, was utilized to digitize landmarks and perform geometric morphometric analysis[1].

3 RESULTS AND DISCUSSION

The geometric scale based on landmarks has been used to distinguish morphological genetic differences between the males of some medical and forensic insects namely *C. megacephala*, *S. carnaria* and *M. domestica* using a set of statistical analyses, which had separated into shape and size investigates, where the percentage of differences in the central size of the wings was very high, 1576.52, 1633.33 and 1126.15, *C. megacephala*, *S. carnaria* and *M. domestica*, respectively., When comparing *C. megacephala* with *S. carnaria* (f = 3.49, P = 0.03 , t = 1.13, P = 0.27, AD = 56.81) , and comparing Ch. *megacephala* with *M. domestica* (f = 2.19, p = 0.15 and t = 15.8, p = 1.77 and AD = 450.35) , and comparing *S. carnaria* with *M. domestica* (f = 7.65, p = 0.00 and t = 10.82, p = 1.61 and AD = 507.17 (Table 1). Comparisons of variation of wing mean Centroid Size between medical and forensic fly species (Figures 2 and 3). Wing shape and size in all species were different among males based on Bonferroni test ($p < 0.01$) (Tables 2 and 3). When comparing the shape of the right wing between males of forensic insects and medical insects, which includes *C. megacephala*, *S. carnaria*, *M. domestica* a significant difference was observed (f = 0.00, p = 0.99). A significant difference was also observed in wing size (f = 0.18, p = 1.00) between the species.

Each box represents the median for the species, distributed between the 25th and 75th percentiles. The blue lines represent the samples for each species. Numbers 1, 2, and 3 correspond to *C. megacephala*, S. *carnaria*, and M. *domestica*, respectively (Fig. 2). In Figure 3, the polygons show group distribution, demonstrating the degree of morphological overlap and distinction.

Increasing knowledge of disease-carrying insects is crucial to understanding vectors, which in turn leads to effective control. Accurately identifying fly genus and species is an important first step in studying them phenotypically and genetically. Although adult flies are relatively easy to genus and species by morphological methods, field specimens are sometimes incomplete, leading to misdiagnosis. The GM technique based on the numbered landmarks on the ends of the veins was used in this study to conduct a dimorphic analysis of three common fly species in Diyala Governorate/Iraq: *C. megacephala*, S. pulchra. Wing size analysis based on the wing central size ratio (MCS) found statistical differences between medical and criminal species. This study results are aligned with a group of previous studies, including a study conducted in Thailand Which used landmark-based morphometric analysis to distinguish twelve different species of flies *Chrysomya megacephala*, *Chrysomya chani*, *Chrysmya pinguis*, *Chrysomya rufifirina*, *Chrysomya villeneuvi*, *Cheysomya ligurriens*, and *Hermetia pulchra*. The study demonstrated that wing

[1] The CLIC package (release 97) is freely available at https://xyom.io

size can be employed for Classification of species [19]. Wing size is an inappropriate factor for differentiating between species within a genus and between genera within same order, as it easily changes according to environmental conditions, especially at breeding sites [19]. Wing shape is a common factor used to distinguish species within the same genus, genera within the same order, and sexual variation because wing shape traits are directly influenced by genetic factors and are less affected by ecological factors than wing size factors. [14]. In this study, shape and size analysis of right wing based on Mahalanobis distances revealed differences between males all species of flies. The geometric morphometric approach enhances the ability to identify dimorphism in fly wings. The geometric morphometric scale was used to distinguish and distinct genera and species, and to differentiate sexes among, twelve different fly species in Thailand: Boettcherisca Peregrine, B. Nathani, Lioproctia Pattoni, L. Ruficornis, L. Saprianovae, Parasarcophaga BreviCornis, P. Antilope, S. Multivillosa, S. Senior Whitei and Senior Whitea Princeps. [19]. The Geometric Morphometric method is also effective in distinguishing between males and females of the same genus in wings, especially in *C. megacephala*, which can be distinguished with over 89% accuracy. This is consistent with a previous study by Sontigun in Thailand, which found that males and females of *C. megacephala* could be easily distinguished from each other by wing size and shape (99% of females and 89% of males had acceptably categorized in compared with the biological sexes by wing size and shape across individuals [19]. This study demonstrated that a landmark-based geometric morphometric approach was effective in identifying and distinguishing *C. megacephala*, S. *carnaria*, and M. *domestica*, and is therefore an easy and inexpensive method to aid in discrimination in circumstances of imperfect Samples that are difficult to distinguied by geometric morphometric analysis. [19]. The research revealed that the geometric morphometric approach was effective and highly accurate in distinguishing between males of *S. carnaria M. domestica C. megacephala*, , and, which is one of the methods to quickly assist in differentiation in cases of incomplete specimens that cannot be classified by molecular methods.

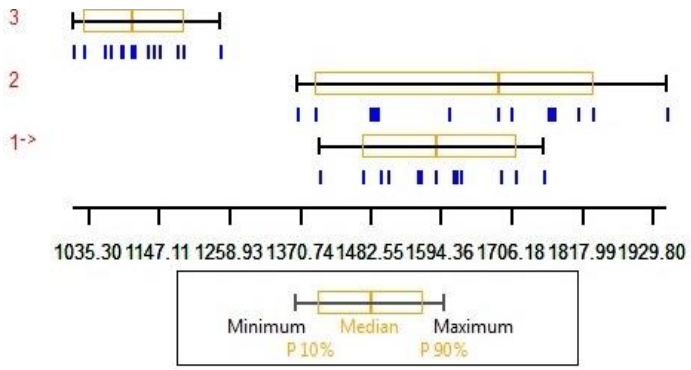


Figure 2: Variation in the central size of the right wing between male medical and forensic insects.

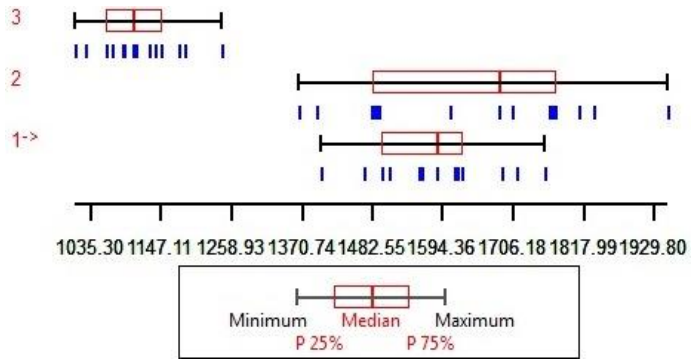


Figure 3: Discriminant analysis plot visualizing the differences in right wing shape among species based on Mahalanobis distances.

Table 1: Statistical analyses of mean of right wings Centroid Size differences between among *C. megacephala, S. carnaria and Musca domestica*.

Species	Mean central size	St.d.	Varience	F-test	p-value	t-test	p-value	A.d.
1 - *C.megacephala*	1576.52	91.27	8330.90	1 vs. 2 3.49	1 vs. 2 0.03	1 vs. 2 1.13	1 vs. 2 0.27	56.81
2 - *S.carnaria*	1633.33	170.59	29102.52	1 vs. 3 2.19	1 vs. 3 0.15	1 vs. 3 15.8	1 vs. 3 1.77	450.35
3 – *M.domestica*	1126.15	61.65	3801.48	2 vs. 3 7.65	2 vs. 3 0.00	2 vs. 3 10.82	2 vs. 3 1.61	507.17

Table 2: Statistical analysis of right-wing shape among species *C. megacephala, S. carnaria and M. domestica* using Model II oneway ANOVA (Arnqvist and Märtensson).

Source	Ss.	df.	Ms.	f.	*P-value*
Side	0.0001	1	0.000087	0.52	0.4744
Side*i	0.0000	2	0.000000	0.00	0.9978
Model	0.0001	5	0.000019	0.13	0.9844
Individual	0.0000	2	0.000009	0.06	0.9395
Residue	0.0053	36	0.000148		

Table 3: Quantitative Statistical analysis of right-wing size among *species C. megacephala, S. carnaria* and *M. domestica* using Model II oneway ANOVA (Arnqvist and Märtensson).

Source	Ss.	df.	Ms.	f.	*P-value*
Side	0.0007	34	0.000020	0.21	1.0000
Individual	0.0014	68	0.000021	0.22	1.0000
Model	0.0032	170	0.000019	0.20	1.0000
Side*i	0.0011	68	0.000017	0.18	1.0000
Residue	0.1167	1224	0.000095		

4 CONCLUSIONS

The findings of this study confirm that geometric morphometrics is a powerful and cost-effective tool for the quantitative genetic differentiation of medical and forensic fly species. By analyzing the right wing morphology of *Chrysomya megacephala*, *Sarcophaga carnaria*, and *Musca domestica*, significant differences in both wing shape and centroid size were detected, validating the reliability of landmark-based morphometric analysis for taxonomic and forensic applications.

The observed variations in Mahalanobis distances and centroid size metrics among the species suggest distinct genetic and phenotypic traits that are consistent across species boundaries. This supports the use of geometric morphometry not only for species identification but also for detecting interspecific and intraspecific morphological diversity, even when traditional morphological markers are inconclusive.

Furthermore, the ability to distinguish specimens using wing morphometrics is particularly valuable in forensic and medical entomology, where sample integrity is often compromised. This technique also provides a complementary or alternative method to molecular identification approaches, especially in resource-limited settings.

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Microalgae as a Biofertilizer on Oat Seed Germination

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Keywords: Biofertilizer, Seed Germination, Spirulina Platensis, Avena Sativa L.

Abstract: Algae are a large and very diverse group of prokaryotic and eukaryotic organisms, autotrophic organisms. These organisms have the ability and potential to produce many secondary metabolites such as proteins, vitamins, amino acids and plant growth regulators and are widely distributed. The effect of different concentrations of Spirulina platensis and Cladophora extract on oat seeds (Avena sativa L.), germination percentage, germination speed, plumule length, radicle length, seedling length, seed vigor, fresh weight and dry weight were studied. Treatment 3 showed the highest germination rates for *S.platensis* extract 88.220%, 2.51333seed day^{-1}, 5.58333 cm, 5.88333 cm, 11.47333 cm, 10110.2, 0.975 g and 0.952 g, while the lowest percentage of treatment 4 was 59.217%, 1.40667, 3.74000 cm, 3.38667 cm, 7.65000 cm, 5084.9, 0.78533 g and 0.37800 g, respectively. While the seed treatment with Cladophora algae extract in treatment 4 showed a germination rate of 84.040%, 2.44667seed day^{-1}, 5.50667 cm, 5.32667 cm, 8333310 cm, 2 9104, 0.91533 g and 0.552167, while the lowest germination rate in treatment 4 was also 45.943%, 1.316637, 0003.39 cm, 3.31000 cm, 7.22667 cm, 2933.9, 0.71733 g and 0.356333 g compared to treatment zero. This study has shown that algae extracts have a positive effect on the germination of oat seeds and that it is possible to produce a commercial and environmentally friendly biofertilizer by using different concentrations of algae extracts.

1 INTRODUCTION

Modern agriculture plays an important role in overcoming the food requirements of the growing world population, which leads to an increased reliance on traditional agriculture that uses chemical fertilizers to increase yields, the main problem that is increasing rapidly now is the excessive use of chemical fertilizers in abundance, including increasing the acidity of the soil due to the decrease in organic matter in it. As well as it kills the beneficial microbes in the soil and responsible for fixing nitrogen in the end these practices lead to a decrease in soil fertility [1], [2].

The rising and increasing global demand for food and increasing environmental impacts due to this intensification of agriculture highlight the critical need for sustainable agricultural methods to improve crop productivity and quality [3]. The global shift towards organic products is driven by the growing demand for sustainable farming practices that promote environmental health, improve soil fertility, and produce food free of harmful chemicals. Agricultural development and management depend heavily on the impact of growth stimulants in boosting crop production, yet chemical growth stimulants pose a major threat to the environment and human health [4].

Algae are a good source of many nutrients such as fiber, minerals, proteins, vitamins and fatty acids that occupy an important place in nutrition, compared to other plants [5].

The presence of an important compound in algae can promote the growth of plants such as auxins, cytokinins and betaines [6]. The latter can affect the development of the root system of the plant [7].

Furthermore, macro and micronutrients can help improve the growth of various vegetables [8], [9]. Oats (*Avena sativa* L.), which is one of the important crops of the gramineae family, has several uses, including as food crops for humans and animals, and the importance of growing oats in recent pots has increased because its grains contain a high percentage of proteins, vitamins, minerals, antioxidants and a high percentage of fiber that is

not soluble [10]. The most productive countries are Russia, the United States of America, Canada and Australia, and 74% of world production is used in animal nutrition in various forms, especially horses and poultry and then ruminants [11]. It has been investigated whether these extracts have a stimulatory effect on seed germination and it is aimed to produce an biostimulant from these extracts.

2 MATERIALS AND METHODS

This experiment was carried out in the winter season of 2024, at the University of Diyala, Laboratories of the College of Education for Pure Sciences (33°40'44.0"N 44°36'00.6"E), which is located about 9.9 km south from the city center, where in 2024 the algae samples were thoroughly and carefully examined and laboratory cleaned of parasites and dust, dried and ground into a fine powder.

2.1 Cultivation and Harvesting

S. platensis isolates were obtained from (Egypt, Cairo, National Research Center, Algae Biotechnology Unit), flask using standard zarrouk culture medium [12], [13], with air presence. The biomass was harvested by centrifugation on day 20 of planting. The biomass was dried in an oven at a temperature of 45 °C for 24 hours and then ground with a grinder and stored at +4 °C. Samples of *Cladophora* algae were obtained locally from Iraq / Wasit Governorate /Al-Ssaouira, Al-Shehaimiya area (30.035840N, 31.205119E).

They were cleaned and washed thoroughly and carefully to get rid of dust and plankton residues, dried at room temperature, ground by grinding and storing at [+4°C.

2.2 Cell Extract

The dried biomass was placed in distilled water at a concentration of 150 $kg.L^{-1}$. To obtain the cell extract with a concentration of 15% W/V, the extract was filtered by Washman filter papers thick 0.34 mm and then a centrifuge was used to remove of the biomass residue at 22° C, and 6000*g for 6 minutes. The resulting extracted liquid was then collected in a vial covered with aluminum foil and stored in a cool room at 4°C. Macro and micro elements of both extracts were measured by (AAS - Atomic Absorption Spectroscopy) In Iraq, the Ministry of Science and Technology. Four solutions of different concentrations were prepared using cell extract [14]:

- T1 , Control 0% W/V (100 ml DIW)
- T2, 10 % W/V (100 ml DIW)
- T3, 20 % W/V (100 ml DIW)
- T4, 30 % W/V (100 ml DIW).

2.3 Seed Collection Center

The seeds of the oat plant were supplied from Iraq and the Ministry of Agriculture Department of Seed Inspection and Certification Baghdad Abu Ghraib district.

2.4 Seed Germination

The germination study of the seeds of the oat plant was conducted using 15 seeds per treatment by 12 experimental units by 4 treatments and 3 replicates. The seeds were placed in Petri dishes containing Whatmann filter leaves soaked in the mentioned treatments and germination was continued to be monitored.

2.5 Measuring Growth Factors

2.5.1 Percentage Measurement Methods

Percentage of germination (%) according to the number of seeds as follows:

$$\textit{Percentage germination} = \textit{number of germinating seeds / total number of seeds} \quad (1)$$

2.5.2 Germination Speed (Seeds / Day)

The germination speed was estimated as follows [15]:

$$\textit{Germination speed} = \textit{number of germinating seeds / days number since the beginning of germination} \quad (2)$$

2.5.3 Measurement of the Length of the Plumule, Radicle and Seedling (cm)

The length of the plumule, radicle and seedling was measured 10 days after cultivating using a graduated tape measure [16].

2.5.4 Measurement of fresh and Dry Weight (g)

The fresh weight of the seedling was estimated using a sensitive balance model Mether PC 400, while the dry weight was calculated after drying the above parts in a 65°C oven until the weight is stable [15].

3 RESULTS AND DISCUSSION

3.1 Chemical Analysis of Algae Extract

Table 1 shows the macro and micro elements found in algae extracts.

Table 1: Chemical analysis of S. *platensis* and *Cladophora* algae extract.

Micro elements (ppm) *S. platensis*					
Mn	Fe	B	Cu	Zn	
380	1110	395	99	1200	
Micro elements (ppm) *Cladophora*					
Mn	Fe	B	Cu	Zn	
210	851	265	76	1110	
Macro elements (%) *S. platensis*					
K2O	S	Ca	Mg	P2O5	N
3.8	1.9	0.9	0.8	7.8	3.9
Macro elements (%) *Cladophora*					
K2O	S	Ca	Mg	P2O5	N
2.8	1.4	0.8	0.9	5.3	2.1

3.2 Germination Percentage

The results of Table 2 show that there are significant differences between the seed activation treatments in the average germination rate, where they show the superiority of the seed stimulation treatment with *S.platensis* extract at a concentration of 20% significantly by giving it the highest average germination rate, which was 88.220% compared to the control treatment (soaking with distilled water), which gave 53.990%, also shows the superiority of the seed stimulation treatment with *Cladophora* extract at a concentration of 20% significantly by giving it the highest germination percentage, which was 84.040% compared to the control treatment, which gave 51.437%, this increase in this treatment was due to its superiority in the average germination speed due to a strong significant relationship between them. Especially since there was a positive significance correlation between the germination percentage and the germination speed, and this was consistent with the study of [17], that the activation of seeds by these extracts leads to a significant increase in the percentage of germination and that the reason for the superiority of this treatment is the role of gibberellic acid in breaking the dormancy of seeds and stimulating germination due to its role in activating the enzymes responsible for germination such as Nuclease, Protease, β-amylase and α-amylase. These enzymes act to digest starch, proteins and nucleic acid and convert them into simple compounds that contribute to the activation of germination. This was confirmed by the results of the study of [18] these results were consistent with [19], [20] and [21].

As for the germination percentage, the results of Table 3 showed the superiority of the treatment 3 and gave the highest germination rate, which was 2.513 seeds/day, compared to the control treatment, which was 1.306 seeds/day when compared to the control treatment, which was 1.306 seeds/day when treated with S. *platensis* extract, while the highest percentage was 2.446 seeds/day when treated with *Cladophora* algae extract, and the lowest percentage was given at treatment 4, which was 1.316 seeds / day.

3.3 Germination Speed

The results of Table 3 showed that the treatment with *S. platensis* extract had a higher percentage of 2.513 seeds/day compared to the control treatment of 1.306 seeds/day than the treatment with *Cladophora* extract, which gave a lower percentage of 2.446 seeds/day compared to the control treatment of 1.343 seeds/day. The reason for this is that the increase in the concentration of *S. platensis* extract led to a decrease in the concentration of toxic ions, which led to an increase in the rates of seed absorption of water and a rise in cellular metabolic reactions related to the performance of the embryo during the germination stage, which reflected positively on the average speed of seed germination, while the increase in the concentration of *Cladophora* algae extract caused an imbalance in the water potential inside and outside the seed, which reduced the rates of seed absorption of water and decreased cellular metabolic reactions that related to the performance of the embryo during the germination stage and reflected negatively on the average speed of seed germination.

3.4 Length of the Plumule (cm)

The results of Table 4 indicate that there are significant differences between the seed activation treatments in the average length of the plumule, the radicle and the length of the seedling, where the results of the study in both extracts showed more than 20% treatment by giving the highest averages for the above-mentioned trait, which was 5.583, 5.506 cm and 11.473 respectively compared to the control treatment, which were 3.556, 2.680 ,6.383 cm, respectively. The results of Table 3 indicate that there are no significant differences between the extracts of both algae. The highest percentage of *Cladophora* extract was recorded, which were 5.506, 5.366 and 10.833 cm respectively when compared to

the control treatment of 3.340, 2.366 and 5.706 cm respectively.

The superiority of the treatment of 10% by giving the highest average length of the plumule, the radicle and seedling to the role of cytokinins are due to the effect of the hormone cytokinin in increasing the absorption of nutrients, including phosphorus, which affected the growth of oat seedlings. The reason for this is due to the role of ascorbic acid in its oxidative enzyme activity, increasing its vegetative growth and encouraging it in the growth of seedling [22].

3.5 Radicle Length (cm)

The results of Table 5 indicate that there are significant differences between the concentrations of seed activation treatments in the average radicle length of the extract of both algae. The highest percentage of the average radicle length characteristic was recorded for *Cladophora* and *S. platensis* at a concentration of 20%, which were 5.883 and 5.326 cm respectively.

The 20% higher treatment is due to the presence of a percentage of hormones and plant growth regulators that increase the processes of cell division and cellular expansion, which they increase the size of the meristematic region as well as increase the number of cells that carry out division [21].

The reason for its superiority is due to the effect of the hormone cytokinin in increasing the absorption of nutrients, including phosphorus, which affected the increase of root growth of the oat plant [23]. The results of Table 4 also showed that there are significant differences in the treatment between the concentrations of the extract of both algae, where it gave the lowest average for the radicle length trait, which were 3.386 and 3.130 cm respectively, due to the increase in the concentration of salts in the extracts, which increases as the concentration increases, and this in turn works to hinder the absorption of nutrients by seeds, which causes nitrogen deficiency, which hinders the proper growth of the plant [24].

3.6 Length of Seedling (cm)

The results of Table 6 indicate that there are significant differences between the seed activation treatments in the average seedling length. The results show that the seed activation treatments were superior in the extract of both algae at a concentration of 20%, which were 11.473 and 10.833 cm respectively, when compared to the control treatment of 6.383 5.706 cm respectively. The reason for this is due to the presence of a proportion of plant hormones and plant growth regulators such as gibberellic acid and cytokinin, which in turn work on cellular expansion and by increasing cell division, which leads to an increase in the size of the meristematic region in addition to increasing the number of cells that carry out the division process. This is consistent with the results of [25].

3.7 Seed Vigor (cm)

The results of Table 7 indicate that there are significant differences between the seed activation treatments in the average seed strength, the highest averages of this trait were recorded in both extracts at a concentration of 20%, reaching 10110.2 and 9103.2 respectively. Perhaps the reason for the superiority of both algae *Cladophora* and *S. platensis* with a concentration of 20% in the seed vigor characteristic was due to their superiority in the percentage of germination and the length of the seed. The strength of the seeds represents the product of multiplying the percentage of germination by the length of the seedling and that any increase in the components of these two traits will lead in the result to a positive increase in the average seeds vigor, while the lowest average of the seed strength trait was for the seed soaking factor to extract both algae at a concentration of 30% due to the low germination ratio as in Table 1 and the average seedling length characteristic in Table 5, which reflected negatively on the average seed vigor trait, and the reason for this is that the product of the multiplying of the germination percentage by the length of the seedling represents the seeds vigor**.**

3.8 Fresh weight (g)

The results of Table 8 indicate that there are significant differences between the seed activation treatments in the average fresh weight trait of the seedling for the extract of both algae at a concentration of 20%.

The results of the study showed that the seed activation treatment in the concentration of 20% in both extracts significantly exceeded the rest of the other treatments by giving it the highest average fresh weight trait of the seed, which were 0.976 and 0.952 g, respectively, when compared with the control treatment, which were 0.383 and 0.373 respectively.

Table 2: Effect of stimulating oat plant seeds with *S. platensis* and *Cladophora* algae extract on average *laboratory* germination percentage (%).

Type of algae	Algae concentration				Average effect of algae
	0	%10	%20	%30	
S. platensis	53.990 d	77.473 b	88.220 a	59.217 c	69.725a
C. glomerata	51.437 e	65.653 c	84.040 a	45.943 f	61.768b
Average concentration effect*	52.714 d	71.563 c	86.130 a	52.580 e	

Table 3: Effect of stimulating oat plant seeds with algae extract on average laboratory germination speed (seed/day).

Type of algae	Algae concentration				Average effect of algae
	0	%10	%20	%30	
S. platensis	1.306 e	2.363 a	2.513 a	1.406 b	1.897 a
C. glomerata	1.343 d	2.193 b	2.446 a	1.316 c	1.825 b
Average concentration effect*	1.325 d	2.278 b	2.480 a	1.361 c	

Table 4: Effect of stimulating oat plant seeds with *Cladophora* and *S. platensis* algae extract on average plumule length (cm).

Type of algae	Algae concentration				Average effect of algae
	0	%10	%20	%30	
S. platensis	3.5566 d	4.273 b	5.583 a	3.740 c	4.288 a
C. glomerata	3.3400 e	4.063 c	5.506 a	3.390 d	4.075 b
Average concentration effect*	3.448 c	4.168 b	5.545 a	3.565 c	

Table 5: Effect of stimulating the seeds of the oat plant with Cladophora and S. platensis algae extract on radicle length cm.

Type of algae	Algae concentration				Average effect of algae
	0	%10	%20	%30	
S. platensis	2.580 g	4.843 b	5.883 a	3.386 e	4.173 a
C. glomerata	2.366 h	4.560 c	5.326 b	3.130 f	3.832 b
Average concentration effect*	2.473 e	4.701 c	5.605 a	3.358 f	

Table 6: Effect of stimulating oat plant seeds with *Cladophora* and *S. platensis* algae extracts on seedling length (cm).

Type of algae	Algae concentration				Average effect of algae
	0	%10	%20	%30	
S. platensis	6.383 g	8.583 c	11.473 a	7.650 c	8.522 a
C. glomerata	5.706 h	7.896 d	10.833 b	7.226 e	7.915 b
Average concentration effect*	6.045 e	8.240 c	11.153 a	8.240 d	

Table 7: Effect of stimulating oat plant seeds with *Cladophora* and *S. platensis* algae extract on seed vigor.

Type of algae	Algae concentration				Average effect of algae
	0	%10	%20	%30	
S. platensis	4373.8 d	5927.6 c	10110.2 a	5083.9 c	6148.9 a
C. glomerata	2933.9 f	3626.4 e	9103.2 b	2933.9 f	5101.0 b
Average concentration effect*	3653.9 d	4777.0 c	9616.7 a	4008.9 b	

Table 8: Effect of stimulating oat plant seeds with *Cladophora* and *S. platensis* algae extract on fresh weight.

Type of algae	Algae concentration				Average effect of algae
	0	%10	%20	%30	
S. platensis	0.383f	0.892 b	0.975 a	0.785 d	0.832 a
C. glomerata	0.616 g	0.821 c	0.915 b	0.717 e	0.767 b
Average concentration effect*	0.500 e	0.857 b	0.945 a	0.751 c	

* Averages with similar letters do not differ significantly at the probability level $p \leq 0.05$.

Table 9: Effect of stimulating oat plant seeds with *Cladophora* and *S. platensis* algae extract on dry weight (g).

Type of algae	Algae concentration				Average effect of algae
	0	%10	%20	%30	
S. platensis	0.373 e	0.473 d	0.952 a	0.378 e	0.544 a
C. glomerata	0.774 b	0.450 e	0.512 c	0.334 f	0.518 b
Average concentration effect*	0.574 b	0.462 d	0.732 a	0.356 e	

* Averages with similar letters do not differ significantly at the probability level $p \leq 0.05$.

The reason for this is due to the high rates of cellular metabolism of seeds soaked with extracts due to their content of plant hormones and growth regulators [26]. Which are responsible for activating hydrolysis enzymes of the nutrients stored in the seeds; that led to increasing the strength of the seeds and stimulating the embryo and then giving seedlings with a high fresh weight [27].

Also, the superiority of this treatment in the fresh weight attribute was due to its superiority in the length of the plumule, radicle and seedling (Tables 3, 4 and 5), which reflects positively on the fresh weight. While the seeds soaked with *Cladophora* and *S. platensis* algae extract recorded the lowest average fresh weight trait in the treatment of 30%, which were 0.717 and 0.758 g respectively. The reason for this is that the use of high concentrations leads to an increase in the concentration of salts and this leads to a failure in germination or the emergence of weak seedlings, which reflects negatively on the fresh weight of the seedling [28].

3.9 Dry Weight (g)

As for the dry weight trait of the seedling, the results of Table 9 show that there are significant differences between the averages of the extract of both algae, which recorded the highest percentage of 0.592 and 0.512 g, respectively, due to the thickness of the root and the number of root hairs when compared with the control treatment, which were 0.373 and 0.374 g, respectively, and the lowest percentage of dry weight in the treatment was recorded 30%, which were 0.378 and 0.334 g, respectively.

4 CONCLUSIONS

Algae are a large and very diverse group of prokaryotic and eukaryotic organisms, autotrophic organisms. Algae extracts can be used to produce organic fertiliser. Stimulating the seed by soaking with algae extract resulted in a significant increase in the average traits analysed, indicating its positive role in breaking the dormancy of the seed and the regular growth of the plant. Also, the use of high concentrations of seaweed extracts when soaking the seeds before cultivation led to negative results and a low percentage of the analysed traits. Treatment 3 showed the highest germination rates for S.platensis extract 88.220%, 2.51333, 5.58333 cm, 5.88333 cm, 11.47333 cm, 10110.2, 0.78533 g and 0.59200 g, while the lowest percentage of treatment 4 was 59.217%, 1.40667, 3.74000 cm, 3.38667 cm, 7.65000 cm, 5084.9, 0.78533 g and 0.37800 g, respectively. This study has shown that algae extracts have a positive effect on the germination of oat seeds and that it is possible to produce a commercial and environmentally friendly biofertilizer by using different concentrations of algae extracts.

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Anatomical Changes in the Tissues of Phragmites Communis Accumulated with Cadmium in the Rivers and Drains of Diyala Governorate

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Keywords: Common Reed (Phragmites Australis), Anatomical Features, Cadmium, Drainage Water, River Water.

Abstract: The problem of pollution of water environments with heavy elements is a major environmental challenge in Iraq in general, including Diyala Governorate. Where the governorate witnessed in recent years a great pollution in rivers and homes due to industrial, agricultural and home waste that contains varying proportions of heavy elements, including cadmium, which is considered one of the most dangerous environmental pollutants due to its high heaven and stability in the environment, where the importance of this research arises to know the effect of cadmium accumulation on the anatomical structure of the wild cane plant (phragmites australis) The developing in rivers and homes, where five sites were studied in Diyala Governorate (Khanaqin, Baquba, Al -Khalis, Al - Al-Muqdadiya, and Balad Ruz). The results of the research indicated that there are no concentrations of cadmium in the water, while we found a clear accumulation of it in the soil, especially in the locations of the yields that recorded much higher concentrations (up to 0.15 mg/kg) compared to the river water soil. In the plant's leg, cadmium concentrations were not detected, indicating that the wilderness has an effective mechanism to stabilize the heavy elements in its roots and limit its transmission to the air parts. The anatomical changes in the tissues of the plants in the waters of the clients also showed compared to other river water. There were a decrease in the thickness of the vascular package by rates ranging between 7.44% and 34.01%, and a larger shrinkage in Qatar, the wood container, with rates of 69.35% in some locations. Because of the environmental stress resulting from the presence of cadmium.

1 INTRODUCTION

The problem of pollution of water environments with heavy elements in Iraq in general and Diyala Governorate in particular is one of the most dangerous contemporary environmental challenges. In recent years, rivers and hostels have become a major future for industrial, agricultural and home waste containing varying proportions of heavy elements, which are considered one of the most dangerous environmental pollutants due to their high toxicity and stability in the environment. This situation requires urgent measures to protect water resources [1]. The continuous accumulation of pollutants in river deposits greatly affects the environmental balance and leads to the accumulation of toxins in the food chain and among the heavy elements [2], cassudm is highlighted as a dangerous pollution as a result of its toxic effect on environmental systems, as it can affect the chemical balance in the water, which increases its risk on living organisms , [3] the [Phragmites australis] .L most important plants used in phytoremediation technology. It also shows high efficiency in the absorption and concentration of specific heavy minerals such as cadmium, which nominates it for polluted water treatment programs [5]. Recent studies indicate that the floating wet land systems planted with wild cane plants have a high efficiency in removing cadmium from running rain water and polluted water, with the ability of the plant to withstand high concentrations of this element without great impact on growth and development, which enhances practical applications for this plant in sustainable environmental treatment techniques[6]. Wild cane is also characterized by a different accumulation of toxic elements in its various organs (roots, stems, leaves), which makes it

an effective biological indicator to monitor the levels of pollution in the aquatic environmental systems. This contrast in the distribution of heavy elements between the plant tissues reflects the various mechanisms to absorb, transfer and store these elements within the plant, and provides a deeper understanding of the physiological and anatomical plants towards environmental stress caused by pollution with heavy minerals [7]. Despite these features, the high accumulation of cadmium in the plant tissues may lead to notable anatomical changes, such as increasing the thickness of cellular walls, changes in vascular tissues, a decrease in the physiological efficiency of the plant [8]. The roots have a high ability to absorb these minerals from the surrounding environment, which causes clear changes in their anatomical structure, [9]. The cadmium accumulation inside the wood contains increases its thickness of its walls and reduces its countries, which negatively affects the efficiency of water and nutrient transport [10]. The pollution also leads to clear anatomical signs that are the deposition of silica and the foresters and deformities in the sects. Green, which leads to a decrease in the production of chlorophyll and reduce the number and size of the gaps [11]. The current research aims to study the anatomical changes in the tissues of the growing wild cane in the rivers and homes of Diyala province as a result of the accumulation of cadmium, and it is expected that the results of this study will contribute to deepening the understanding of this plant's response to stress resulting from this element, and evaluating the possibility of its use in environmental monitoring programs and plant treatment For polluted water, the results will also help in determining the relationship between the levels of pollution with cadmium and anatomical changes in the tissues of this plant, which enhances the possibility of its use as a vital indicator of environmental pollution with these elements.

2 MATERIALS AND METHODS

2.1 The Selection of Study Sites

This study was conducted in five districts belonging to the Diyala/Iraq Governorate, where several sites were chosen along the rivers and homes that are exposed to various sources of pollution with heavy elements. The selection process was carefully done to ensure accurate representation of varying pollution levels, with a focus on areas affected by industrial, agricultural and home waste. The various environmental factors that may affect the distribution of heavy metals in soil and water have been taken into account.

2.2 Sampling Collection

The samples were collected on 10/2/2023 until 11/15/2023 from the districts of Diyala Governorate, where the sites were carefully chosen to ensure geographical and environmental diversity. The vegetable samples were collected, then washed, cleaned and placed in clean plastic bags with the site's registration and date on it, and then took samples of water from each site using sterile plastic packages of 1 liter, and added a few drops of concentrated nitric acid (HNO_3) to control the pH as well as to prevent the precipitation of heavy metals and the packages were tightly closed, and finally the soil samples were collected from the bottom of the samples The vegetation that was taken to study at a depth ranges between 0-30 cm using a manual tool and was placed in plastic bags with the registration of the sample number and the location that is site and the date, and the temperature and the PH in the water were measured directly for all the sites from which the sample was taken, and all samples were transferred to the laboratory for the necessary laboratory analyzes (see Table 1) [12] .

Table 1: PH and temperature for the five sampling sites.

Location	Water Type	PH	Temperature (°C)
Khanaqin	River water	7.03	22
	Drainage water	7.63	21
Baquba	River water	7.50	19
	Drainage water	7.83	20
Al-Khalis	River water	7.22	25
	Drainage water	7.71	24
Al-Muqdadiya	River water	7.18	22
	Drainage water	7.49	24
Balad Ruz	River water	7.39	20
	Drainage water	7.55	21

2.3 Chemical Analysis of Samples

After completing the collection of samples and transferring them to the laboratory, placed in the electric oven (Oven), where the vegetable samples were placed at a temperature of 60 ° C for four days, while the soil was placed at a temperature of 100 ° C for 48 hours, in order to dry them from moisture and then the leg samples were grinded by a ceramic mortar, and they were stamped across an sieve with a 2 mm diameter.

500 mg of each sample was taken and placed in a glass gep, adding 9 ml of nitric acid (HNO_3) concentration of 65%, 1 ml of hydrogen peroxide (H2O2 30% concentration), root and leg samples while 8 ml of nitric acid (HNO_3), 65% and 2 ml of hydrogen peroxide (H2O2) is added Focus 30%, for paper samples.

500 mg of each dried soil sample was weighed and placed in a glass spike added 9 ml of nitric acid (HNO_3 concentration), of 65%, and 1 ml of hydrogen peroxide (H2O2 30%concentration).

Then the samples were transferred to the microbial digestive system at a temperature of 180 °C for 30 minutes (see Table 1). The samples were nominated using filtration and the concentrations of heavy elements were measured using the ICP -OES device based on the method approved by [13].

2.4 Preparation of Plant Samples and Laboratory Procedures

2.4.1 Preparing Anatomical Clips

The vegetable samples were extracted from the alcohol solution (70%), and was washed with distilled water to remove any residue of the alcohol, samples were cut to make very thin luminous clips to the extent of reaching a bright anatomical clip showing the tissues and cells clearly and with a thickness ranging from (5-7 cm) using a sharp dissection code under the anatomy microscope, the clips were transferred to a minor solution Diluted with distilled water with a concentration of 5 % to get rid of chlorophyll dye for 5-10 minutes. Glycerin solution, then gently put the slices to avoid the formation of air bubbles, After completing the preparation of the sample, and its golden pladium, the microscopic features were examined using the FE-SEM [15].

2.4.2 Preparation of Plant Sample Sections

The vegetable clips of the leg were prepared using the hand securityment, samples were cut into parts of 4-6 cm from specific areas, then fixed in a solution

Table 2: Cadmium concentration in water, soil and leg for wild cane plants in the five sites.

Location	Water Type	Soil	Stem	Mean±std	p-value
Khanaqin	River water	0.07	ND	0.02±0.04	0.015*
	Drainage water	0.15	ND	0.05±0.09	
Baquba	River water	ND	ND	0.00±0.00	0.043*
	Drainage water	0.15	ND	0.05±0.09	
Al-Khalis	River water	0.08	ND	0.03±0.05	0.022*
	Drainage water	0.15	ND	0.05±0.09	
Al-Muqdadiya	River water	0.01	0.01	0.00±0.01	0.008**
	Drainage water	0.13	ND	0.05±0.07	
Balad Ruz	River water	0.01	ND	0.00±0.01	0.062
	Drainage water	0.08	ND	0.03±0.05	

*Significant at probability level $p < 0.05$ **Significant at probability level $p < 0.01$

(F.A.A), which contains 50 ml of ethanol alcohol (95%), 10 ml of formalin, 5 ml of olive acid, and 35 ml of distilled water, for a period of 48 hour. After that, the samples were washed twice with ethanol alcohol (70%) to remove the effects of the stabilizer, and then kept in alcohol with the same concentration until the anatomy is performed [14].

2.5 Statistical Analysis

Data was analyzed using SPSS (version 22), where the arithmetic average and standard deviation were calculated to describe the variation of values of cadmium concentration in water and soil and the legs of the wild reed plant within different environments. To assess and impact on the anatomical qualities of the plant tissues, the contrast analysis (Anova) was used to compare the differences between different sites, with the adoption of ($P < 0.05$) as a standard to determine the significance Statistics.

3 RESULTS

Table 2 shows the results of cadmium concentration in the water, soil and the stem of wild the cane plants. Cadmium was not been detected in water samples in all locations, whether in river water or wastewater. The means (± standard deviation) showed a clear variation between wastewater and river water, as wastewater in all sites was recorded higher compared to river water. The wastewater was recorded in the locations of Khanaqin, Baquba and Al -Khalis, the highest value (0.05 ± 0.09), while the pure site recorded the highest value for river water (0.03 ± 0.05) (see Fig. 1).

Cadmium concentrations in the soil ranged from undetected at the Baquba site to 0.15 ppm in drainage water soil at several sites. As for the concentration of cadmium in the stem, most sites have recorded uncomfortable values, except for the stem of the plant in the river waters at the Muqdadiya site (0.01) parts per million.

Statistical moral tests revealed the presence of moral differences ($P < 0.05$) between river water and wastewater in four of the five locations, with the highest moral at the Al -Muqdadiya site ($P = 0.008$), while Balad Rose site did not show a significant difference ($P = 0.062$).

Table 3 shows the presence of clear anatomical and moral changes in the growing wild cane in the waters of the locally compared to its counterpart in clean water, where a decrease in the thickness of the vascular package ranged between 7.44% in the Baquba site and 34.01% in the site of Muqdadiyah. As for the diameter of the wood container, it witnessed a greater decrease, as the rate of decrease reached 69.35% in the Baquba site, followed by Muqdadiya and Blarages sites by 25.00% (Fig. 2).

Table 4 illustrates the wood pot in the leg of the wild cane in the five sites based on the measurements of the electronic microscope scanned in clean rivers and the waters of the clients. All sites showed a decrease in the diameter of the wood pot in the developing plants in the waters of the locally compared to river water. The Baquba website recorded the highest rate of decrease (52.6%), followed by the Baldrouz sites (35.1%), then Sharean (29.5%). Al -Khalis site also recorded the lowest decrease (19.4%), followed by Khanaqin (21.4%). The average decrease in Qatar was the wood container for all sites (31.6%)) (Fig. 3 and 4).

Table 3: The percentage of change in the anatomical measurements of the leg of the cane plant within the study sites.

Location	Anatomical criterion	River water	Drainage water	Percentage change (%)
Khanaqin	Vascular bundle thickness	103.4	86.3	-16.54
	Xylem vessel diameter	2.5	2.3	-8.00
Baquba	Vascular bundle thickness	111.5	103.2	-7.44
	Xylem vessel diameter	6.2	1.9	-69.35
Al-Khalis	Vascular bundle thickness	105.5	93.5	-11.37
	Xylem vessel diameter	2.4	1.9	-20.83
Al-Muqdadiya	Vascular bundle thickness	88.5	58.4	-34.01
	Xylem vessel diameter	4.4	3.3	-25.00
Balad Ruz	Vascular bundle thickness	118.2	106.5	-9.90
	Xylem vessel diameter	3.2	2.4	-25.00

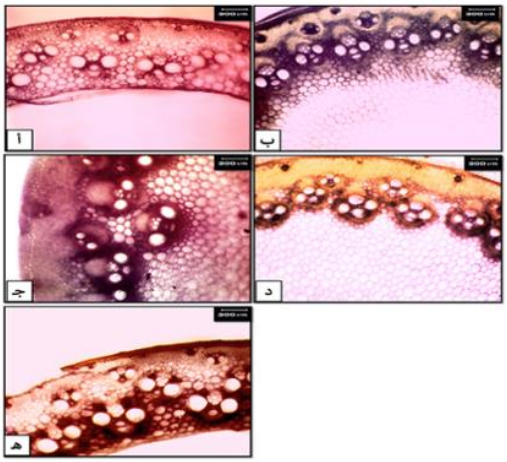


Figure 1: Stem from clean water.

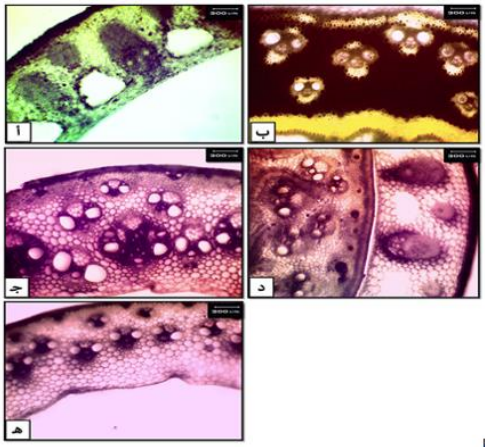


Figure 2: Stem from drainage water.

Table 4: The diameter of the wood bowl in the leg of the wild cane based on the e-microscopic microscope measurements (SEM).

Location	Xylem vessel diameter in river water	Xylem vessel diameter in drainage water	Difference	Percentage decrease (%)
Khanaqin	2.8	2.2	0.6	21.4
Baquba	9.7	4.6	5.1	52.6
Al-Khalis	3.6	2.9	0.7	19.4
Al-Muqdadiya	4.4	3.1	1.3	29.5
Balad Ruz	5.7	3.7	2.0	35.1
Average	5.24	3.30	1.94	31.6

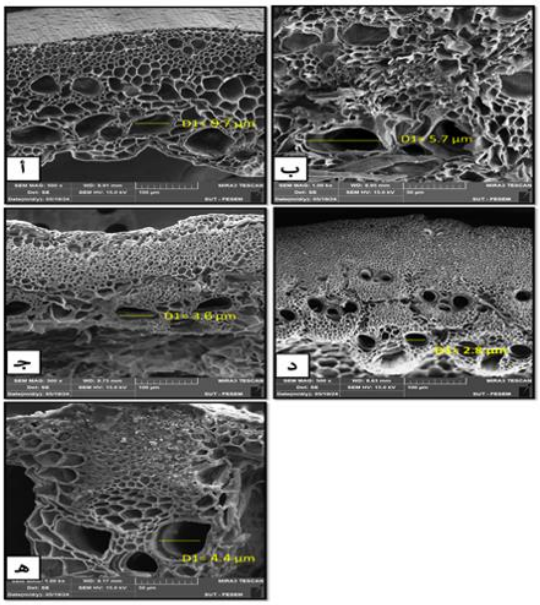


Figure 3: SEM images of stem cross-sections water.

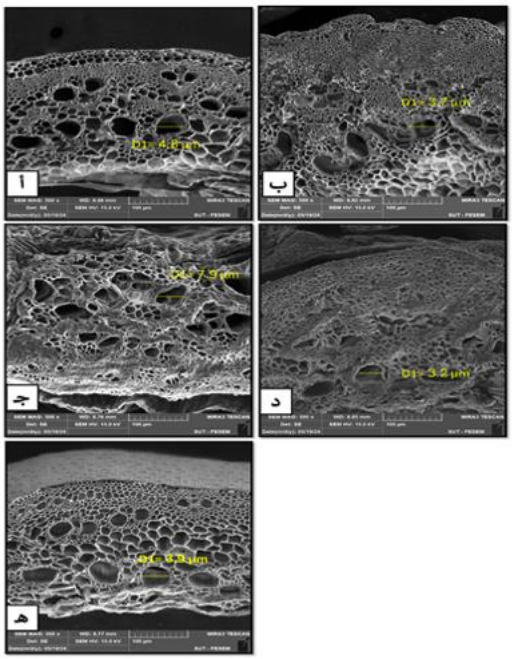


Figure 4: SEM images of stem cross-sections from the river from drainage water.

4 DISCUSSION

The results of the study showed that the concentration of cadmium in the leg of the wild cane was zero in all locations except for the site of

Muqdadiyah in the river waters, where the concentration reached 0.01 mg/kg. This indicates that the wild cane plant tends to stabilize the heavy elements in the roots instead of transferring them to the air parts, which corresponds to a study that emphasized the ability of water plants to reduce the accumulation of cadmium in the air tissue [16], in addition, another study found that some aquatic plants have the ability to enhance the stabilization of heavy metals in the roots through complex physiological mechanisms such as the production of proteins that It is associated with cadmium [17]. Another study also showed that the acidic soil increases the cadmium installation in the roots, which limits its transition to the air parts [18]. The current study also indicates a noticeable decrease in the thickness of the vascular package and the diameter of the wood bowl in the developing plants in the waters of the yield compared to river water. The average decrease in the thickness of the vascular package was 34.01% in Muqdadiya, while the decrease in the diameter of the wood container was 69.35% in Baquba according to optical microscope measurements, while the SIM microscopic measurements (SEM) that amounted to 52.6%. Exposure to cadmium leads to the contraction of wooden vessels and inhibiting the activity of the vascular Campium, which negatively affects the transportation of water and nutrients, which was confirmed by a recent study on the chickpea plant, where a decrease in the diameter of the wooden vessels and its number was observed, as a result of the accumulation of cadmium in the vascular tissues [19]. Another study also showed that cadmium reduces the synthesis of cellulose and ligans, which leads to a decrease in the thickness of the vascular package and the contraction of the vascular diameter in the peas as a result of anatomical changes and vascular deformities caused by cadmium [20]. On the other hand, a recent study conducted on the willow plant indicates that some plants develop compensatory mechanisms to improve water transportation and reduce the effect of cadium on wooden vessels by enhancing the root mass and expanding the size of the secondary vessels [21]. A recent study also confirmed that the use of microbial sedimentation technology with ochrobactrum splash. POC9 can effectively reduce the vital availability of cadmium in the soil and its toxic effect on plants.

5 CONCLUSIONS

The study revealed that most types of juices and soft drinks available in the local markets in Baghdad contain levels of heavy elements that fall within the permissible limits according to the standards of the World Health Organization. However, recording abuses in the concentrations of some elements such as silver (AG), chromium (CR), and aluminum (AL) in some samples indicates the need to be cautious, as the average concentration of silver (0.2746) parts per million exceeding the permissible limit 0.1, and the risk index reached 1.569143, while the risk index of aluminum reached 2.346857 as a result of the dose exceeding The permitted daily. As for the lanes of arsenic, cadmium and chrome remained within the safe borders, while the cumulative index of health risks to all elements reached 5,1855, indicating the possibility of health risks when repeated consumption of these products. The study recommends tightening control over these products and educating consumers of the need to moderate the consumption of soft juices and drinks and choose healthy alternatives when possible.

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Freshwater Gastropoda in Diyala River: A Study on Diversity, Distribution, and Environmental Variation

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Keywords: Freshwater Gastropods, Snails Diversity, Environmental Factors, Diyala River.

Abstract: This field study investigated the diversity, spatiotemporal distribution, and environmental relations of freshwater gastropods in the Diyala River (Iraq). Monthly surveys were conducted from September 2024 to March 2025 at three stream sites (Mahroot, Al-Saria, Rose), recording gastropod densities and water quality variables (temperature, pH, dissolved oxygen, and electrical conductivity). Six gastropod species were identified: *Physa acuta*, *Melanopsis costata*, *Theodoxus jordani*, *Melanoides tuberculata*, *Bellamya bengalensis*, and *Lymnaea* sp., with species richness varying from 4 to 6 across sites. The most abundant taxa were *Melanoides tuberculata* and *Bellamya bengalensis*. In contrast, *Melanopsis costata* and *Theodoxus jordani* were exclusive to Al-Saria in late winter. Community structure changed seasonally, with *Melanoides tuberculata* peaking in autumn and *Bellamya bengalensis*, *Lymnaea* sp., and *Physa acuta* increasing in winter. Temperature ranged from ~29°C to ~9°C, with slight changes in pH (7.2–8.8), dissolved oxygen (6.3–8.8 mg/L), and conductivity (520–680 µS/cm). Results highlight the value of combining seasonal biological surveys with physico-chemical monitoring for biodiversity conservation and biomonitoring.

1 INTRODUCTION

The phylum Mollusca is considered one of the most important phyla belonging to invertebrate animals. Generally, the animals of this phylum are characterized by their soft, unsegmented bodies and a calcareous shell (shell) consisting of a head, foot, visceral mass, and mantle [1]. The phylum Mollusca, in terms of the number of described species, is one of the largest and most common groups, ranking second after Arthropoda and Vertebrates [2]. Mollusks are highly heterogeneous in terms of size, shape, and adaptation to various environments (terrestrial, marine, aquatic), except for air, nutrition, and behavior, in addition to the significant radiation exposure this phylum experienced during the Cambrian period [3] and others. Mollusks have a high adaptability, as they can be found in almost all types of water bodies and are present in (Stygobiont) animals (for example, animals that live in water-saturated caves). In many natural water bodies, the class Gastropoda is considered one of the most important classes belonging to the phylum Mollusca, as it includes more than 25,000 genera and groups such as snails, clams, and bivalves. Gastropods are characterized by a well-defined head and foot and asymmetrical organs, a feature resulting from the unique torsion process that occurs during genetic development [4]. The types of snails that live in freshwater vary significantly in their adaptation to the different biotic and abiotic factors that control their distribution and abundance in space and time. Therefore, the goal is to understand how the communities of different species living in freshwater are structured and how environmental factors affect their distribution [5]. Freshwater snails have immense health and veterinary importance because they serve as intermediate hosts for parasitic infections in humans and animals, such as schistosomiasis, fascioliasis, and many other trematode infections. Many of these species belong to the class Gastropoda. Additionally, species from the families Pilidae and Thiaridae have been recorded as habitats for trematode larvae [6]. Therefore, identifying them has become extremely important for zoologists and malacologists [7]. The dynamics of serious parasitic diseases worldwide are influenced by several factors, such as climate change and temperature, dissolved oxygen, and rainfall patterns, in addition to important physiological factors, which can affect the distribution and abundance of snail species and consequently the transmission of diseases [8]. The

dual-gene perforated mollusks are characterized by complex life cycles that involve intermediate hosts (mollusks and vertebrates) and a final host to complete their life cycle [9]. In addition to the commercial importance of aquatic snails for human consumption, they are also considered very important from an economic, ecological, and environmental monitoring perspective due to their unique biological characteristics and their ability to adapt to changing environments [10].

2 MATERIAL AND METHODS

2.1 Study Site

Diyala River is a major River in Iraq, one of the most important tributaries of River Tigris. It is a main water resources of Iraq that provides supplies for drinking and agriculture purposes. Many cities are closely situated along its banks. Unfortunately, the river gradually became dump site for fluid and solid wastes produced from agricultural and industrial activities [11].The site that chosen Regarded as the down stream part of Diyala river, particularly at the Diyala Dam at Al Sudor area, the river is divided to several branches of small size Rivers including Al-Rose, Mahroot,and Al-Saria [12]

2.2 Collection and Identification of the Sample

The samples were collected monthly with 3 replicates from three Sites (map 1), including the Al-saria stream in the Baqubah district, the Mahroot stream in the Muqdadiyah district, and the Rose stream in the Baladruz district. And this is for the period from the beginning of Sep. 2024 until March 2025. Samples were collected at 1-2 meter from the river bank with depth 0.5 meter by use quadrate (1*1) m and pick up the snails were then transported to the laboratory in plastic containers with some of the

Figure 1 illustrates the Diyala River Basin, highlighting the locations of the study sites.

River water to preserve them for a longer period during transportation. Preserved samples of Mollusks were identified according to the shell features, and in some cases depending on internal parts. Well-known key references were used in this process: [13]

Environmental factors were measured monthly directly by used WalkLAB instrument at all study sites, including temperature, pH, electrical conductivity, dissolved oxygen.

2.3 Statistical Analysis

To determine the differences between environmental factors in each month and sites and Pearson's correlation analysis between density of species and environmental factors, use of the Statistical Analysis System(SAS).

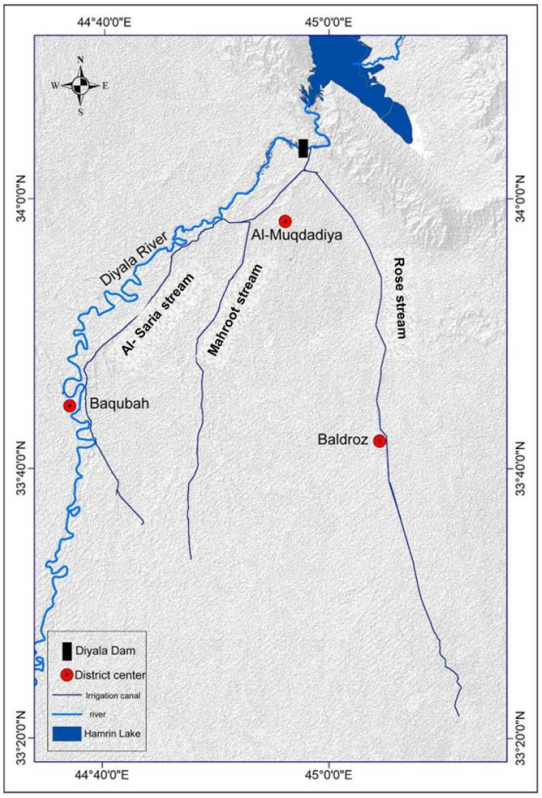


Figure 1: Diyala river.

3 RESULTS AND DISCUSSION

3.1 Ecological Study

As it appear in Figure 2 environmental factors during the study period at the three sites (Mahroot, Al-Saria, and Al-Rose) showed variations between months and sites. A gradual decrease in water temperatures was observed from the autumn months to the winter months, with the lowest temperature recorded in February (9.8°C at the Al-Saria site). This decrease is likely to have an adverse effect on the physiological and reproductive activities of snails, as temperature is a determining factor in the presence and growth of mollusks [14]

As for pH values, they ranged from moderate to weakly alkaline, as is the case in all Iraqi water bodies, with the highest level recorded in Al-Saria Creek during September (8.85). These values are suitable for many freshwater snail species, most of which thrive in environments with a pH between 7 and 9, due to the presence of calcium ions necessary for building their shells [15]. The significant increase in pH values at the Mahroot site during January (8.64) provided more stable conditions for the growth of species such *as B. bengalensis* and *M. tuberculata*, which were abundant during this period.

Dissolved oxygen (DO) concentrations varied slightly between sites and months, with the highest values recorded in November (8.8 mg/L in Al-Rose). Lower temperatures are known to lead to water retaining more oxygen [16], which may explain the increased density of some lung snail species in the colder months, particularly *P. acuta* and *Lymnaea sp*., which are often found in well-ventilated environments.

Electrical conductivity (EC) values varied between months and sites, ranging from 520 µS/cm (in Al-Rose during September) to 680 µS/cm (in Mahroot) during March. These changes reflect variations in ion concentrations, which may be due to changes in surface runoff or evaporation levels, as well as rainfall and the leaching of sediments from adjacent river areas. Some species, such as *M. tuberculata*, are known to tolerate a wide range of electrical conductivity values [17], which explains their continued presence at the Mahroot site despite changing conditions.

3.2 Biological Study

The results of the snail survey revealed differences in the density and distribution of freshwater snail species. Six species were recorded: Physa acuta, Melanopsis costata, *Theodoxus jordani*, *Melanoides tuberculata*, *Bellamya bengalensis*, and *Lymnaea sp*.

Melanoides tuberculata recorded the highest density at the Mahrot site during the first months (September and October), reaching 80 and 78 individuals/m², respectively, before declining significantly in December and January. This may be due to the invasive nature of this species and its ability to withstand varying environmental conditions. Recent studies have indicated that this species is an invasive species capable of rapidly colonizing new environments due to its high adaptability to varying environmental conditions, such as high temperatures and conductivity [18], [19].

B. bengalensis showed relatively high densities at the Mahroot and Rose sites in December (50 and 20 individuals/m², respectively), indicating a preference for mid-winter conditions. This aligns with findings by [20] in Southeast Asia, where the species favored moderate temperatures and stable oxygen levels.

Physa acuta, a widespread species, peaked during colder months (e.g., 12 individuals/m² at Rose in March), consistent with its tolerance for unstable conditions and moderate water quality (Fig. 3) [21].

Rare species (Melanopsis costata, Theodoxus jordani) occurred at low densities exclusively at Al-Sari in February–March, likely due to specific habitat requirements linked to benthic sediments and water quality [22]. In contrast, Lymnaea sp. thrived across sites, peaking at Rose (20 and 17 individuals/m² in February/March), demonstrating adaptability to low temperatures (Table 1) [23].

Pearson's correlation analysis between the monthly densities of the studied snail species and the average values of the environmental factors revealed variations in the direction and strength of the relationship. The results showed a strong relationship between *P.acuta* and electrical conductivity ($r = 0.895$, $p = 0.0065$), indicating that increased concentrations of dissolved ions in the water positively affect the density of this species. Physa acuta is known for its high adaptability to chemically variable environments and its ability to exploit resources in waters rich in dissolved substances.

The relationship between *Physa acuta* and other environmental factors, such as temperature, pH, and DO, was not statistically significant. Although the negative relationship with temperature ($r = -0.688$, $p = 0.0872$), it may indicate that this species prefers cooler temperatures, which is consistent with observations of seasonal distribution during the study period.

As for the other species, *Melanoides tuberculata* and *Bellamya bengalensis*, the statistical analysis results recorded some moderate relationships with environmental factors, but they cannot be considered statistically significant ($p > 0.05$). This may be due to their unbalanced distribution among sites or their influence by local environmental factors that were not measured in this study.



Figure 2: Monthly average of ecological factors of study sites from September 2024 to March 2025.

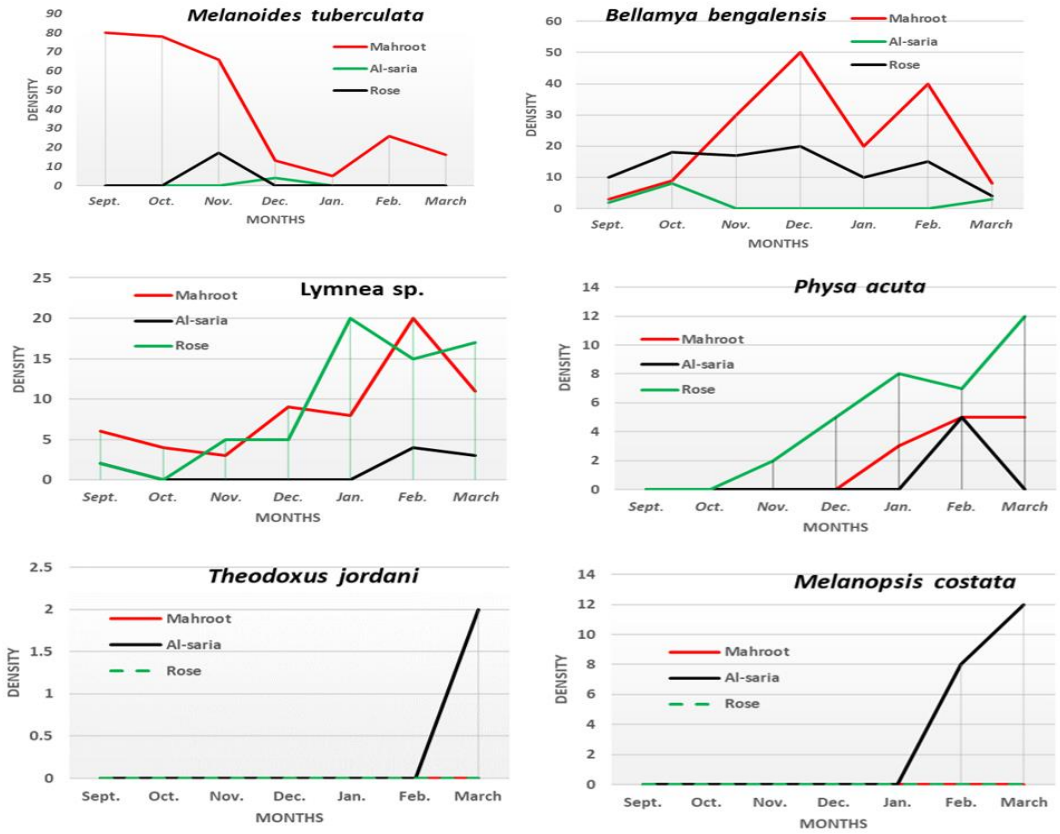


Figure 3: Monthly Average of density of freshwater gastropods in the studied sites from September 2024 to March 2025.

Table 1: Pearson's correlation analysis between the densities of the snail species and the environmental factors.

Species	Temperature	pH	DO	EC
Physa acuta	-0.688	0.339	0.08	0.703
Melanopsis costata	-0.411	-0.134	0.08	0.703
Theodoxus jordani	-0.121	-0.396	-0.133	0.555
Melanoides tuberculata	0.624	-0.397	0.002	-0.717
Bellamaya benglensis	-0.491	0.115	0.806	-0.354
Lymnea sp	-0.626	0.51	-0.058	0.882

4 CONCLUSIONS

This study highlights the diversity of freshwater Gastropod communities in the Diyala River, based on field surveys conducted monthly from September 2024 to March 2025 at three sites along the Diyala River. Six species were recorded, including *Melanoide tuberculat*, which had its highest density at Mahrut in early autumn (80 and 78 individuals/m² in September and October, respectively), demonstrating its ability to invade studied environments and its resilience to high temperatures and electrical conductivity. In contrast, *Physa acuta* and *Lymnaea* showed increased density during the colder months, particularly at the Rose site in February and March, where *Lymnaea* reached 20 individuals/m², indicating a preference for lower temperatures and higher oxygen levels. *Melanopsis costata* and *Theodoxus jordani* were only recorded in the Saria area in late winter, confirming their environmental sensitivity and potential exposure to habitat changes. Pearson correlation analysis revealed

a statistically significant positive relationship between *Physa acuta* density and electrical conductivity ($r = 0.895$, $p = 0.0065$), confirming this species' ability to adapt to changing environmental conditions at the selected sites. The results of this study emphasize the importance of combining biological monitoring with the assessment of certain environmental parameters to better understand species distribution and distribution patterns, as well as ecosystem health. These findings underscore the value of these communities as bioindicators of environmental stress.

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