Bioactive Compounds and Antifungal Activity of Sesame Seed Oil and Chaetomium Globosum Extracts Against Fusarium Solani

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Keywords: Sesame Seed Oil, Endophytic Fungi, GC-MS Analysis, Bioactive Metabolites, Antifungal Activity, Fusarium Solani.

Abstract Plant-derived compounds and endophytic fungi are eco-friendly alternatives for controlling phytopathogenic fungi.This study aimed to identify bioactive compounds in sesame (Sesamum indicum L.) seed oil and the endophytic fungus *Chaetomium globosum*, and evaluate antifungal activity against *Fusarium solani*, the causal agent of vascular wilt in peanut plants under in vitro conditions.same oil and fungal extracts were chemically analyzed to determine bioactive constituents, and antifungal activity was evaluated against *Fusarium solani* using four concentrations (0, 2.5, 5, and 7.5 mL L^{-1}). Chemical analysis revealed the presence of 10 compounds in sesame seed oil, with iridomyrmecin (21.79%) as the major component, followed by oleic acid (16.67%), palmitic acid (14.53%), and sesamin (8.99%). Similarly, 10 compounds were identified in the *Chaetomium globosum* extract, with thymol (8.13%) as the dominant compound, along with linolenic acid (4.74%) and hesperetin (3.58%). As for the antifungal activity, the results showed that the sesame oil extract at concentrations of 5 and 7.5 ml/L exhibited strong inhibitory activity against the growth of the pathogenic fungus, with a percentage of 100%. The concentration of 2.5 ml/L showed an inhibitory capacity of 44.44% compared to the control treatment, where the inhibition percentage was 0%. The results showed that the endophytic fungus extract at a concentration of 7.5 ml/L exhibited strong inhibitory activity against the growth of the pathogenic fungus, with a percentage of 100%. The concentrations of 2.5 and 5 ml/L recorded inhibitory capacities of 77.77% and 83.33% respectively, compared to the control treatment, where the inhibition percentage was 0%. These results indicate the high efficiency of sesame seed oil extract at concentrations (5, 7.5) ml L^{-1}, and the endophytic fungus *Chaetomium globosum* extract at a concentration of (7.5) ml L^{-1}, in demonstrating their antifungal effectiveness, confirming the possibility of utilizing them as promising natural sources in combating pathogenic fungi.

1 INTRODUCTION

Field peanut belongs to the family Fabaceae and is known by several common names such as poor man's almond, groundnut, and peanut. Despite the nut-like appearance of its seeds, it is botanically classified as a legume. Field peanut has contributed significantly to reducing malnutrition associated with protein deficiency, which has led to its increased cultivation in many regions worldwide [1]. Fungal diseases represent one of the major challenges facing farmers, as crop losses resulting from plant diseases may exceed 50% in tropical and subtropical regions [2]. Among the fungi responsible for root rot disease is the genus Fusarium. Root rot disease in field peanut has been attributed to more than 11 species of Fusarium, with *Fusarium solani* reported as the most prevalent species. It has been observed that all isolated species are capable of causing the disease [3]. The fungus *Fusarium solani* was historically classified within the division Deuteromycota (imperfect fungi), while according to modern classification it has been placed within the phylum Ascomycota. *Fusarium solani* commonly grows saprophytically in soil or on plant debris and decomposing organic matter. However, it may become an opportunistic parasite on living plant tissues, causing a range of root diseases, including root rot [4].

Sesame (Sesamum indicum L.) is considered one

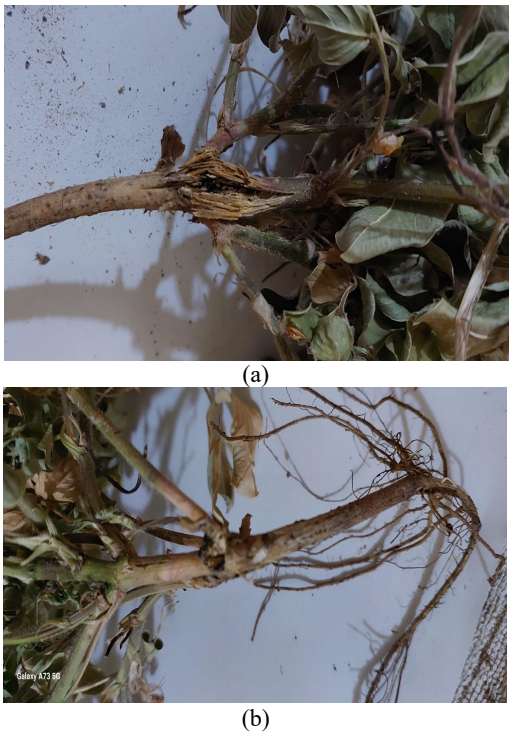
of the important oilseed crops cultivated widely around the world and belongs to the family Pedaliaceae. Sesame seeds contain a wide spectrum of biologically active compounds, most notably sesamin, sesamol, and sesaminol, in addition to terpenoids, anthraquinones, naphthoquinones, polyphenols, phytosterols, and flavonoids [5]. The high oxidative stability of sesame oil is attributed to the presence of lignans and tocopherols, which act as effective natural antioxidants [6].

The fungus *Chaetomium globosum* is one of the widely distributed endophytic fungi and is characterized by its strong ability to produce a diverse range of secondary metabolites. This fungus plays an important ecological role by actively contributing to the decomposition of organic materials, particularly plant compounds rich in cellulose, thereby enhancing nutrient cycling in various ecosystems. It is widely distributed in different environments, as species of the genus Chaetomium occupy diverse ecological niches and are often isolated from soil, seeds, and decomposing plant residues [7]. *Chaetomium globosum* has received considerable attention due to its ability to produce a variety of bioactive compounds effective against numerous plant pathogens, including fungi and nematodes [8]. In addition, *Chaetomium globosum* is capable of parasitizing fungal hosts and colonizing plant roots, which may enhance plant growth and strengthen plant defense responses [9].

2 MATERIALS AND METHODS

2.1 Isolation and Identification Pathogenic Fungus

With the aim of isolating the fungus causing root and stem base rot disease in field pistachio plants, samples were collected from infected field pistachio plants from a number of fields located in the Jalula district, northeast of Baquba, the center of Diyala Governorate in Iraq. 69 infected plants were collected from four different areas of Jalawla district, including Marjana, Kli, and Sheikh Baba, and placed in plastic bags and transported to the Mycology Laboratory, Department of Life Sciences, College of Education for Pure Sciences, for the purpose of isolating the pathogenic fungus *Fusarium solani*. The infected part was taken from the area where the stem meets the root, as shown in Figure 1. It was washed with water to remove dirt and cut into small pieces of 0.5 mm in length. Then it was immersed in a 2% sodium hypochlorite solution for 4 minutes to remove surface contaminants. After that, it was washed with sterile distilled water three times and transferred to Petri dishes containing SDA medium. Then the dishes were placed in the incubator at a temperature of 25 ±2 °C for a week, after which they were purified until fungal growth appeared [10].



(a)

(b)

Figure 1:Symptoms of fungal infection causing a) stem rot and b) root rot in peanut plants

2.2 Collection of Sesame Seeds

Sesame (Sesamum indicum L.) seeds were collected directly from plants grown in fields located in the villages of Shurween, Sulaymani, Al-Uboor, and Sankar, which belong to Al-Mansouriyah District in Diyala Province, situated approximately 110 km northeast of Baghdad, the capital of Iraq, during the period from October to December 2024.

2.3 Isolation of Endophytic Fungi from the Seeds of Sesamum Indicum L.

For endophytic fungi from sesame (Sesamum indicum L.) seeds, healthy seeds were selected and then surface-sterilized by immersion in a 2% sodium hypochlorite solution for 3 minutes. The seeds were washed three times with sterile distilled water and transferred to Petri dishes containing SDA medium. The plates were incubated at 25 ± 2 °C for one week

until fungal growth appeared. Once fungal growth was evident, a small amount from the edge of the fungal colony was removed and transferred to a new Petri dish with SDA, prepared according to the manufacturer's instructions. The plates were incubated at 25 ± 2 °C for one week while the growth was checked to purify the fungal isolates, which were obtained in pure colonies for subsequent identification. Isolates were characterized morphologically based on the procedure introduced by [10].

2.4 Preparation of the Oil Extract from Sesame Seeds

The oil extract from sesame (Sesamum indicum L.) seeds was prepared after washing the seeds with water to remove adhering dust and impurities, followed by drying. After drying, the seeds were ground using an electric grinder to obtain a fine powder. The extraction process was carried out by weighing 50 g of the powdered seeds and dissolving them in 400 mL of absolute ethanol at a ratio of 5:7.5 (w/v) using a Soxhlet apparatus. The extraction process continued for 6 hours, yielding approximately 5 mL of oil. This procedure was repeated to obtain the required quantity of oil. The extract was then subjected to a rotary evaporator to remove the solvent. Finally, the obtained oil extract was stored in dark glass bottles until use [11].

2.5 Preparation of Fungal Extract for Endophytic Fungus of *Chaetomium globosum*

The fungus *Chaetomium globosum* was purified on Sabouraud Dextrose Broth (SDB) after the medium had been prepared in 250 mL flasks and sterilized by autoclaving. Each flask was inoculated with five discs (0.5 mm in diameter) taken from freshly grown fungal colonies. The flasks were then incubated in a shaking incubator at 25 ± 2 °C for 28 days, with continuous monitoring of fungal growth. After incubation, the filtrate was prepared by passing the liquid culture medium through medical gauze, followed by filtration using Whatman No.1 filter paper with the aid of a vacuum pump [12]. The filtrate was subsequently sterilized using a Millipore membrane filter with a pore size of 0.22 µm. Thereafter, ethyl acetate was added to the culture as an organic solvent in a volume equal to that of the filtered culture medium to obtain the fungal extract. The mixture was shaken for 10 minutes, then left undisturbed until two layers formed. The upper layer, which contained the extracted bioactive compounds, was separated using a separatory funnel. The extract was then concentrated using a rotary evaporator to obtain a dry or concentrated extract, which was used in subsequent experiments. Finally, the dried extract was stored in dark glass bottles away from sunlight and kept in a refrigerator until use [13].

2.6 Identification of Bioactive Compounds Using GC-MS

Bioactive compounds present in the oil extract of sesame seeds and the fungal extract of *Chaetomium globosum* were identified using Gas Chromatography-Mass Spectrometry (GC-MS) (Shimadzu GC-MS 2010, Japan) according to the method described by Ramautar and Zhang [14]. The separation conditions were as follows: the capillary column (DM-5M) used had dimensions of 30 m ×0.25 mm × 0.25 µm; the carrier gas was helium (99.99%) with a flow rate of 1 mL min^{-1}; the injector temperature was 280 °C; the GC inlet temperature was 200 °C; the injection mode was split with an injection volume of 1 µL; the total run time was 30 minutes; the system pressure was 100 kPa; and the oven temperature program was set at 80 °C for 2 minutes, followed by an increase of 10 °C per minute until the oven temperature reached 300 °C.

2.7 Testing the Antagonistic Effect of Sesame Oil Extract and *Chaetomium globosum* Extract against the Pathogenic Fungus *Fusarium Solani* in Vitro

The method of [15] was followed to test the effect of concentrations of 0, 2.5, 5, and 7.5 ml/L^{-1} of the fungal extract *Chaetomium globosum* on the fungus *Fusarium solani*. The fungal extracts were mixed with soluble SDA culture medium cooled to 50°C, with three replicates per concentration. After the culture medium solidified, a 5 mm diameter disc of the pathogenic fungus *Fusarium solani*, taken from a 7-day-old fungal colony growing on dextro-saproid SDA agar, was placed in the center of the plate. Two types of controls were used: a positive control, in which the fungal extract was added, and a negative control, which included a concentration of 0 ml/L. The plates were incubated at 25 ± 2°C for 7-10 days. The diameter of the growing colony (average diameter of two perpendiculars) was then measured, and the results were recorded using a ruler according to the equation provided in [reference to relevant work/method [16] The method of [17] was followed

in preparing the sesame oil extract. The oil extract was mixed with SDA culture medium. The oil was obtained by extraction using a Soxhlet device. The sesame oil extract was taken at a concentration of (0, 2.5, 5, 7.5) ml/L-1. The mixing method was used for the oil extract with the culture medium, with the addition of 5 ml/L of (TWEEN 20) per 100 ml/L, with continuous shaking to form a homogeneous emulsion between the oil extract and the culture medium. The medium was poured into 9 cm diameter Petri dishes (three dishes for each concentration). Then, each dish was inoculated in its center with a 5 mm disc of the pathogenic fungus taken from the edge of a seven-day-old colony. All dishes were incubated at a temperature of 25-28 degrees Celsius. Then, the growth diameters of the colonies were measured when the fungal growth reached the edge of the dish for comparison and according to the inhibition percentage [16]. The antifungal activity of sesame seed oil and *Chaetomium globosum* extracts was evaluated using the radial growth method. Each treatment was performed in three independent replicates (triplicates) at each concentration. The percentage of fungal growth inhibition was calculated using the formula:

$$Inhibition\ (\%) = \frac{C - T}{C} \times 100.$$

Where C is the radial growth of the fungus in the control plate, and T is the growth in the treated plate.

3 RESULTS AND DISCUSSION

3.1 Results of the Isolation of Endophytic Fungi from Sesame Seeds

Results Table 1 shows the endophytic fungi that were isolated from the sesame seed samples. To obtain the isolates, a total of 35 sesame seed samples were cultured resulting in the isolation of 43 different fungal isolates. Among the isolates, the endophytic fungus *Chaetomium globosum* was identified and accounted for 20.93% of the total fungal isolates obtained from sesame seeds. It agrees with [18] that *Chaetomium globosum* can be isolated from sesame seeds as one of the endophytic fungi associated with this plant, And Figure 2 shows the endophytic fungus *Chaetomium globosum* isolated from sesame seeds under a microscope.

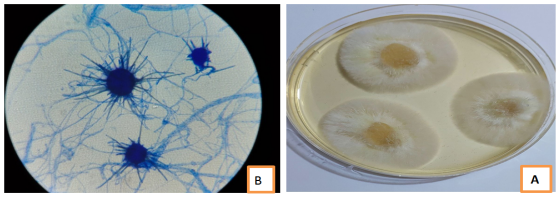


Figure 2: Morphological characteristics of *Chaetomium globosum* isolated from sesame seeds: a) fungal colony and b) microscopic observation of ascocarps at 40× magnification.

3.2 Results of GC-MS Analysis of the Oil Extract from Sesame Seeds

GC-MS analysis of the oil extract from sesame (Sesamum indicum L.) seeds showed 10 biochemical compounds present in 10 chromatographic peaks Table 2 and Figure 3. Among the identified compounds, Iridomyrmecin was found to have the highest relative abundance with a concentration of 21.79%. The results showed that the fatty acid group was the most variable, with 4 compounds that collectively represented 40.31% of the total identified compounds. The one compound from the lignans comprised 8.99% of the whole. Additionally, one compound from the ester group (3.42%), one from the aldehyde group (4.59%). The phytosterol group was represented by one compound at 2.65%, the ketone group at 2.02% of the compounds in the sesame-seed oil extract.

3.3 Efficiency of the Oil Extract of Sesamum indicum L. in Inhibiting *Fusarium solani*

The results obtained in Table 4 and Figure 5 showed that the oil extract of sesame seeds exhibited a high inhibitory activity against pathogenic fungus *Fusarium solani* at (5, 7.5) mL L^{-1} with inhibition at 100%, While the concentration (2.5) ml L^{-1} showed an inhibitory effect of 44.44% compared to the control treatment, in which the inhibition rate was 0%. [19] also confirmed the role of sesame oil in the inhibition of many plant pathogenic fungi, including Fusarium spp. The antifungal properties of sesame are associated with its chemical ingredients, in particular the presence of iridomyrmecin, one of its main constituents.

Table 1: Results of the isolation of endophytic fungi from sesame seeds.

Plant	No. of samples	No. of fungal isolates	No. of *Chaetomium globosum* isolates	Frequency (%)	Occurrence (%)
Sesamum indicum L.	35	43	9	25.71%	20.93%

Table 2: Identified chemical compounds in the oil extract of sesame (Sesamum indicum L).

No.	Compound type	Area %	RT	Formula	Name
1	Terpene	21.79	24.624	$C_{10}H_{16}O_2$	Iridomyrmecin
2	Fatty Acid	16.67	24.658	$C_{18}H_{34}O_2$	9-Octadecenoic acid, (E)-
3	Fatty Acid	14.53	22.900	$C_{16}H_{32}O_2$	n-Hexadecanoic acid
4	Lignan	8.99	32.743	$C_{20}H_{18}O_6$	Sesamin
5	Fatty Acid	6.19	26.183	$C_{18}H_{34}O_3$	Ricinoleic acid
6	Aldehyde	4.59	14.289	$C_6H_6O_3$	5-Hydroxymethylfurfural
7	Ester	3.42	24.637	$C_{24}H_{30}O_5$	Phthalic acid, 4- methoxybenzyl octyl ester
8	Fatty Acid	2.92	24.752	$C_{18}H_{36}O_2$	Octadecanoic acid
9	Phytosterol	2.65	34.341	$C_{29}H_{50}O$	γ-Sitosterol
10	Ketone	2.02	30.358	$C_{11}H_{18}O$	Decahydro cyclooctenone

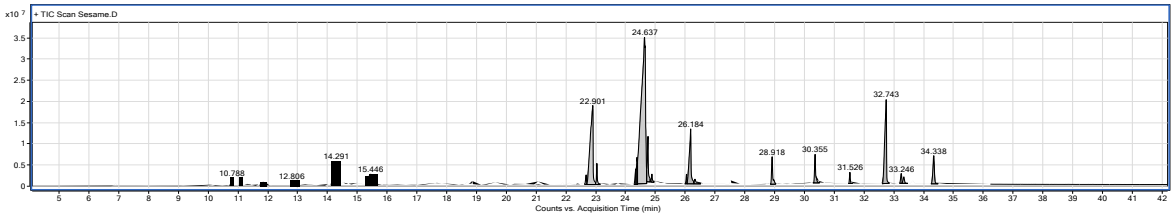


Figure 3: Chromatographic peaks of the oil extract from sesame (Sesamum indicum L.) seeds using.

Table3: Identified chemical compounds in the fungal extract of *Chaetomium globosum* using GC-MS.

No.	Compound type	Area %	RT	Formula	Name
1	Terpenoid	8.13	14.101	$C_{10}H_{14}O$	Thymol
2	Fatty Acid	4.74	23.502	$C_{18}H_{30}O_2$	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
3	Flavonoid	3.58	30.264	$C_{16}H_{14}O_6$	Hesperetin
4	Fatty Acid	2.93	21.806	$C_{16}H_{32}O_2$	n-Hexadecanoic acid
5	Diketopiperazine	2.13	19.939	$C_7H_{14}N_2O_2$	Pyrrolo[1,2-a]pyrazine-1,4- dione, hexahydro-
6	Coumarin	0.78	26.135	$C_7H_{18}O_5$	8-(2,3-Dihydroxy-3- methylbutyl) coumarin derivative
7	Diketopiperazine	0.71	21.793	$C_{10}H_{14}N_2O_2$	Octahydrodipyrrolo[1,2-a:1',2'- d]pyrazine-5,10-dione
8	Diketopiperazine	0.55	19.701	$C_8H_{12}N_2O_2$	3-Methyl-hexahydro- pyrrolo[1,2-a]pyrazine-1,4-dione
9	Aldehyde	0.46	10.070	C_8H_8O	Benzeneacetaldehyde
10	Ester	0.33	5.021	$C_7H_{14}O_2$	Propanoic acid, butyl ester

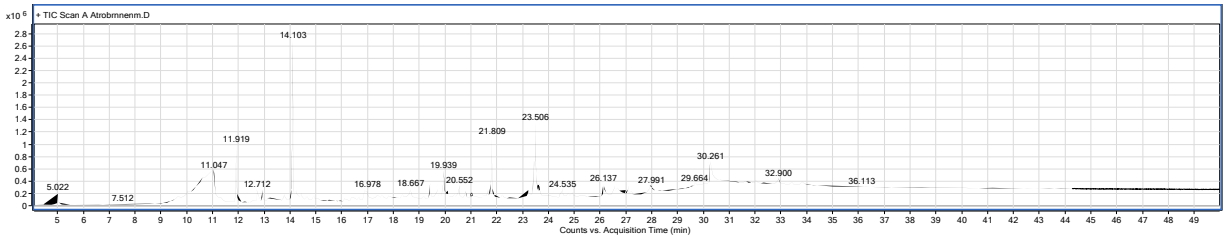


Figure 4: Chromatographic peaks of the fungal extract of *Chaetomium globosum* using GC-MS.

Table 4: In vitro effect of sesame oil extract on the inhibition percentage of the pathogenic fungus *Fusarium solani*.

Oil extract of sesame (Sesamum indicum L.) seeds	0 mL L^{-1}		2.5 mL L^{-1}		5 mL L^{-1}		7.5 mL L^{-1}	
	Growth rate (mm)	Inhibition (%)	Growth rate (mm)	Inhibition (%)	Growth rate (mm)	Inhibition (%)	Growth rate (mm)	Inhibition (%)
Fusarium solani	90	0	55.56	44.44	0	100	0	100
Extract of the fungus *Chaetomium globosum*	0 mL L^{-1}		2.5 mL L^{-1}		5 mL L^{-1}		7.5 mL L^{-1}	
	Growth rate (mm)	Inhibition (%)	Growth rate (mm)	Inhibition (%)	Growth rate (mm)	Inhibition (%)	Growth rate (mm)	Inhibition (%)
Fusarium solani	90	0	22.23	77.77	16.67	83.33	0	100

Table 5: In vitro effect of the fungal extract of *Chaetomium globosum* on the inhibition percentage of the pathogenic fungus *Fusarium solani*.

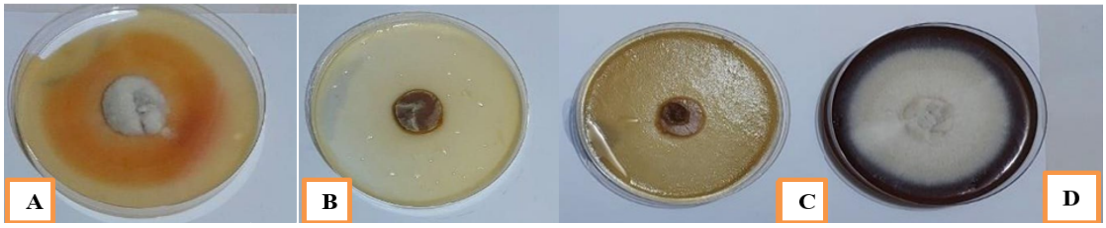


Figure 5: Diameters of the inhibition zones of the pathogenic fungus *Fusarium solani* as affected by oil extract of sesame seeds at concentrations of a) 2.5 mL L^{-1}, b) 5mL L^{-1}, c) 7.5 mL L^{-1} and d) 0 mL L^{-1}.

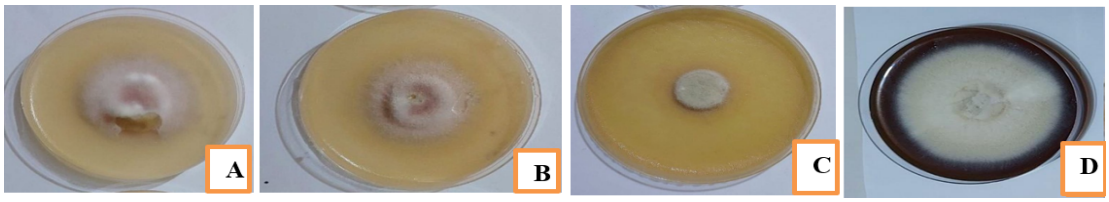


Figure 6: Diameters of the inhibition zones of the pathogenic fungus *Fusarium solani* as affected by the fungal extract of *Chaetomium globosum* at concentrations of a) 2.5 mL L^{-1}, b) 5mL L^{-1}, c) 7.5 mL L^{-1} and d) 0 mL L^{-1}.

Recent work has shown the utility of iridoids for affecting fungal enzymatic systems and limiting hyphal elongation [20]. In addition, fatty acids (9-octadecenoic acid and n-hexadecanoic acid) promoted the inhibiting effect mainly due to the fact that these increase the permeability of the fungal cell membrane and therefore leak cellular contents and contribute to cell death [21]. Additionally, the addition of lignans, including sesamin (8.99%), may activate the extract's defensive functions, since they have been reported to inhibit the synthesis of mycotoxins through the disturbance of fungal gene expression [22].

3.4 Results of GC-MS Analysis of the Extract of *Chaetomium globosum*

The results of analyzing the extract of the fungus C. globosum using Gas Chromatography-Mass Spectrometry (GC-MS) showed the identification of several active compounds, as illustrated in Table 3 and Figure 4. Ten compounds were identified, distributed over 10 chromatographic peaks. The compound Thymol, belonging to the terpene group, recorded the highest abundance among the compounds, reaching 8.13%, while the ester compound recorded the lowest abundance at 0.33%. Two fatty acid compounds were also identified with a total percentage of 7.67%, and three compounds from the Diketopiperazines group (cyclic peptides) with 3.39%, and one flavonoid compound with 3.58%. Additionally, one aldehyde compound was identified at 0.46%, and one coumarin compound at 0.78%.

3.5 Efficacy of *Chaetomium globosum* Fungal Extract in Inhibiting *Fusarium solani*

The results obtained in Table 5 and Figure 6 showed that the fungal extract of *Chaetomium globosum* exhibited a high inhibitory effect against the pathogenic fungus *Fusarium solani* at a concentration

of 7.5 mL L^{-1}, While at concentrations of (2.5, 5) ml L^{-1}, the inhibitory capacity was recorded at 77.77% and 83.33%, respectively, compared to the control treatment in which the inhibition rate was 0%. [23] reported the role of *Chaetomium globosum* in inhibiting the growth of Fusarium spp. [24], while demonstrated the potential use of this biocontrol fungus in suppressing the growth of *Fusarium solani* under laboratory conditions. The inhibitory activity of the endophytic fungal extract of *Chaetomium globosum* can be attributed to its distinctive chemical composition, particularly its high content of thymol. Recent studies have confirmed that the antifungal activity of this compound is due to its ability to disrupt the permeability of the fungal cytoplasmic membrane, thereby impairing mitochondrial function and essential enzymatic processes [25]. Furthermore, GC-MS analysis revealed the presence of the flavonoid hesperetin, which is considered one of the most potent natural inhibitors of cell membrane biosynthesis enzymes, and interferes with ergosterol biosynthesis pathways in fungi [26]. In addition, the fungal extract contained the fatty acid 9,12,15-octadecatrienoic acid, where unsaturated fatty acids play an important role in antifungal activity by integrating into the lipid bilayer of the fungal cell membrane, leading to membrane destabilization, increased permeability, and leakage of vital cellular contents [27].

4 CONCLUSIONS

Based on the results of chemical analysis using GC-MS, it was revealed that a diverse range of secondary metabolites is present in both extracts. The oil extract of sesame (Sesamum indicum L.) seeds contained 10 compounds, with the terpenoid compound iridomyrmecin being the most abundant at 21.79%, followed by essential fatty acids such as oleic acid (16.67%) and palmitic acid (14.53%), in addition to the lignan sesamin (8.99%).

In contrast, the extract of the endophytic fungus *Chaetomium globosum* exhibited comparable chemical diversity, with 10 identified compounds, the most prominent being the phenolic compound thymol (8.13%), linolenic acid (4.74%), and the flavonoid hesperetin (3.58%). These complex compounds including terpenoids, phenolics, and fatty acids act in a biological synergistic manner, where fatty acids enhance the permeability of the fungal cell membrane, facilitating the entry of terpenoid and phenolic compounds such as iridomyrmecin and thymol, which in turn disrupt enzymatic processes and interfere with cell wall biosynthesis .These findings are consistent with recent studies indicating that the integration of plant extracts with endophytic fungal systems enhances the efficiency of biological control and achieves complete inhibition of plant pathogens.

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