Data-Driven Evaluation of Natural and Chemical Pesticides for Tick Control

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Abstract: Ticks are external parasites that are hemophagic. They are considered serious health risks to animals and humans. This study investigates whether a natural extract from beetles could be a highly effective natural pesticide against two species of Hyalomma ticks, comparing it to two common chemical pesticides, Cypermethrin and Ivermectin. Using laboratory experiments. Fourier transform infrared spectroscopy (FT-IR) was conducted to identify the peaks of both the beetle extract (Cicindelidae) and currently used pesticides. The results of the current study referred to the presence of 9 peaks in the beetle extract (Cicindelidae), which indicated the presence of C-O bonds at peaks 1270.45 cm^{-1} and 1112.19 cm^{-1}, which have the ability to kill ticks through their effect on the nervous system. 9 peaks were also identified for the Cypermethrin pesticide, elucidating the presence of a carbonyl group at peak 1740.31 cm^{-1} only, while the number of peaks in the Ivermectin pesticide was 9. 12 peaks showed the presence of the carbonyl group at peak 1712.34 cm^{-1} only. While using of the gas chromatography-mass spectrometry (GC-MS) technique obtained the presence of 22 chemical compounds with different retention times in the beetle extract. The active compounds are octadecenamide, myristic acid, methyl ester, nonanoic acid, and tricosene. While the pesticides showed the presence of 29 chemical compounds in the pesticide Cypermethrin, including deltamethrin, and 31 chemical compounds in the pesticide Ivermectin, including metalaxyl and triethanolamine, which are effective against ticks. The results of laboratory experiments based on several concentrations of beetle extract (Cicindelidae) 25, 50, 75 and 100 mg/ml. The concentration of 100 mg/ml within 48 hours achieved a 100% killing rate when compared to the locally used pesticides .This study concludes that tiger beetle extract is a very promising, natural acaricide that could be a viable alternative for current chemical treatments.

1 INTRODUCTION

Ticks, belonging to the Ixodidae family, are blood-feeding external parasites that are of great importance in the medical and veterinary fields. They are serious pests that affect human and animal health, both directly and indirectly, leading to significant economic losses in livestock and public health problems [1]. Their danger extends beyond bloodsucking; they act as vectors for a wide range of pathogens, including viruses, bacteria, and protozoan parasites [2]. An anticoagulant present in tick saliva, injected into the host during tick feeding, suppresses the host's immune response and facilitates the transmission of pathogens, including hemorrhagic fevers [3]. Tick infestations, particularly with *Hyalomma anatolicum* and *Hyalomma excavatum*, is a significant risk to threaten livestock health and productivity. The growing resistance to traditional synthetic acaricides, searching medicinal plants and natural extracts, such as insects, as safer and environmentally friendly alternatives is critical for sustainable tick control [4]. Current tick control methods rely on traditional methods limited to the excessive and irregular use of pesticides. This may the emergence of resistant tick strains, in addition to resulting in environmental pollution and the accumulation of these pesticides in various animal and plant products, rendering them unfit for human use, raising crucial concerns about food safety and the environment [5].

In light of these challenges, global attention has grown in the search for natural alternatives that effective and environmentally friendly for tick control and safe to use. Extract from plants and other organisms have emerged as promising sources of bioactive compounds with acaricidal properties [6], and reduce its resistance to antibiotics [7].

Among these organisms, increasing interest had been attracted to those belonging to the Cicindelidae Family in various research areas. These predator beetles are recognized for producing a wide range of defensive chemical compounds to protect themselves from natural enemies and microbes. Recent research has confirmed that these defensive secretions are not just simple deterrents, but rather a mix of complex chemicals containing compounds such as alkaloids and benzoquinones that display antimicrobial and insecticidal activity [8].

Bioprospecting for natural products from arthropods, including beetles, is a promising management for developing a new generation of biopesticides. These natural compounds often characterized biodegradable and have unique modes of action, reducing the riskiness of resistance development compared to synthetic pesticides. Therefore, studying compounds produced by tiger beetles [9].

2 MATHERIALS AND METHODS

2.1 Sample Collection

Manually collection of ticks specimens (*H. excavatum and H. anatolicum*) was conducted from cattle using forceps and alcohol cautiously to prevent loss of mouth-parts, The information, such as infestation sites and the number of ticks per animal, was recorded from May to July 2025 in Baqubah District (Bani Saad, Buhriz, and Al-Abbara sub-districts), Diyala Governorate, Iraq. Tiger beetles (Cicindelidae) were collected from orchards in Buhriz and Al-Abbara, Diyala Governorate, using light traps between February and April 2025 for extraction purpose

2.2 Preparation of Extract

The extraction process was carried out through the following steps using Soxhlet:

1) Washing and sterilization. Fifty grams of adult beetles (Cicindelidae) samples were taken, washed with 70% methanol and sterilized using a steam autoclave at 15 psi for 10 min.
2) Grinding. The sterilized beetles were ground using an electric grinder.
3) Filtration and Soxhlet extraction. The ground material was placed in filter paper (9 cm) and extracted using a Soxhlet apparatus with 400 ml of hexane for 24 hours.
4) Concentration. The extract was separated from the solvent using a rotary evaporator for 8 minute.
5) Storage. The concentrated extract was stored in dark containers for FTIR, GC-MS analysis, and laboratory bioassays to test its effect on ticks [10].
6) Preparation of concentrations. Multiple concentrations (25, 50, 75, and 100 mg/ml) were prepared for bioassays

Figure 1: Stages of the extraction process of chemical compounds from (Cicindelidae).

2.3 Experimental Design

Ten ticks (5 males and 5 females) were placed in Petri dishes, with three replicates for each concentration of the beetle extract. The ticks were sprayed with the prepared extract. Mortality rates and time of death were recorded, starting from zero time and at intervals of 0.5, 1, 2, 3, 6, 12, 24, 48, and 72 hours [11], [12].

2.4 Chemical Analysis

The chemical analysis of the Cicindelidae extract was carried out using, Fourier-transform infrared spectroscopy (FTIR), Conducted with a Shimadzu spectrometer (Japan) at the laboratories of the Ministry of Science and Technology. Gas Chromatography–Mass Spectrometry (GC-MS) Performed using an Agilent Technologies GC-MS 7890 system (USA) at the laboratory department of Basra Oil Company. The process began at 40 °C, with a 10 °C/min increase up to 300 °C. Helium carrier gas was maintained at 1 ml/min. One microliter of extract was injected, and peaks were identified using the [NIST] database and device memory library [13], [14].

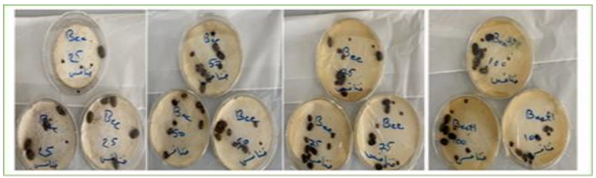

Figure 2: Mortality rates of ticks (Hyalomma excavatum and Hyalomma anatolicum) using different concentrations (25, 50, 75, 100 mg/ml) of (Cicindelidae) extract.

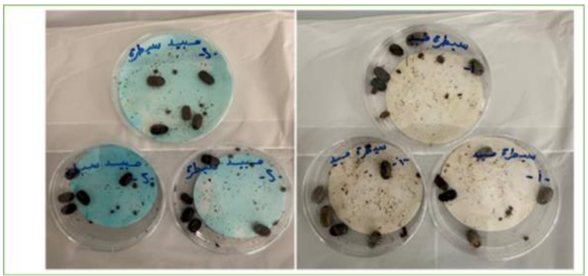

Figure 3: Mortality rates of ticks (Hyalomma excavatum and Hyalomma anatolicum) using different concentrations (25, 50, 75, 100 mg/ml) of the insecticides Ivermectin and Cypermethrin.

3 RESULTS

3.1 FTIR Chemical Analysis

3.1.1 Cicindelidae Extract

Fourier transform infrared (FTIR) spectroscopy results indicate the presence of nine peaks, each representing the following:

- The characteristic carbonyl peak (C=O) at 1737.10 cm^{-1}: This perfectly matches the absorption band of the ester.
- The presence of C-O bonds at 1270.45 cm^{-1} and 1112.19 cm^{-1}: These bonds are essential in the ester structure.
- The presence of aliphatic chains (C-H) at 2925.33 cm^{-1} and 2854.72 cm^{-1}, in addition to bending vibrations at 1464.57 cm^{-1} and 1384.91 cm^{-1}: This confirms the organic nature of the compound and the presence of alkyl moieties.

The peak at 3435.54 cm^{-1} (-OH group) may indicate that the ester contains an additional hydroxyl group (hydroxyester), as shown in Table 1 and Figure 4. This confirms the presence of chemicals that affect the tick's nervous system, leading to its death.

3.1.2 Cypermethrin

FTIR results of Cypermethrin revealed nine peaks, including, Carbonyl (C=O) at 1740.31 cm^{-1} (ester absorption).C–O bond at 1122.22 cm^{-1}. Aliphatic C–H chains at 2956.50, 2925.31, and 2854.82 cm^{-1}, with bending vibrations at 1465.78 cm^{-1} and 1384.51 cm^{-1}.Hydroxyl (-OH) group at 3431.17 cm^{-1} as shown in Table 2 and Figure 5.

Table 1: Peaks of chemical compounds in Cicindelidae extract using FTIR.

Peak Number	X (cm-1)	Y (%T)
1	3435.54	66.58
2	2925.33	62.99
3	2854.72	66.84
4	1737.10	70.69
5	1631.90	69.34
6	1464.57	69.77
7	1384.91	69.51
8	1270.45	70.31
9	1112.19	68.11

Table 2: FTIR peaks of chemical compounds in Cypermethrin.

Peak Number	X (cm-1)	Y (%T)
1	3431.17	65.60
2	2956.50	64.33
3	2925.31	55.46
4	2854.82	62.01
5	1740.31	69.65
6	1631.77	68.50
7	1465.78	67.69
8	1384.51	68.83
9	1122.22	68.01

3.1.3 Ivermectin

FTIR results of Ivermectin showed twelve peaks, including, Carbonyl (C=O) peak at 1712.34 cm^{-1} (carboxylic acid absorption). Broad O–H peak at 3413.39 cm^{-1}, confirming hydroxyl group presence. C–O bonds at 1204.91 cm^{-1} and 1114.42 cm^{-1}.Long aliphatic C–H chains at 2925.59 cm^{-1} and 2854.74 cm^{-1}, with bending vibrations at 1465.81, 1406.39, 1384.73 cm^{-1}, and CH_2 rocking at 722.12 cm^{-1}. As shown in Table 3 and Figure 6.

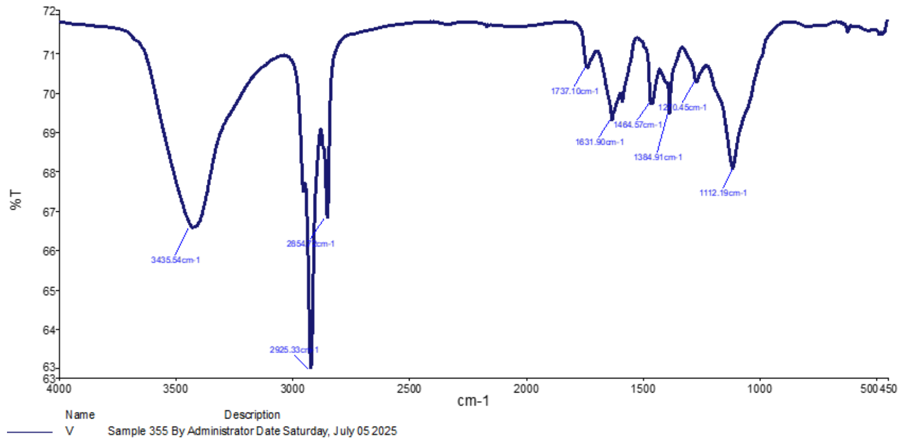

Figure 4: FTIR peaks of chemical compounds in Cicindelidae extract.

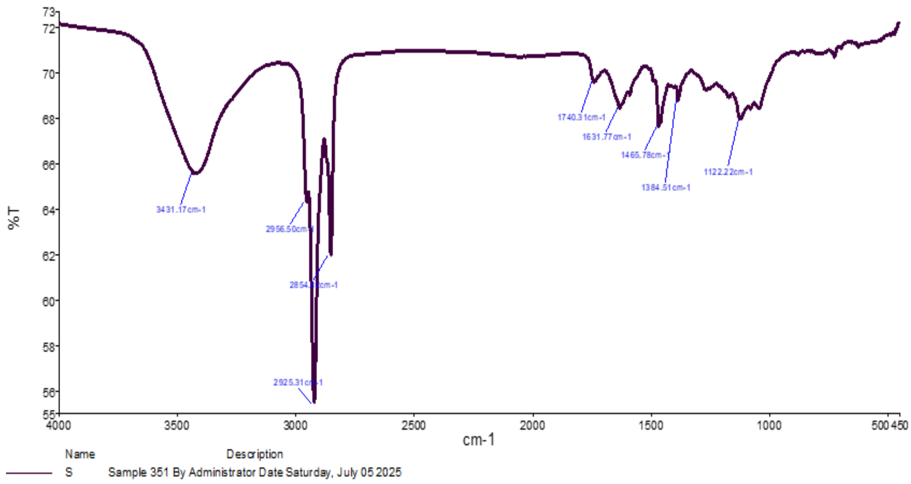

Figure 5: FTIR peaks of Cypermethrin.

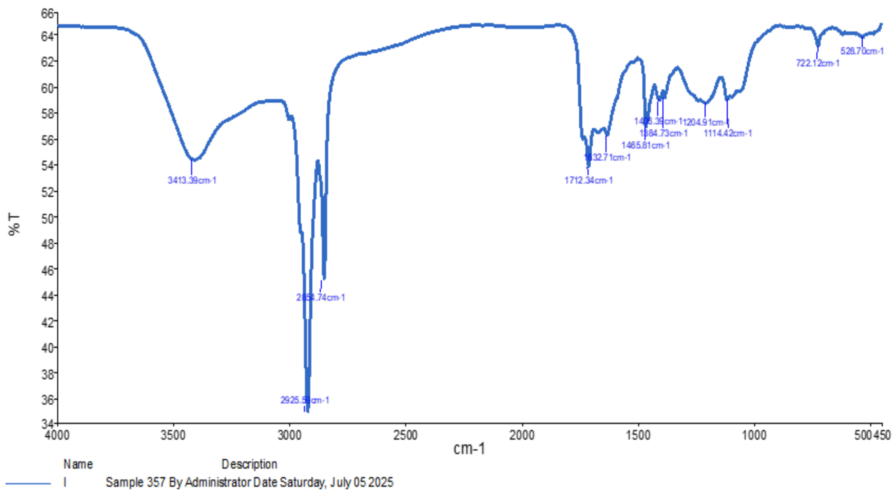

Figure 6: FTIR peaks of Ivermectin.

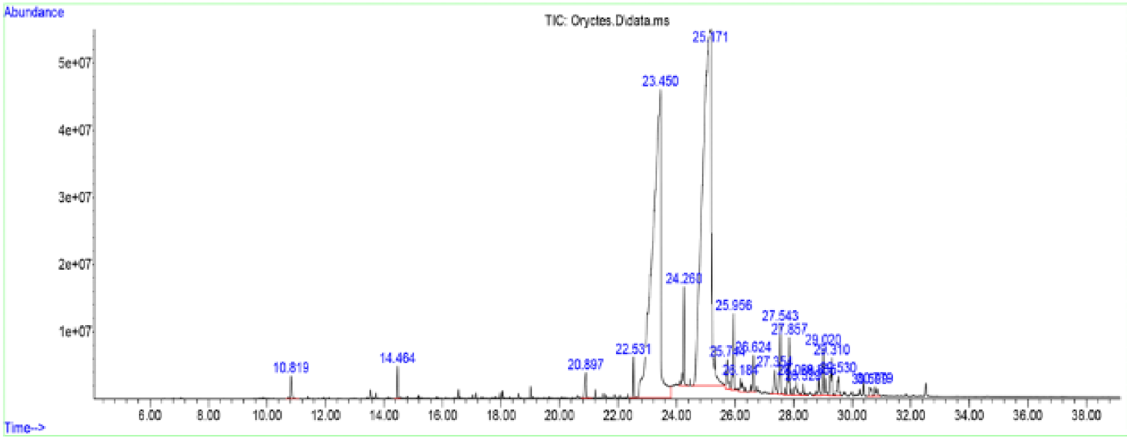

Figure 7: GC–MS peaks of Cicindelidae extract.

Table 3: FTIR peaks of chemical compounds in Ivermectin.

Peak Number	X (cm-1)	Y %T)
1	3413.39	54.45
2	2925.59	34.93
3	2854.74	45.26
4	1712.34	53.98
5	1632.71	56.38
6	1465.81	57.06
7	1406.39	59.11
8	1384.73	59.27
9	1204.91	58.88
10	1114.42	59.14
11	722.12	63.27
12	528.70	63.95

3.2 GC–MS Chemical Analysis

3.2.1 Cicindelidae Extract

GC–MS analysis revealed 22 compounds with different retention times. Key bioactive compounds included, Tetradecanoic acid (Myristic acid) (RT = 20.894), Hexadecanoic acid, methyl ester (RT = 25.319), Nonanoic acid (RT = 23.452), Octadecenamide (RT = 26.626) In Table 4 and Figure 7.

Table 4: The effective chemical compounds for killing ticks in the extract of (Cicindelidae) using GC-MS technology.

N	Name	M.W	R.T.	% Area
1	Octadecenamide	281	26.626	0.5561407
2	Tetradecanoic acid (Myristic acid)	228	20.894	0.5805823
3	Hexadecanoic acid, methyl ester	270	25.319	0.5376797
4	Nonanoic acid	158	23.452	28.199424
5	Tricosene	322	25.953	1.0774844

3.2.2 Cypermethrin

The results obtained using Gas Chromatography–Mass Spectrometry (GC–MS) showed the presence of 29 chemical compounds with different retention times in Cypermethrin. Among them were, Deltamethrin with a retention time of 31.668, peak area of 6,062,972, and a percentage of 1.0579107. Benzaldehyde, 3-phenoxy with a retention time of 20.335, peak area of 3,296,525, and a percentage of 0.5752012.1-Propanone, 2,2-dimethyl-1-(4-phenoxyphenyl) with a retention time of 30.644, peak area of 2,233,281, and a percentage of 0.3896788. As shown in Table 5 and Figure 8.

Table 5: Active compounds in Cypermethrin by GC–MS.

N	Name	M.W	R.T.	% Area
1	Deltamethrin	505	31.668	1.0579107
2	Benzaldehyde, 3-phenoxy	198	20.335	0.5752012
3	1-Propanone, 2,2-dimethyl-1-(4-phenoxyphenyl)	254	30.644	0.3896788

3.2.3 Ivermectin

The results obtained using Gas Chromatography–Mass Spectrometry (GC–MS) revealed the presence of 29 chemical compounds with different retention times in Ivermectin. Among them were, Metalaxyl with a retention time of 22.665, peak area of 4,941,909, and a percentage of 0.9699. Benzyl Alcohol with a retention time of 11.285, peak area of 253,899,806, and a percentage of 58.05527. Triethanolamine with a retention time of 7.752, peak area of 35,864,584, and a percentage of 8.200589. As shown in Table 6 and Figure 9.

Table 6: Active compounds in Ivermectin by GC–MS.

No	Name	M.W	R.T.	% Area
1	Metalaxyl	279	22.665	0.9699
2	Benzyl Alcohol	108	11.285	58.05527
3	Triethanolamine	149	7.752	8.200589

3.3 Adult Immersion Test

An adult immersion test was used to evaluate the effect of different concentrations (25, 50, 75, and 100 mg/ml) of tiger beetle (Cicindelidae) extract on the mortality rates of tick (Hyalomma) over different time periods (1, 3, 6, 12, 24, and 48 hours).

The results, shown in Table 7 and Figures 2 and 3, showed an increase in the mortality rates of both tick species over time in a concentration-dependent manner. In contrast, groups treated with currently used acaricides (Ivermectin and Cypermethrin) showed only a slight mortality rate, confirming the superior performance of the Cicindelidae extract against the tested tick species (Fig. 10).

A one-way ANOVA revealed statistically highly significant differences between the treatment groups, with an F-value = 48.92 and a P-value < 0.0001. This indicates that the extract and the pesticides had distinctly different effects on tick mortality, as shown in Table 8.

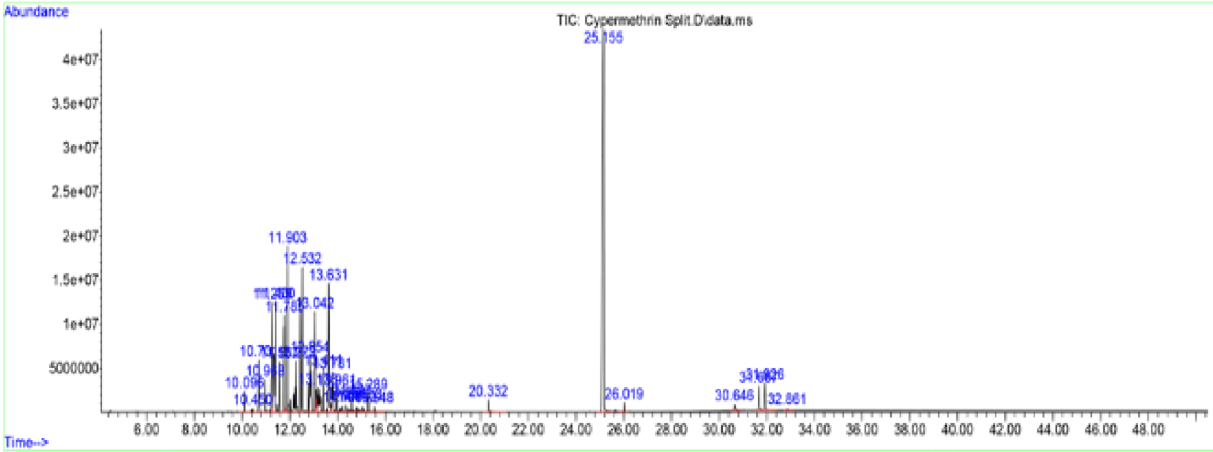

Figure 8: GC–MS peaks of chemical compounds in Cypermethrin.

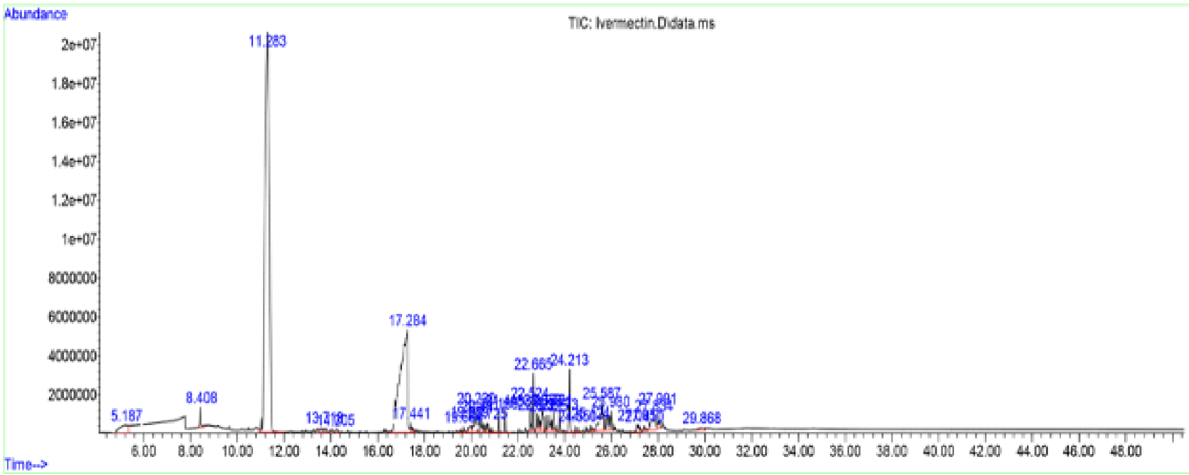

Figure 9: GC–MS peaks of Ivermectin.

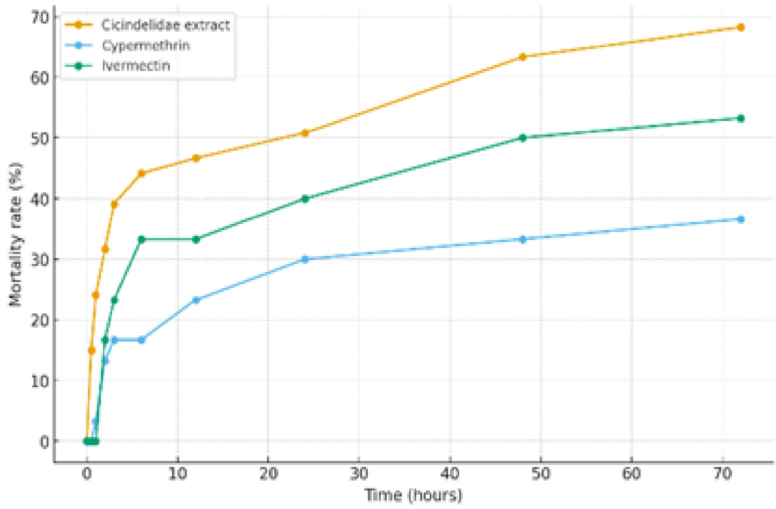

Figure 10: Tick mortality over time by treatment.

Table 7: Mortality rates of ticks (Hyalomma excavatum and Hyalomma anatolicum) using different concentrations and exposure times of extract (Cicindelidae), cypermethrin, and ivermectin.

Time (hours)	Extract 25%	Extract 50%	Extract 75%	Extract 100%	Cypermethrin	Ivermectin
0	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
1	20.00%	20.00%	23.30%	63.30%	13.30%	13.30%
3	23.30%	26.70%	33.30%	73.30%	16.70%	16.70%
6	30.00%	30.00%	40.00%	86.60%	23.30%	23.30%
12	30.00%	36.60%	50.00%	86.70%	30.00%	33.30%
24	43.30%	50.00%	60.00%	100.00%	33.30%	40.00%
48	43.30%	53.30%	76.60%	100.00%	36.60%	53.30%

Table 8: Showing the significant difference between the extract (Cicindelidae), and the pesticides cypermethrin and ivermectin.

Source of Variation	Between Groups	Within Groups	Total
Sum of Squares	24580.67	17720	42280.67
Degrees of Freedom	5	174	179
Mean Square	4916.13	101.84	
F-value	48.92	-	-
P-value	<0.0001	-	-

Table 9: showing the means and standard deviation for the extract (Cicindelidae), cypermethrin, and ivermectin pesticides.

Treatment Group	Mean	Standard Deviation	Sample Size
Extract 25%	43.30%	± 2.5%	30
Extract 50%	53.30%	± 3.1%	30
Extract 75%	76.60%	± 4.2%	30
Extract 100%	100.00%	± 0.0%	30
Cypermethrin	36.60%	± 2.8%	30
Ivermectin	53.30%	± 3.5%	30

The means and standard deviations of mortality rates after 48 hours of exposure were calculated, as shown in Table 9 it is noted that the 100% extract concentration achieved the highest mortality rate, while cypermethrin had the lowest.

Post-hoc comparisons (Tukey's test) showed the following:

- The 100% Extract was significantly superior to all other groups ($P < 0.001$). while;

- The 75% Extract was significantly superior to Cypermethrin ($P < 0.001$) and the 25% Extract ($P = 0.003$).in the other hand;
- No significant difference was found between the 50% Extract and Ivermectin ($P = 0.985$), indicating comparable efficacy between them, as shown in Table 10.

Table 10: Showing a comparison between the concentrations of extract (Cicindelidae), cypermethrin, and ivermectin.

Comparison	Difference	P-value
100% vs Cypermethrin	63.40%	<0.001
100% vs Ivermectin	46.70%	<0.001
100% vs 25%	56.70%	<0.001
75% vs Cypermethrin	40.00%	<0.001
75% vs 25%	33.30%	0.003
50% vs Ivermectin	0.00%	0.985

Based on the results and LC50 values, the treatments were ranked as shown in Table 11.

Table 11: Showing the final order of treatment used as an effective pesticide against ticks (Hyalomma excavatum and Hyalomma anatolicum).

Rank	Treatment	Efficacy	Recommendation
1	Extract 100%	Excellent	⭐⭐⭐⭐⭐
2	Extract 75%	Very Good	⭐⭐⭐⭐
3	Extract 50%	Average	⭐⭐⭐
4	Ivermectin	Average	⭐⭐⭐
5	Extract 25%	Poor	⭐⭐
6	Cypermethrin	Very Poor	⭐

To determine the effectiveness of different concentrations, the median lethal concentration (LC50) was evaluated. LC50 represents the concentration required to kill 50% of the study sample within 24 hours. Based on the dose-response curve derived from the data, approximate values were as follows: For Ciscindilidae extract, the median lethal concentration was approximately 45%. This indicates high efficacy, as a moderate concentration is sufficient to achieve a 50% mortality rate.while for Ivermectin, The median lethal concentration was approximately 48%, demonstrating good efficacy, slightly lower than the 50% extract concentration, according to the comparison results.

Cypermethrin acaricide, recorded the highest median lethal concentration (greater than 50%), confirming its relative weakness compared to other treatments at medium and low concentrations, which is consistent with its "very weak" classification.

The LC50 analysis reflects the higher efficacy of the Cicindelidae extract, achieving a lethal effect at a lower concentration compared to the conventional chemical pesticides used in the study, highlighting its strength as a natural acaricide.

4 DISCUSSION

The results of this study confirm the acaricidal efficacy of tiger beetle (Cicindelidae) extract against the resistant ticks Hyalomma excavatum and Hyalomma anatolicum, suggesting its potential use as a natural alternative to chemical acaricides, The extract demonstrated significantly higher efficacy than Cypermethrin and Ivermectin at 100% concentration, achieving complete tick mortality within 24 hours, compared to much lower mortality rates observed with the chemical acaricides over the same period, These results align with the global trend toward searching safe and environmentally friendly alternatives for pest control, particularly given the increasing occurrence of resistance of ticks to chemical acaricides and their negative effects on the environment and human health [5], [6].

Chemical analysis of the tiger beetle extract using GC/MS obtained the presence of compounds such as Tricosene and Nonanoic acid in high quantities. These compounds are known for their various biological properties, including insecticidal and acaricidal activities. For instance, Tricosene has been notified to possess insecticidal and acaricidal properties [13]. Similarly, fatty acids like Nonanoic acid exhibit pest control effectiveness by disrupting cell membrane functions [15]. Furthermore, other compounds with impacts on ticks were identified, including Tetradecanoic acid (Myristic acid), a saturated fatty acid. Some studies have reported that fatty acids and their derivatives may have acaricidal or repellent activity against ticks [16]. Additionally, Isopropyl myristate, an ester of meristic acid, is commonly used in flea and tick control products for pets [17]. Ester (known as methyl palmitate), a fatty acid ester. Research indicates that some methyl esters of fatty acids show acaricidal performance [17], and this compound has been stated in plant extracts with tick-killing properties. Octadecenamide is a fatty acid amide. Some studies propose that fatty acid amides may have insecticidal or acaricidal activity. Particularly, 9Z-octadecenamide has been determined in certain organisms and shown to have antimicrobial and antioxidant activities, which may indicate broader biological characteristics.

In comparison, the synthetic acaricides used in this study, Ivermectin and Cypermethrin, obtained lower efficacy [18]. Cypermethrin is a pyrethroid insecticide known for its efficiency against a wide range of insects and ticks, but it faces challenges because the development of resistance [1]. A prominent chemical related to Cypermethrin is Deltamethrin, a widely used synthetic pyrethroid insecticide, known for its high acaricidal performance and repellent effect. Deltamethrin is extensively used for tick control in livestock and pets, obtain effectiveness against both adult ticks and larvae [19]. However, studies indicate that tick resistance to Deltamethrin has arisen in some areas, necessitating alternative or integrated control strategies [20]. Benzaldehyde, 3-phenoxy- is an intermediate component used in the synthesis of pyrethroid insecticides such as Cypermethrin and Fenvalerate [21]. Some studies propose that benzaldehyde derivatives may have repellent activity against insects and ticks [22]. While not an acaricide itself, its role in insecticide formulations makes it important in tick control. Propanone, 2,2-dimethyl-1-(4-phenoxyphenyl)- is part of some pyrethroid There is limited direct information about its effect on ticks when used alone; however, its presence in tick-control formulations suggests it may contribute to the overall efficacy, possibly as a synergist or component enhancing the active ingredient's effect. Ivermectin is an anthelmintic and insecticidal drug widely used; however, its efficacy may vary, and some tick species may develop resistance [23]. One of its notable components is Benzyl Alcohol. Benzyl Alcohol is an organic compound used as a solvent, preservative, and antiseptic in many products [24]. Recent studies have shown that benzyl alcohol possesses acaricidal activity against certain tick species, including lethal effects on adult ticks, larvae, and eggs. It may also have a synergistic impact when used with other tick acaricides, enhancing their overall activity [25]. Finally, the high levels of nonanoic acid and the presence of other compounds such as octadecinamide and methylhexadecanoic acid ester (present in lower concentrations in the extract) give the extract its tick-killing properties, as nonanoic acid possesses tick-control capabilities. Nonanoic acid, when combined with other fatty acids, appears to act as an effective organic insecticide for livestock and general insect control. Without any systemic action or long-term soil residue, it works by interfering with cell function and inducing oxidative stress in target organisms by rupturing cell membranes, removing the waxy cuticle, and causing rapid desiccation (drying out) and cell death, thus facilitating the penetration of pesticides. These compounds, particularly nonanoic acid, are short-chain fatty acids and natural products, perfectly aligned with the principles of the Green Revolution. It is characterized by its low toxicity; wearing gloves is usually sufficient to avoid skin irritation. It is also approved by the US Food and Drug Administration (FDA) as non-carcinogenic and of negligible toxicity; this agree with [26], [27]

5 CONCLUSIONS

The high performance of (Cicindelidae) extract, long with its biological features, Fourier transform infrared spectroscopy (FT-IR) was conducted to identify the peaks of both the beetle extract (Cicindelidae) and currently used pesticidesThe results of the current study referred to the presence of 9 peaks in the beetle extract (Cicindelidae), which indicated the presence of C-O bonds at peaks 1270.45 cm^{-1} and 1112.19 cm^{-1}, which have the ability to kill ticks through their effect on the nervous system, While using of the gas chromatography-mass spectrometry (GC-MS) technique obtained the of 22 chemical compounds with different retention times in the beetle extract, (presence of nonanioc acid, octadecinamide, and methyl hexadecanoic ester together gave the insect extract highly effective tick-killing properties compared to the synthetic pesticides used in the comparison), makes it a promising candidate for the development of unique biopesticides. Such biopesticides are often biodegradable and possess novel modes of action, decreasing the risk of resistance development compared to synthetic acaricides. Moreover, the use of natural extracts helps reduce environmental pollution and the health risks related to chemical pesticides.

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