ANTAGONISTIC ACTIVITY OF ENDOPHYTIC FUNGI ISOLATED FROM *ALOE VERA* LEAVES AGAINST SOME PLANT PATHOGENIC FUNGI

M. A. A. Al Nuaimy **S. N. Hawar**
Researcher **Assist. Prof.**
Dept. Biol. Coll. Educ. Ibn Al-Haitham, University of Baghdad
mohamadali.alnuaimy73@gmail.com **dr.sumaiya.bio@gmail.com**

ABSTRACT

The present study was aimed to test the antagonistic activity of endophytic fungi isolated from *Aloe vera* leaves against a number of plant-pathogenic fungi. The results showed that the highest antagonistic activity was exerted by *Penicillium chermesinum* against the pathogenic fungus *Rhizoctonia solani*, with an inhibition rate of 78.57%. Also, *Talaromyces verruculosus* and *P. chermesinum* showed their highest antagonistic activities against pathogenic fungus *Fusarium* 1 with inhibition rates of 61.79% and 61.53%, respectively, while *T. verruculosus* showed an inhibition rate of 62.91% against the pathogenic fungus *Fusarium* 2. Most of the endophytic fungi showed the least percentage of inhibition against *Macrophomina phaseolina* compared to the rest of the pathogenic fungi. The fungal filtrates showed a concentration-dependent inhibition rate against the plant pathogens, with a range of 0.00-48.13%. The highest inhibitory activity of *Aspergillus niger* was recorded at a concentration of 60% against *Fusarium* 2, *R. solani*, and *Fusarium* 1, with rates of 48.13, 37.39, and 36.66%, respectively. The results also showed the ineffectiveness of all fungal filtrates against the pathogenic fungus *M. phaseolina*. Chemical analysis of the filtrates of the endophytic fungi which showed antagonistic activity against pathogenic fungi was also conducted by using GC-MS. The results showed the presence of effective compounds with biological effects, such as Pentadecanoic acid, Oleic Acid, Limonene, cis-Vaccenic acid, Hexanedioic acid, bis(2-ethylhexyl) ester, Cycloheptasiloxane, and tetracamethyl-.

Key words: dual culture, growth inhibition, mycoparasitism, competition, GC-MS assay.
***Part of M.Sc. Thesis of the first author.**

الفعالية التضادية للفطريات المستنبتة المعزولة من اوراق نبات الألوفيرا تجاه بعض الفطريات الممرضة للنبات

محمد علي عبد الرزاق النعيمي **سمية نعيمة حوار**
باحث **استاذ مساعد**
قسم علوم الحياة– كلية التربية للعلوم الصرفة (ابن الهيثم)– جامعة بغداد

المستخلص

استهدفت الدراسة الحالية اختبار الفعالية التضادية للفطريات المستنبتة المعزولة من اوراق نبات الألوفيرا *Aloe vera* تجاه عدد من الفطريات الممرضة للنباتات. اظهرت النتائج ان أعلى فعالية تضادية اظهرها الفطر المستنبت *Penicillium chermesinum* تجاه الفطر الممرض *Rhizoctonia solani* بنسبة تثبيط 78.57%، كما اظهر الفطران المستنبتان *Talaromyces verruculosus* ، *P. chermesinum* اعلى فعالية تضادية تجاه الفطر الممرض 1 *Fusarium* بنسبة تثبيط 61.79% و 61.53% على التوالي، بينما اظهر الفطر المستنبت *T. verruculosus* تجاه الفطر المرضي 2 *Fusarium* نسبة تثبيط بلغت 62.91%، واظهرت اغلب الفطريات المستنبتة اقل نسبة تثبيط تجاه الفطر الممرض *Macrophomina phaseolina* مقارنة ببقية الفطريات الممرضة. بينت نتائج تأثير رواشح الفطريات المستنبتة تجاه الممرضات النباتية فعالية تثبيطية بنسبة تراوحت بين 0.00–48.13% وكانت نسبة التثبيط تتناسب طرديا مع زيادة تركيز رواشح الفطريات المستنبتة، وكانت اعلى فعالية تثبيطية لراشح الفطر *Aspergillus niger* بتركيز 60% تجاه الفطريات الممرضة 2 *Fusarium*، *R. solani* و1 *Fusarium* بنسبة 48.13، 37.39، 36.66% على التوالي، وكذلك اظهرت النتائج عدم فعالية جميع رواشح الفطريات المستنبتة تجاه الفطر الممرض *M. phaseolina*. كما اظهرت نتائج التحليل الكيميائي لرواشح الفطريات المستنبتة والتي اظهرت فعالية تضادية تجاه الفطريات الممرضة بفحص GC–MS انها تمتلك مركبات فعالة ذات تأثيرات بيولوجية مثل Pentadecanoic acid، Oleic Acid، Limonene، cis-Vaccenic acid، Hexanedioic acid, bis(2-ethylhexyl) ester، Cycloheptasiloxane, tetradecamethyl- .

الكلمات المفتاحية: الزراعة المزدوجة، تثبيط النمو، التطفل الفطري، التنافس، فحص كروماتوغرافيا الغاز– مطياف الكتلة.
***البحث مستل من رسالة الماجستير للباحث الاول.**

Received: 22/1/2023, Accepted:14/5/2023

INTRODUCTION

Endophytic fungi are microorganisms that live in a mutualistic relationship inside plant tissues without causing any pathological symptoms, the fungi obtain protection and nutrients from the host plants, and in return they can contribute to their growth and absorption of nutrients, they can also improve tolerance of plants to abiotic and biotic stresses and increase their resistance to insects and pests, endophytic fungi produce biologically active compounds similar to those found in the host plant, such as quinones, lignans, alkaloids, lactones, isocoumarins, steroids, and phenols, these compounds have pharmacological properties such as, antiviral, antifungal, anti-inflammatory, antitumor and antiparasitic, antidiabetic and immunosuppressant (9, 54). Endophytic fungi prevent damages to plants by their ability to release enzymes, antibiotics, hydrogen cyanide, and volatile compounds that inhibit pathogen's activities and induce systemic resistance (42). Global trends are shifting towards reducing the use of chemical pesticides due to their severe negative impacts on human health and surrounding ecosystems, with biological control methods are being used to control diseases (39, 50). Biocontrol agents (BCAs) are usually fungal or bacterial strains isolated from the plant's phyllosphere, endosphere, or rhizosphere, and they play an important role in controlling plant pathogens (43). Some Rhizosphere bacteria have been used as biological control agents to inhibit and kill the pathogenic fungi *Rhizoctonia solani* and *Fussarium solani* (8, 53). Some fungi have been used as biocontrol agents against some plant pathogens. For example, *Trichoderma* spp. was employed in inhibiting the growth of *Erwinia carotovera*, which causes soft rot of potato tubers (46). The fungus *Fusarium chlamydosporum* which causes agent of decline date palm offshoots was treated with arbuscular mycorrhizal fungi (34). The insect *Tribolium confusum* which causes economic damage to stored wheat flour was treated with *Fusarium proliferatum* and *Beauveria bassiana* (3). *Glomus* spp. fungus were used as a biological control agent against the fungus *Rhizoctonia solani* and root-knot nematode *Meloidogyne javanica* causing Rhizoctonia-Meloidogyne complex disease in the Chickpea (6). Antagonism is a phenomenon in which antagonistic organisms suppress or interfere with the normal growth, development, and activity of plant pathogens in their vicinity (20). Antagonism can manifest itself in several mechanisms of biological control of disease, including antibiosis (a metabolite or antibiotic that is inhibited by the antagonist is produced), mycoparasitism (in which the antagonist obtains some or all of its nutrients from the fungal host), induced resistance (induction of plant defense response against plant pathogens), and promotion of plant growth while reducing disease effects, as well as through microbial hormones such as indole acetic acid and gibberellic acid (50). *Pseudomonas fluorescens* and *Azotobacter chroococcum* bacteria were used to induce systemic resistance in the barley plant against barley yellow dwarf virus (1). *Aloe vera* is considered as one of the most powerful and well-known medicinal plants, being used for more than 5,000 years, it is used in cosmetics, especially to treat burns and sunburns, help in healing wounds, and combat cell aging, the plant is also used to strengthen the immune system and improve blood circulation. Its leaves contain many vitamins, minerals, enzymes, amino acids, natural sugars, and other bioactive compounds with skin-conditioning, laxative, antimicrobial, anti-inflammatory, antioxidant, aphrodisiac, anthelmintic, and antifungal properties (22). Silver nanoparticles coated with *A. vera* gel extract were used as antibacterial agent (21). The most active compounds found in *A. vera* are aloe-emodin, aloin, aloesin, emodin, and acemannan. (41). Given the negative effects on human health and the environment as a result of the use of chemical pesticides to control plant pathogens, and due to the global trend towards the use of environmentally friendly biocontrol agents to control plant pathogens, the current study aimed to test the antagonistic activity of endophytic fungi isolated from *A. vera* leaves against some plant pathogenic fungi and analyze the alcohol extract of the filtrates of endophytic fungi using GC-MS device.

MATERIAIS AND METHODS

Obtaining endophytic fungi

The endophytic fungi were isolated from the leaves of *A. vera* plant and identified phenotypically and molecularly as described in our previous study (7). They include the following species : *Penicillium chermesinum, Paecilomyces variotii, Aspergillus terreus, Alternaria solani, Aspergillus flavus, Aspergillus niger, Neoscytalidium dimidiatum, Aspergillus quadrilineatus, Talaromyces verruculosus, Talaromyces radicus, Aspergillus niveus.* The identified isolates were kept in potato dextrose agar (PDA) culture media in the refrigerator and then used in the current study.

Obtaining plant-pathogenic fungi

The identified types of plant-pathogenic fungi (*Fusarium* 1, *Fusarium* 2, *Rhizoctonia solani*, *Macrophomina phaseolina*) were obtained from Department of Plant Protection at the College of Agriculture, University of Baghdad, and the isolates were kept PDA culture media in the refrigerator until used.

Testing the antagonistic activity of endophytic fungi isolated from *A. vera* leaves against plant pathogenic fungi using the dual culture method.

A disc of fungal growth with a diameter of 5 mm was taken from fresh fungal cultures, at the age of 7 days, using a sterile cork puncture from each of the endophytic fungi isolated from the *A. vera* leaves and one of the plant-pathogenic fungi. The two discs were placed on Potato Dextrose Agar (PDA) medium in one petri dish, at a distance of 1 cm from the edge and about 6 cm between them. A comparison plate (control) was used for the pathogenic fungus alone. The plates were incubated at a temperature of 27 ± 2 ° C for 7 days. The diameter of the pathogenic fungus colony was calculated (average of two perpendicular diameters) in the different treatments. The antagonistic activity of the endophytic fungi isolated was estimated on the basis of the rate of growth inhibition of the pathogenic fungus, according to the following equation: Growth inhibition % = {D1 – D2)/D1 }×100 Where D1 = the diameter of the colony of the pathogenic fungus alone (control), D2 = the diameter of the colony of the pathogenic fungus in the dual culture (36). It was observed whether the antagonistic interaction between the endophytic fungus and the pathogenic fungus was in the form of growth inhibition (the formation of a growth inhibition zone between them), if the growth of one on top of the other, or if the growth of the two fungi was stopped when one of them came into contact with the other. The results of this experiment were analyzed based on the growth inhibition rating scale from 0 - 4 approved by (52) and shown below:

0 = no growth inhibition, 1 = 1 - 25% growth inhibition, 2 = 26 - 50% growth inhibition, 3 = 51 - 75% growth inhibition, 4 = 76 - 100% growth inhibition.

Effect of endophytic fungi filtrates against some plant-pathogenic fungi

1/ Preparation of endophytic fungi filtrates

The endophytic fungal isolates that showed high antagonistic activity were selected by dual culture method. They included *A. niger*, *Penicillium chermesinum*, *Paecilomyces variotii*, *A. flavus* and *T. verruculosus*. Conical flask 250ml containing 150ml of potato dextrose broth (PDB) were inoculated with two agar plugs (5mm) from 7 day old fungal inoculated at 28C°,125rpm for 14 days. Fungal biomass were separated using a whatman filter paper (no.1) , Millipore Filter with a diameter of 0.22 microns was used to eliminate spores and obtain a sterile cell-free endophytic fungi filtrate (28).

2/ Testing the effect of endophytic fungi filtrates against the growth of some plant-pathogenic fungi.

The endophytic fungal filtrates prepared in the previous steps were added to the warm sterile PDA medium at a temperature of about 45 °C - 50 °C with three concentrations of 20%, 40% and 60%. The mixture was shaken well and poured into sterile Petri dishes. Sterilized PDA medium was placed alone in Petri dish as a control. After solidification of the media, each plate was inoculated with a 5 mm diameter disc taken from the edge of one of the pathogenic fungal colonies under test (*Fusarium 1, Fusarium 2, R. solani, M. phaseolina*). The inoculated plates were incubated at a temperature of 27 ° C for 7 days. The diameter of the pathogenic fungus colony was calculated and the inhibition

percentage was extracted according to the following equation:

Percentage of inhibition % = (control colony diameter - treatment colony diameter) / (control colony diameter) x 100 (49).

Detection of the organic compounds present in the extract of endophytic fungi filtrates using the GC-MS device

1/ Preparation of the extract of endophytic fungi filtrates.

The filtrate of each fungus, as prepared previously, was placed in a separating funnel and ethyl acetate was added in a ratio of (1:1) volume: volume. In order to separate the (upper) organic solvent and (lower) filtrate layers, the organic solvent layer was collected in small glass vials for the purpose of examination by GC-MS (47).

2/ GC-MS assay of fungal culture filtrate extract

A mass spectrometer (Agelint Company; 7820A; USA) was used in the laboratories of the Ministry of Industry and Minerals. 1µl of pre-prepared fungal filtrate extract was injected. The mass of organic compounds was obtained and their spectra were compared with standard mass spectral libraries (NIST 11.L). The conditions for the analysis were as follows:

Analytical Column: Agelint HP-5msUltra Ineit (30m Length × 250 µm inner dimeter × 0.25µm film thickness). Injection volume: 1µl. Pressure: 11.933 psi. GC Inlet Line Temperature:250C°. Aux heaters Temperature: 310C°. Carrier Gas: He 99.99% . Injector Tempertaure: 250 C°. Injection Type: Splitless. and oven program was: Tempertaure Ramp1 60 C° hold to 3 min, Ramp2 60 C° to 180 C° 7C°/min ,Ramp3 180 C° to 280 C° 8 C° /min, Ramp4 280 C° hold to 3 min.

RESUITS AND DISCUSSION

Testing the antagonistic activity of endophytic fungi against some plant-pathogenic fungi by dual culture method

The results shown in Table 1, reveal a variation in the antagonistic activity each of the endophytic fungi isolated from *A. vera* against each of the fungal pathogens of the plant. The two endophytic fungal species of *T. verruculosus* and *Penicillium chermesinum* howed the highest antagonistic activity against the Pathogenic fungus *Fusarium* 1 (inhibition rates of 61.79 and 61.53%, respectively, scale 3). As for the antagonistic activity against the pathogenic fungus *Fusarium* 2, *T. verruculosus* endophytic fungal species showed the highest values, within scale 3: with inhibition rate of 62.91%. As for the antagonistic effects against the pathogenic fungus *R. solani*, the endophytic species *Penicillium chermesinum* showed the highest antagonistic ability (78.57% inhibition rate, scale 4). As for the antagonistic activity of the endophytic fungi against the pathogenic fungus *M. phaseolina*, most of them showed lower rates of inhibition than those showed against *Fusarium* 1, *Fusarium* 2 and *R. solani*; the species *A. flavus*, *A. niger*, *Paecilomyces variotii*, *Penicillium chermesinum*, and *A. niveus* exerted inhibition rates ranging between the highest of 49.11% and the lowest of 39.60%; scale 2.

Table 1. Endophytic fungi isolated from *A. vera* leaves and the percentage of growth inhibition of plant-pathogenic fungi tested by dual culture method in PDA medium at a temperature of 27 ± 2 ° C for 7 days

Endophytic fungi isolates	Pathogenic fungi and growth inhibition percentage %			
	Fusarium 1	*Fusarium* 2	*Rhizoctonia solani*	*Macrophomina phaseolina*
Penicillium chermesinum	61.53 ±0.00 a	44.82 ±1.99 c	78.57 ±0.00 a	41.17 ±3.39 bc
Paecilomyces variotii	47.43 ±3.39 c	55.17 ±0.00 b	48.84 ±5.16 cd	42.13 ±4.27 bc
Aspergillus terreus	28.97 ±1.12 f	31.03 ±1.99 ef	35.71 ±2.06 ef	11.76 ±0.00 f
Alternaria solani	30.73 ±2.21 f	29.42 ±1.01 f	34.04 ±1.03 ef	16.27 ±0.85 ef
Aspergillus flavus	31.02 ±1.14 f	58.61 ±3.98 ab	53.56 ±4.12 c	49.11 ±2.97 a
Aspergillus niger	53.48 ±0.00 b	47.12 ±3.04 c	63.45 ±0.52 b	47.05 ±1.70 ab
Neoscytalidium dimidiatum	30.76 ±0.00 f	36.20 ±0.00 de	42.85 ±0.00 de	23.52 ±0.00 d
Aspergillus quadrilineatus	36.66 ±1.12 e	32.64 ±1.01 ef	25.00 ±00 gh	20.67 ±1.69 de
Talaromyces verruculosus	61.79 ±1.14 a	62.91 ±0.49 a	41.18 ±7.21 def	20.58 ±0.00 de
Talaromyces radicus	32.71 ±1.11 ef	41.37 ±1.98 cd	23.24 ±1.03 h	14.70 ±0.00 ef
Aspergillus niveus	42.30 ±2.21 d	44.82 ±3.98 c	32.14 ±4.12 ghf	39.60 ±2.54 c
LSD value	4.653 *	6.564 *	9.662 *	6.356 *

* The numbers in the table represent the mean of three replicates ± the standard error.
* Means bearing different letters within one column are significantly different and those bearing similar letters are not significantly different among themselves at the probability level of P≤0.05.
* The highest mean takes the letter a, followed by the one with the letter b downwards, and so on.
* The mean that carries two or more letters is not significantly different from the averages that carry the same letters.

This process of antagonism between endophytic fungi isolated from the leaves of *A.vera* and plant-pathogenic fungi, the results of which are shown in Figure 1-4, indicates the emergence of various cases of antagonism, as follows:

1- Endophyic fungus growing on the pathological fungus. The occurrence ratio of this case of antagonism was 54.54% Figure 1 (1B, 1D, 1E, 1G, 1H, 1K), Figure 2 (2B, 2C, 2F, 2G, 2L), Figure 3 (3B, 3C, 3D, 3E, 3F, 3I, 3J, 3L), Figure 4 (4C, 4E, 4I, 4K, 4L). This antagonistic activity appeared to be varied according to the different endophytic fungus and pathogenic fungus. The growth of one fungus on top of the other, as shown by the dual culture method, indicates mycoparasitism, which is the ability of fungi to grow and feed on other fungi (30).

2- Pathogenic fungus growing on the endophytic fungus. This case of antagonism appeared at an occurrence rate of 9.09% Figure 4 (4D). Shows that the pathogenic fungus *M. phaseolina* a showed strong activity for growth over the endophytic fungus *T. radicus*. In addition, as shown in Figure 4 (4G, 4H, 4J), the same pathogenic fungus showed strong growth efficacy over the two endophytic fungi of *A. terreus* and *A. quadrilineatus*, and weaker growth efficacy over the endophytic fungus *Alternaria solani*. This indicates the effectiveness of the pathogenic fungus *M. phaseolina* as a mycoparasite against the *T. radicus*, *A. terreus*, *A. quadrilineatus* and *Alternaria solani*.

3- Endophytic fungus and pathogenic fungus grow towards each other and the growth stops at the point of contact. The occurrence ratio of this case was 20.45%, as shows in Figure 1 (1C, 1F, 1L), Figure 2 (2H, 2I, 2J), Figure 3 (3G) , and Figure 4 (4B, 4F). These two kinds of fungi showed competition between them for space and food, and the diameters of their occurrence varied depending on the speed and rate of their growth (12). The pathogenic fungus *M. phaseolina* appeared as the strongest competitor among the tested pathogenic fungi against the endophytic fungi under study.

4- Growth inhibition zone between the endophytic fungus and pathogenic fungus.

This case of antagonistic activity was shown by 15.9% of cases, as shown in Figure 1 (1I, 1J), Figure 2 (2D, 2E, 2K), and Figure 3 (3H,3K). The diameter of the inhibition zones varied between the endophytic fungus and pathogenic fungus. The endophytic fungus *A. niger* caused a larger growth inhibition zone of the pathogenic fungus *Fusarium* 2 than that caused by A. quadrilineatus Figure 1 (1I, 1J). On the other hand, the endophytic fungus *A. quadrilineatus* caused a larger growth inhibition zone of the pathogenic fungus *R. solani* than that cause by *A. niveus* showed Figure 3 (3H, 3K). Also, the endophytic fungus *T. radicus* caused a larger inhibition zone, as compared to those caused by *A. flavus* and *A. quadrilineatus*, against the growth of the pathogenic fungus *Fusarium* 1 Figure 2 (2D, 2E, 2K). This zone between the two growing fungi implies antibiosis, which is important as it indicates the effectiveness of the endophytic fungus in producing mycotoxins or antibiotics to inhibit the growth of the pathogenic fungus (48). From the results of the antagonistic activities of the endophytic fungi isolated from *A. vera* plant against some of the plant-pathogenic fungi on the solid medium in the dual culture, demonstrate that some of the endophytic fungi showed the ability to compete with the pathogenic fungi for space or food. Also, some showed the capability of fungal parasitism on the fungal pathogens, while others showed their ability to produce antibiotics against plant pathogenic fungi. Based on these different activities, endophytic fungi can be used efficiently in biological control against fungal pathogens. The results of the antagonistic efficacy shown in this study are consistent with those of (45) and(26) and (48) in terms of the comparable growth inhibition rates of these pathogenic fungi and others caused by endophytic fungi isolated from different plants.

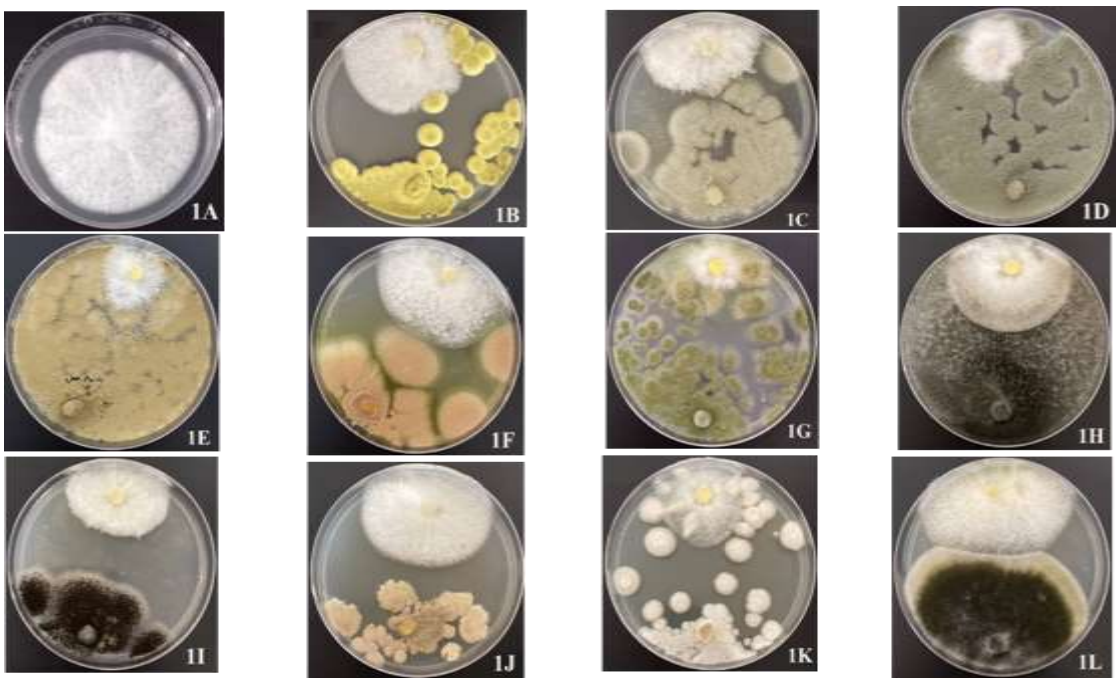


Figure 1. Antagonism of endophytic fungi isolated from the *A.vera* leaves against the pathogenic fungus *Fusarium* 2 by dual culture method and grown in PDA medium at a temperature of 27 ± 2 °C for 7 days (the pathogenic fungus in the upper part of the images).

Description : (1A) Colony of fungus pathogen *Fusarium* 2 growing alone on PDA medium (control), (1B) *T. radicus* vs *Fusarium*2, (1C) *Penicillium chermesinum* vs *Fusarium*2, (1D) *T. verruculosus* vs *Fusarium*2, (1E) *Paecilomyces variotii* vs *Fusarium*2, (1F) *A. terreus* vs *Fusarium*2, (1G) *A. flavus* vs *Fusarium*2, (1H) *Neoscytalidium dimidiatum* vs *Fusarium*2, (1I) *A. niger* vs *Fusarium*2, (1J) *A. quadrilineatus* vs *Fusarium*2, (1K) *A. niveus* vs *Fusarium*2, (1L) *Alternaria solani* vs *Fusarium*2.

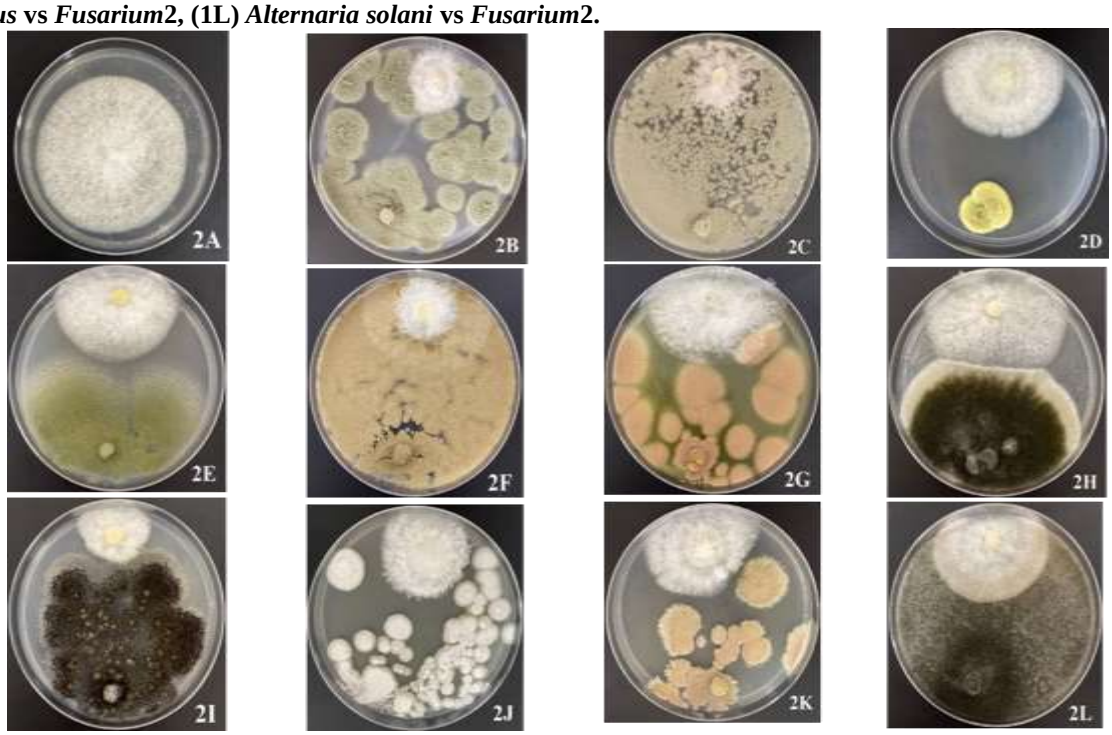


Figure 2. Antagonism of endophytic fungi isolated from the *A.vera* leaves against the pathogenic fungus *Fusarium* 1 by dual culture method and grown in PDA medium at a temperature of 27 ± 2 °C for 7 days (the pathogenic fungus in the upper part of the images).

Description : (2A) Colony of fungus pathogen *Fusarium* 1 growing alone on PDA medium (control), (2B)) *T. verruculosus* vs *Fusarium*1, (2C) *Penicillium chermesinum* vs *Fusarium*1, (2D) *T. radicus* vs *Fusarium*1, (2E) *A. flavus* vs *Fusarium*1, (2F) *Paecilomyces variotii* vs *Fusarium*1, (2G) *A. terreus* vs *Fusarium*1, (2H) *Alternaria solani* vs *Fusarium*1, (2I) *A. niger* vs *Fusarium*1, (2J) *A. niveus* vs *Fusarium*1, (2K) *A. quadrilineatus* vs *Fusarium*1, (2L) *Neoscytalidium dimidiatum* vs *Fusarium*1.

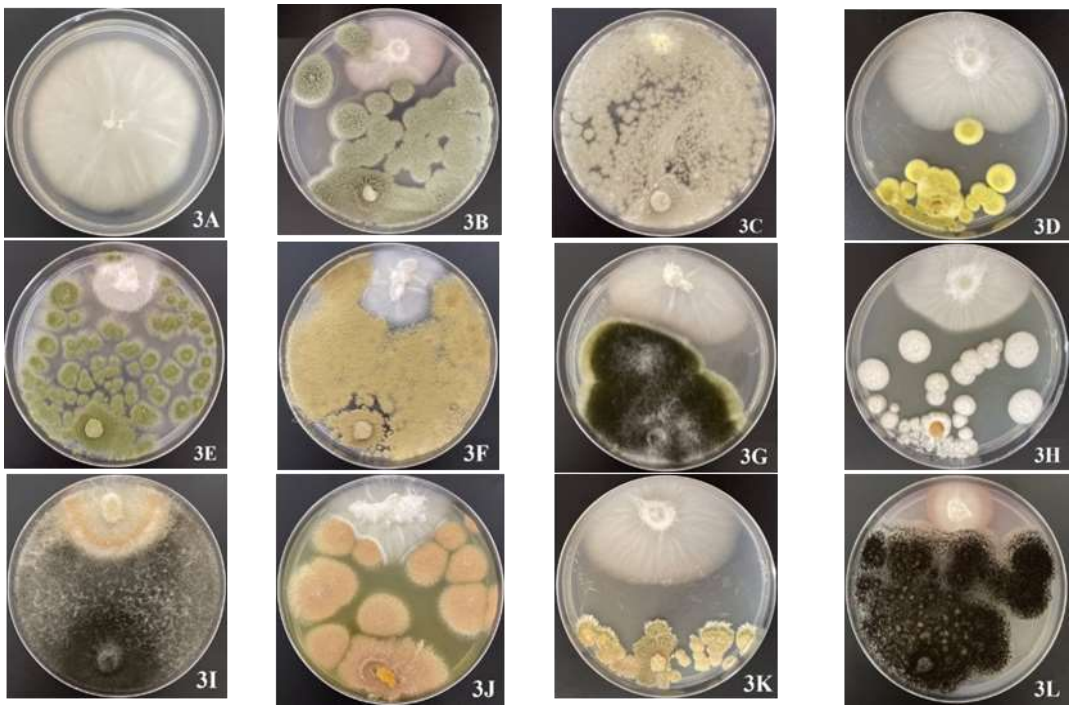


Figure 3. Antagonism of endophytic fungi isolated from the *A.vera* leaves against the pathogenic fungus *R. solani* by dual culture method and grown in PDA medium at a temperature of 27 ± 2 °C for 7 days (the pathogenic fungus in the upper part of the images).

Description : (3A) Colony of fungus pathogen *R. solani* growing alone on PDA medium (control), (3B) *T. verruculosus* vs *R. solani*, (3C) *Penicillium chermesinum* vs *R. solani*, (3D) *T. radicus* vs *R. solani*, (3E) *A. flavus* vs *R. solani*, (3F) *Paecilomyces variotii* vs *R. solani*, (3G) *Alternaria solani* vs *R. solani*, (3H) *A. niveus* vs *R. solani*, (3I) *Neoscytalidium dimidiatum* vs *R. solani*, (3J) *A. terreus* vs *R. solani*, (3K) *A. quadrilineatus* vs *R. solani*, (3L) *A. niger* vs *R. solani*.

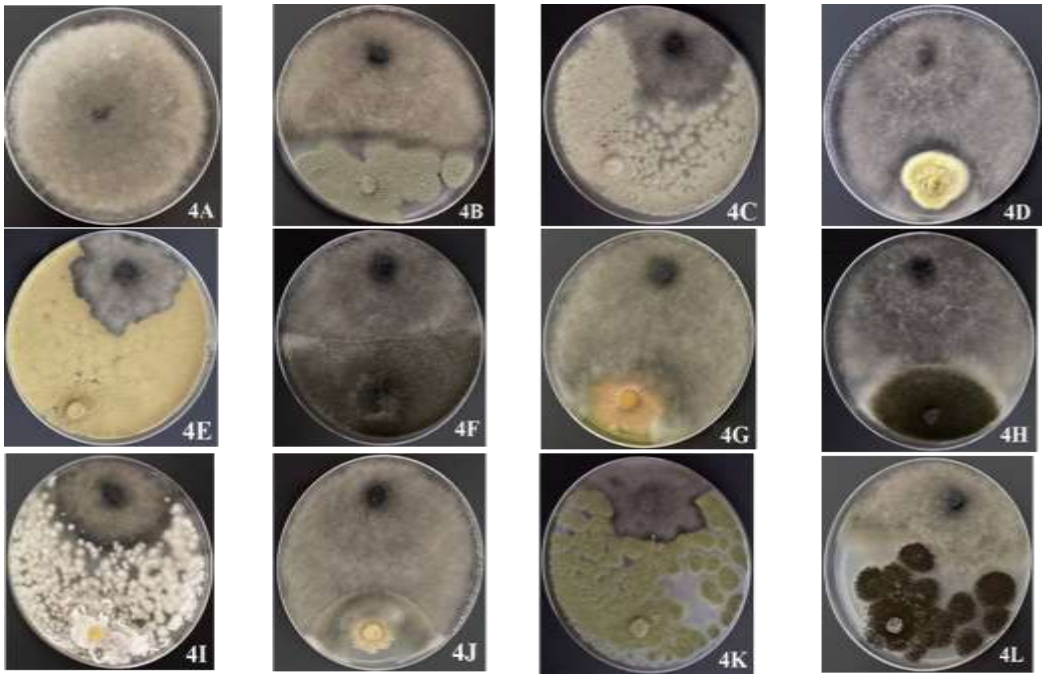


Figure 4. Antagonism of endophytic fungi isolated from the *A.vera* leaves against the pathogenic fungus *M. phaseolina* by dual culture method and grown in PDA medium at a temperature of 27 ± 2 °C for 7 days (the pathogenic fungus in the upper part of the images).

Description : (4A) Colony of fungus pathogen *M. phaseolina* growing alone on PDA medium (control), (4B) *T. verruculosus* vs *M. phaseolina*, (4C) *Penicillium chermesinum* vs *M. phaseolina*, (4D) *T. radicus* vs *M. phaseolina*, (4E) *Paecilomyces variotii* vs *M. phaseolina*, (4F) *Neoscytalidium dimidiatum* vs *M. phaseolina*, (4G) A. *terreus* vs *M. phaseolina*, (4H) *Alternaria solani* vs *M. phaseolina*, (4I) *A. niveus* vs *M. phaseolina*, (4J) *A. quadrilineatus* vs *M. phaseolina*, (4K) *A. flavus* vs *M. phaseolina*, (4L) *A. niger* vs *M. phaseolina*.

Effects of endophytic fungal filtrates on the growth of some plant-pathogenic fungi

The results show in Table 2 and Figure 5 demonstrate a variation in the inhibitory effects on the growth of pathogenic fungi, depending on the endophytic fungus and pathogenic fungus. In general, the endophytic fungal filtrate showed inhibition efficacy with a rate ranging between 0.00-48.13%, in a concentration-dependent manner. Also, the highest activity was recorded for the filtrate of *A. niger* at a concentration of 60%, as it inhibited the growth of *Fusarium* 2, *R. solani*, and *Fusarium* 1 with rates of 48.13, 37.39, and 36.66%, respectively. (18) found that the concentration 60% of *Paecilomyces sp.* filtrate is the highest effect against the radial growth of *Rhizoctonia solani*. In comparison with the antagonistic activities of the same endophytic fungi against the same pathogenic fungi obtained using the dual culture method Table 1, where all the endophytic fungi showed higher inhibition activities against pathogenic fungi, with a rate ranging between 20.58-78.57 %, especially against the pathogenic fungi *M. phaseolina*. The reason is perhaps that the concentrations of endophytic fungi filtrates (20%, 40%, 60%) contain fewer antibiotic compounds; thus, they had little or no effect on the pathogenic fungi. While, in the antagonism in the dual culture experiments, the fungi were growing, forming colonies, and producing secondary metabolites that circulate in the solid medium and directly affect the pathogenic fungi. These results indicate that some endophytic fungi, especially those that showed high antagonistic activities, can be used in agricultural applications as biocontrol agents against fungal plant-pathogens (4).

Table 2. Effects of endophytic fungal filtrates on the growth of some plant-pathogenic fungi

Endophytic fungi isolates and concentration of their filtrates		Pathogenic fungi and growth inhibition percentage %			
Endophytic fungi isolates	Concentration of filtrate	*Fusarium* 1	*Fusarium* 2	*R. solani*	*M. phaseolina*
Penicillium	20%	12.50 ±0.00 ef	16.66 ±0.00 g	5.74 ±1.15 i	0.00 ±0.00
chermesinum	40%	21.52 ±5.68 d	16.66 ±0.00 g	24.13 ±0.00 de	0.00 ±0.00
	60%	33.33 ±0.00 ab	18.51 ±0.00 g	29.88 ±0.00 c	0.00 ±0.00
Paecilomyces	20%	8.33 ±0.00 f	17.27 ±0.61 g	10.34 ±0.00 h	0.00 ±0.00
variotii	40%	15.96 ±0.69 e	19.74 ±1.23 fg	18.38 ±1.14 f	0.00 ±0.00
	60%	25.00 ±0.00 d	32.09 ±1.23 c	24.13 ±0.00 de	0.00 ±0.00
Aspergillus	20%	11.10 ±0.69 ef	18.51 ±0.00 g	8.73 ±1.01 h	0.00 ±0.00
flavus	40%	15.96 ±0.69 e	22.22 ±0.00 ef	24.13 ±0.00 de	0.00 ±0.00
	60%	24.30 ±0.69 d	35.79 ±1.23 b	33.90 ±0.57 b	0.00 ±0.00
Aspergillus	20%	26.38 ±1.39 cd	28.38 ±1.23 d	13.79 ±1.23 g	0.00 ±0.00
niger	40%	30.55 ±1.39 bc	35.79 ±1.23 b	36.20 ±1.72 ab	0.00 ±0.00
	60%	36.66 ±1.92 a	48.13 ±3.71 a	37.39 ±0.00 a	0.00 ±0.00
Talaromyces	20%	21.52 ±1.38 d	23.45 ±0.61 e	9.76 ±1.14 h	0.00 ±0.00
verruculosus	40%	23.61 ±1.39 d	33.33 ±0.00 bc	22.41 ±0.00 e	0.00 ±0.00
	60%	33.33 ±0.00 ab	35.79 ±1.23 b	26.43 ±1.15 d	0.00 ±0.00
LSD value		5.039 *	3.631 *	2.463 *	0.00 NS

* The numbers in the table represent the mean of three replicates ± the standard error.

* Means bearing different letters within one column are significantly different and those bearing similar letters are not significantly different among themselves at the probability level of P≤0.05.

* The highest mean takes the letter a, followed by the one with the letter b downwards, and so on.

* The mean that carries two or more letters is not significantly different from the averages that carry the same letters

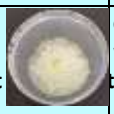
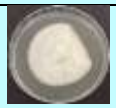
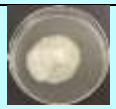
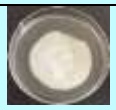
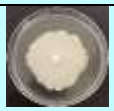
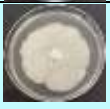
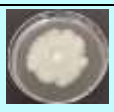
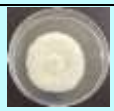
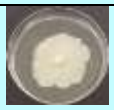
Endophytic fungi isolates	Concentrations of filtrates of endophytic fungi	Pathogenic fungi			
		Fusarium 1	*Fusarium* 2	*R. solani*	*M. phaseolina*
		control without treatment 	control without treatment 	control without treatment 	control without treatment 
Penicillium chermesinum	20%				
	40%				
	60%				
Paecilomyces variotii	20%				
	40%				
	60%				
Aspergillus flavus	20%				
	40%				
	60%				
Aspergillus niger	20%				
	40%				
	60%				
Talaromyces verruculosus	20%				
	40%				
	60%				

Figure 5. Antagonism of endophytic fungi filtrates against pathogenic fungi, grown in PDA medium at 27 ± 2 °C for 7 days

GC-MS assay of endophytic fungi filtrate extract: GC-MS was employed to analyze the chemical compounds of the extracts of the endophytic fungi filtrates. Figure 6 indicates the presence of 20 peaks that diagnosed the presence of 20 active compounds, table 3 shows the compounds obtained from *Penicillium chermesinum* filtrate extract. Figure 7 indicates the presence of 20 peaks that diagnosed the presence of 20 active compounds, table 4 shows the compounds obtained from the extract of the filtrate of *Paecilomyces variotii*. Figure 8 also indicates the presence of 20 peaks that diagnosed the presence of 20 active compounds, table 5 shows Compounds obtained from *A. flavus* filtrate extract. Figure 9 indicates the presence of 5 peaks that diagnosed the presence of 5 active compounds, table 6 shows the compounds obtained from *A. niger* filtrate extract. Figure 10 indicates the presence of 15 peaks that diagnosed the presence of 15 of the active compounds, table 7 shows the compounds obtained from *T. verruculosus* filtrate extract.

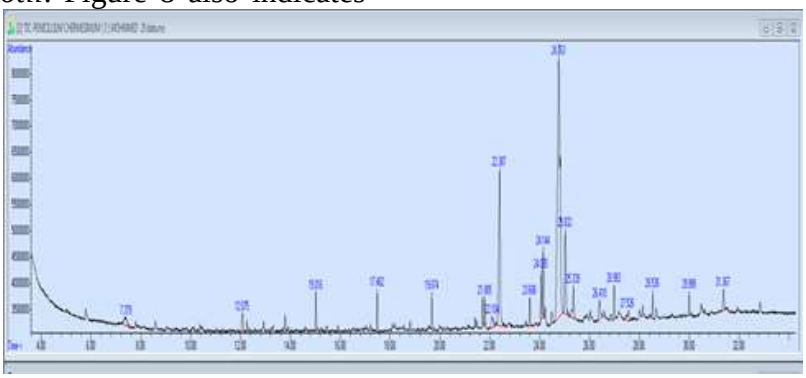


Figure 6. Chromatographic analysis of the active compounds in *Penicillium chermesinum* filtrate extract and their retention time in GC-MS

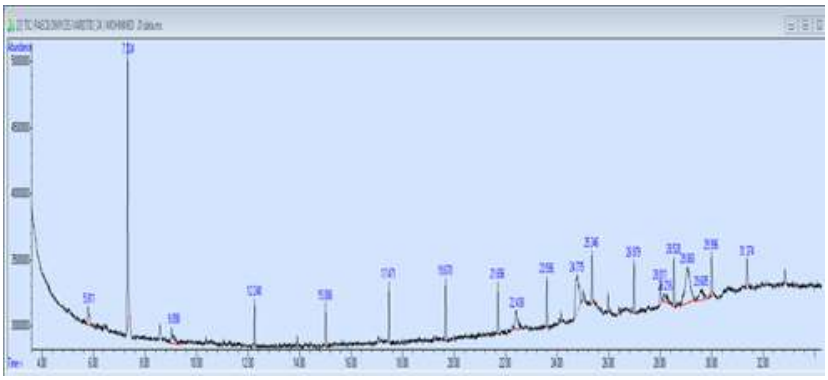


Figure 7. Chromatographic analysis of the active compounds in *Paecilomyces variotii* filtrate extract and their retention time in GC-MS

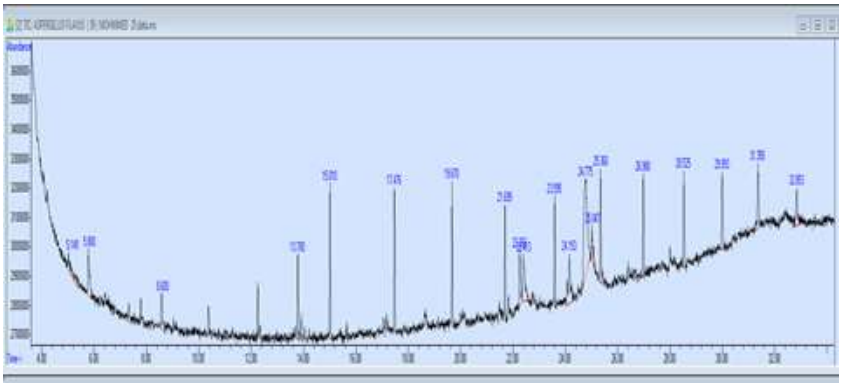


Figure 8. Chromatographic analysis of the active compounds in *A. flavus* filtrate extract and their retention time in GC-MS

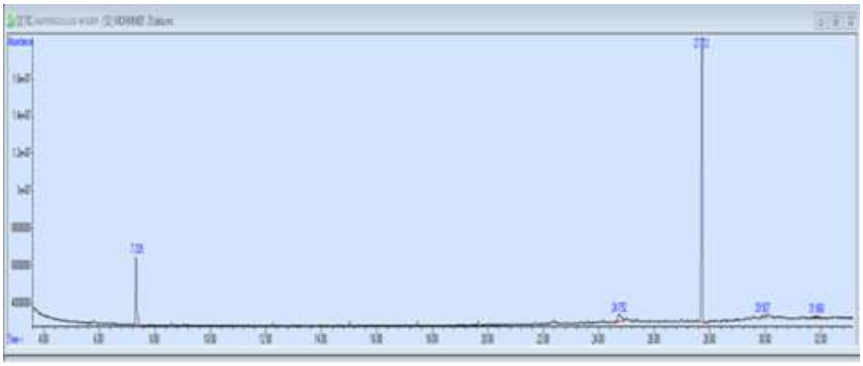


Figure 9. Chromatographic analysis of the active compounds in *A. niger* filtrate extract and their retention time in GC-MS

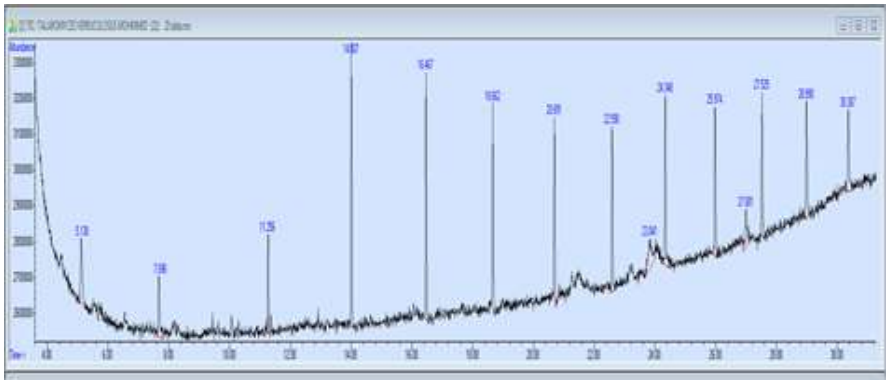


Figure 10. Chromatographic analysis of the active compounds in *T. verruculosus* filtrate extract and their retention time in GC-MS

Table 3. Active compounds of *Penicillium chermesinum* filtrate extract, retention time, and surface area percentage in GC-MS assay

Peak	Retention time (min)	Area %	Chemical compound
1	7.379	1.93	Docosanoic acid 1-methyl-butyl ester
2	12.075	1.38	1-Dodecanol
3	15.016	1.80	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-
4	17.482	1.94	Cyclooctasiloxane, hexadecamethyl-
5	19.674	1.78	2-(2',4',4',6',6',8',8'-Heptamethyltetrasiloxan-2'-yloxy)-2,4,4,6,6,8,8,10,10-nonamethylcyclopentasiloxane
6	21.805	1.52	Pentadecanoic acid, 14-methyl-, methyl ester
7	22.104	1.55	2,2'-(1,4-Piperazinediyl)bis(N-(4-methoxyphenyl)succinimide)
8	22.387	15.11	Pentadecanoic acid
9	23.606	1.54	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-
10	24.055	2.65	9,12-Octadecadienoic acid, methyl ester
11	24.144	3.60	9-Octadecenoic acid (Z)-, methyl ester
12	24.763	46.05	Oleic Acid
13	25.032	6.63	Octadecanoic acid (Stearic acid)
14	25.339	2.44	3-Isopropoxy-1,1,1,7,7,7-hexamethy l-3,5,5-tris(trimethylsiloxy)tetrasiloxane

Table 4. Active compounds of *Paecilomyces variotii* filtrate extract, retention time, and surface area percentage in GC-MS assay

Peak	Retention time (min)	Area %	Chemical compound
1	5.811	2.99	Methyl 5-acetyl-2-methoxybenzoate
2	7.324	18.03	Limonene
3	9.098	3.85	Ethanedicarboxamide, N-allyl-N'-(2,5-dimethylphenyl)-2,2'-(1,4-Piperazinediyl)bis(N-(4- methoxyphenyl)succinimide)
4	12.248	2.69	Cyclohexasiloxane, dodecamethyl-
5	15.006	2.48	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-
6	17.471	3.46	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane
7	19.670	3.78	2-(2',4',4',6',6',8',8'-Heptamethyltetrasiloxan-2'-yloxy)-2,4,4,6,6,8,8,10,10-nonamethylcyclopentasiloxane
8	21.696	3.20	1-Monolinoleoylglycerol trimethylsilyl ether
9	22.438	3.88	Heptane, 1-(ethenylthio)-
10	23.596	3.25	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane
11	24.775	10.87	cis-Vaccenic acid
12	25.346	4.31	3-Isopropoxy-1,1,1,7,7,7-hexamethy l-3,5,5-tris(trimethylsiloxy)tetrasiloxane
13	26.979	3.33	1,1,1,3,5,7,9,11,11,11-Decamethyl-5-(trimethylsiloxy)hexasiloxane
16	28.528	3.72	3-Trimethylsilyloxystearic acid, trimethylsilyl ester
19	29.996	3.52	Hexasiloxane, tetradecamethyl-

Table 5. Active compounds of *A. Flavus* filtrate extract, retention time, and surface area percentage in GC-MS assay

Peak	Retention time (min)	Area %	Chemical compound
1	5.149	3.10	Ethyl Acetate
2	5.800	3.95	7-Methylxanthopteridine
3	8.600	2.79	Benzene-1,2-dicarboxylic acid, bis(4-acetylphenyl) ester
4	13.780	3.22	Hexanoic acid, hexyl ester
5	15.010	5.65	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-
6	17.476	3.46	Cyclooctasiloxane, hexadecamethyl-
7	19.670	5.21	2-(2',4',4',6',6',8',8'-Heptamethyltetrasiloxan-2'-yloxy)-2,4,4,6,6,8,8,10,10-nonamethylcyclopentasiloxane
8	21.699	5.00	1-Monolinoleoylglycerol trimethylsilyl ether
9	22.366	3.65	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester
10	22.413	5.23	Heptane, 1-(ethenylthio)-
11	23.595	4.68	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane
13	24.775	18.12	cis-Vaccenic acid / Oleic Acid
14	25.047	2.51	Heptane, 1-(ethenylthio)-
15	25.360	5.82	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-
16	26.980	4.45	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane
17	28.525	4.67	Hexasiloxane, tetradecamethyl-
18	29.993	3.90	Heptasiloxane, hexadecamethyl-

Table 6. Active compounds of *A. niger* filtrate extract, retention time, and surface area percentage in GC-MS assay

Peak	Retention time (min)	Area %	Chemical compound
1	7.339	17.22	Limonene
2	24.752	6.52	cis-Vaccenic acid
3	27.712	71.44	Hexanedioic acid, bis(2-ethylhexyl) ester
4	29.927	2.66	Pyrimidine, 4,6-dimethoxy-5-nitro-

Table 7. Active compounds of *Talaromyces verruculosus* filtrate extract, retention time, and surface area percentage in GC-MS assay

Peak	Retention time (min)	Area %	Chemical compound
1	5.138	5.37	Acetophenone, 3'-(trimethylsiloxy)
2	7.696	4.69	Benzaldehyde, 2,4-bis(trimethylsiloxy)-
3	11.256	4.28	Cyclohexasiloxane, dodecamethyl-
4	14.007	9.82	Cycloheptasiloxane, tetradecamethyl-
5	16.467	9.00	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-
6	18.662	8.14	3-Trimethylsilyloxystearic acid, trimethylsilyl ester
7	20.691	7.12	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane 1,1,1,3,5,7,9,11,11,11-Decamethyl-5-(trimethylsiloxy)hexasiloxane
8	22.590	7.69	Heptasiloxane, hexadecamethyl-
9	23.841	5.81	4-Spirohexanone, 5,5-dichloro-
10	24.348	8.75	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane
11	25.974	7.14	Cyclononasiloxane, octadecamethyl-
13	27.525	6.41	Cyclooctasiloxane, hexadecamethyl-
14	28.990	6.40	Hexasiloxane, tetradecamethyl-

Pentadecanoic acid was reported to have toxic effects on MCF-7/SC human breast cancer cells, leading to cell cycle arrest and apoptosis (51). Pentadecanoic acid and pentadecanal are anti-biofilm agents for *Candida albicans* and *Klebsiella pneumonia*; that is, they have the ability to prevent the formation and destabilize the structure of dual-species biofilm (15). Oleic acid was used to design ufasomes (unsaturated fatty acid vesicles), which are monolayer spherical colloidal carriers used for encapsulating the antifungal drug Itraconazole (ITZ). A microbiological study confirmed the ability of the produced ufasomes to inhibit phospholipase and proteinase produced by *C. albicans* (16). Oleic acid has antifungal

activities against three Gram-positive bacteria (11). Octadecanoic acid has anti-cancer and anti-microbial activities (29). Trans-13-Octadecenoic acid is among the chemical compounds resulting from the GC-MS analysis of the alcoholic filtrate extract of the fungus *Beauveria bassiana* and showed repellent activity against insects that infect some plants, such as cotton (2). The results also showed the presence of Limonene compound, which is used to control agricultural pests due to its antifungal and insecticidal activities (14). One of the active compounds present in the fungal filtrate extract is cis-vaccenic acid, which is known for its antibacterial and hypolipidemic effects (35). The compound 3-Isopropoxy-1, 1, 1, 7, 7 ,7 - hexamethyl-3, 5, 5-tris (trimethylsiloxy) tetrasiloxane is one of the compounds with antimicrobial activity for rice pathogens, such as *Magnaporthe oryzae* and *Xanthomonas oryzae* (44). Octasiloxane, 1, 1, 3, 3, 5, 5 ,7, 7,9,9,11, 11,13, 13, 15, 15- hexadecamethyl- showed antimicrobial activities (33). Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl- had a broad spectrum of activities against *Escherichia coli* (13). The compound 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester showed antifungal activities against the plant-pathogenic fungus *Fusarium oxysporum* (32). The compound 1-Monolinoleoylglycerol trimethylsilyl ether is among the compounds present in the alcoholic extract of cyanobacteria, the extract showed antioxidant and antimicrobial activities and it induces apoptosis in human liver cancer cell lines *in vitro* (10). Hexanedioic acid, bis(2-ethylhexyl) ester is one of the compounds illustrated by the GC-MS assay of the alcoholic extract of *Lasiodiplodia pseudotheobromae*, the extract showed antibacterial activities against both Gram-positive and Gram-negative bacteria (23). The compound -Pyrimidine, 4,6-dimethoxy-5-nitro was among the compounds detected by GC-MS examination of the methanolic extract of *Crotalaria longirostrata* leaves, the extract showed activity against the nymph insect pest *Bactericera cockerelli* (31). Cycloheptasiloxane, tetracamethyl- showed antifungal activities (38) as well as antioxidant, antimicrobial and cytotoxic properties against a range of microbes (24). Hexasiloxane, tetracamethyl- is commonly used in medications, antacids and laxatives (40). It is one of the compounds obtained from the alcoholic extract of the flower buds of *Dianthus caryophyllus*, the extract showed antibacterial activities against the pathogenic bacteria *E. coli* and *S. aureus* (25). The compound 1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane is among the compounds present in the extract of the stem and fruits of *Berberis vulgaris*, which showed antibacterial activities against caries-causing bacteria (27). 3-Trimethylsilyloxystearic acid is among the compounds present in the methanolic extract of *Orobanche crenata*, the extract showed strong antioxidant properties and inhibition of the spread of human hepatocellular carcinoma (HepG2), human prostate cancer (PC3), human breast cancer (MCF-7), and human colon carcinoma (HCT-116) (19). The results of the current study indicate that the bioactive compounds indicated by the GC-MS assay of the alcoholic extract of endophytic fungi filtrate isolated from *A. vera* leaves are among the compounds responsible for the antagonistic activities against the plant-pathogenic fungi under study. Endophytic fungi also produce extracellular enzymes as a mechanism of pathogen resistance (5). Extracellular enzymes have great therapeutic potential in clinical microbiology (17). These enzymes can be used in biotechnological applications (37). The production of bioactive compounds by endophytic fungi in plant tissues is affected by several factors, including humidity, temperature, light, geographical location of the host plant, age of the host plant tissues, and genotypes of both the endophytic fungus and the host plant (39).

Conclusions

A.vera has a wide range of medicinal and therapeutic effects, as the leaves of the plant contain effective compounds that have different biological properties. The results showed that the endophytic fungi present in the leaves of *A. vera* have a role in the therapeutic and medicinal effects of the plant through its production of biologically effective compounds that have many therapeutic and medicinal effects. This provides promising

potential for using these compounds in the therapeutic and cosmetic aspects in the future.

REFERENCES

1. Abbas, A. K. and M. M. Jarjees. 2017. Induction of systemic resistance against barley yellow dwarf virus using *pseudomonas fluorescens* & *azotobacter chroococcum*. Iraqi Journal of Agricultural Sciences, 48 (1): 368-374. https://doi.org/10.36103/ijas.v48i1.456

2. Abdullah, R. R. 2019. Insecticidal activity of secondary metabolites of locally isolated fungal strains against some cotton insect pests. J. Plant Prot. & Pathol. 10(12): 647-653.

3. Al-Ani, L. K. T., M. I. Yonus, B. A. Mahdii, M. A. Omer, J. K. Taher, S. F. A. Albaayit amd S. B. Al-Khoja . 2018. First record of use *Fusarium proliferatum* fungi in direct treatment to control the adult of wheat flour *Tribolium confusum*, as well as, use the entomopathogenic fungi *Beauveria bassiana*. Ecology, Ecol. Environ. Conserv. 24(3):1043-1047.

4. Ali, B. Z. and A. A. M. Alfayed. 2014. endophytic fungi from leaves and twigs of *Albizia lebbeck* and their antifungal activity. Ibn Al-Haitham j. pure appl. sci. 27(3): 24-33.

5. Ali, B.Z. and H. M. Kadhoam. 2014. endophytic fungi of myrtle (*Myrtus communis*) and their extracellular enzyme activity. ibn al-haitham j. pure appl. sci. 27(3): 152-163.

6. Aljuboori, F. K., B. Y. Ibrahim and A. H. Mohamed. 2022. Biological control of the complex disease of *Rhizoctonia solani* and root-knot nematode *Meloidogyne javanica* on chickpea by *Glomus* spp. and *Pseudomonas* sp. Iraqi journal of agricultural sciences. 53(3): 669-676. https://doi.org/10.36103/ijas.v53i3.1577

7. Al-Nuaimy, M.A. and S. N. Hawar. 2024. isolation and identification of endophytic fungi from *Aloe vera* leaves and chemical analysis of the alcoholic extract of the leaves using HPLC, GC, and GC-Mass devices. Ibn Al-Haitham J. Pure Appl. Sci. 34. In press.

8. Anwar, Sh., F. Mahmood, N. A. Tahir, and G. F. Salih. 2022. Secondary compounds released by rhizospheric bacteria exhibit fungistatic effects against phytopathogenic fungus. Iraqi Journal of Agricultural Sciences, 53(5): 1174-1183. https://doi.org/10.36103/ijas.v53i5.1631

9. da Silva, S. G. M., B. A. de Melo, M. T. dos Santos, R. R. S. Rios, C. M. D. S. A. Santos, K. A. L. R. Júnior, F. C. de. Maranhão, T. M. C. Santos and A. B. Fraga. 2022. Endophytic fungi: Benefits for plants and biotechnological potential. Res. Soc. Dev. 11(4): 27008.

10. Dash, S., S. Parida, B. Sahoo and B. Rath. 2022. *In Vitro* Screening of Antioxidant, Antimicrobial and Anticancer Activities of Cyanobacteria Found Across Odisha Coast, India. Res. Sq. 1-19.

11. Dilika, F., P. D. Bremner and J. J. M. Meyer. 2000. ntibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*: a plant used during circumcision rites. Fitoterapia, 71(4): 450-452.

12. Diwan, H. M. k. 2014. Antimicrobial and Enzymatic Activity of Endophytic Fungi Isolated From Myrtle (*Myrtus communis* L.). M.Sc. Thesis, Coll. of Educ. for Pure Scie. - Ibn Al-Haitham, Univ. of Baghdad: Iraq, pp:131.

13. Ezez, D., N. Mekonnen and M. Tefera. 2023. Phytochemical analysis of *Withania somnifera* leaf extracts by GC-MS and evaluating antioxidants and antibacterial activities. Int. J. Food Prop. 26(1): 581-590.

14. Feng, J., R. Wang, Z. Chen, S. Zhang, S. Yuan, H. Cao and W. Yang. 2020. Formulation optimization of D-limonene-loaded nanoemulsions as a natural and efficient biopesticide. Colloids Surf. A Physicochem. Eng. Asp. 596: 124746.

15. Galdiero, E., A. Ricciardelli, C. D'Angelo, E. de Alteriis, A. Maione, L. Albarano and E. Parrilli. 2021. Pentadecanoic acid against *Candida albicans-Klebsiella pneumonia* biofilm: Towards the development of an anti-biofilm coating to prevent polymicrobial infections. Res Microbiol. 172(7-8): 103880

16. Hashem, S. M., M. K. Gad, H. M. Anwar, N. M. Saleh, R. N. Shamma and N. I. Elsherif. 2022. Itraconazole-Loaded Ufasomes: Evaluation, Characterization, and Anti-Fungal Activity against *Candida albicans*. Pharmaceutics, 15(1): 26.

17. Hawar, S. N. 2022. Extracellular Enzyme of Endophytic Fungi Isolated from *Ziziphus spina* Leaves as Medicinal Plant. Int. J. Biomaterials, 2022(ID 2135927): 1-9.

18. Hawar, S. N., Z. K. Taha, A. S. Hamied, H. S. Al-Shmgani, G. M. Sulaiman and S. E.

Elsilk. 2023. Antifungal Activity of Bioactive Compounds Produced by the Endophytic Fungus *Paecilomyces* sp.(JN227071. 1) against *Rhizoctonia solani*. *Int. J. Biomater*. 2023(ID 2411555): 1-8.

19. Hegazy, M. G., A. M. Imam and B. E. Abdelghany. 2020. Evaluation of cytotoxic and anticancer effect of *Orobanche crenata* methanolic extract on cancer cell lines. Tumor Biol. 42(5):1-11.

20. Heydari, A. and M. Pessarakli. 2010. A review on biological control of fungal plant pathogens using microbial antagonists. J. Biol. Sci. 10(4): 273–290.

21. Ibrahim, O. M. S., A.M. Abed and N. Z. Yahea. 2020. Evaluation of Biological Activity of Greenly Synthesized Silver Nanoparticles Using *Aloe Vera* Gel Extract As Antibacterial Agent In-Vitro and In-Vivo. Iraqi Journal of Agricultural Science, 51(6): 1706-1715. https://doi.org/10.36103/ijas.v51i6.1199

22. Jadhav, A. S., O. A. Patil, S. V. Kadam and M. A. Bhutkar. 2020. Review on *Aloe Vera* is used in medicinal plant. Asian J. Pharm. Sci. 10(1): 26-30.

23. Jalil, M. T. M. and D. Ibrahim. 2022. Volatile Bioactive Compounds from *Lasiodiplodia pseudotheobromae* IBRL OS-64, an Endophytic Fungus Residing in the Leaf of *Ocimum sanctum*. HAYATI J. Biosci. 29(5): 570-585.

24. Jesy, E. J. and B. Jose. 2017. Antioxidant, antimicrobial and cytotoxic activities of alcoholic leaves extracts of *Spatholobus parviflorus* (Roxb. Ex Dc.) Kuntze. Int. J. of Pharm. and Phy. Res. 9(7): 1029-1034.

25. Kamaluldeen, A. J. and M. E. Al-Defiery. 2021. Antibacterial Activity Of *Dianthus Caryophyllus* L. Against Antibiotic Resistant Pathogenic Bacteria. Nat. Vol. Essent. Oils J.Nevo, 8(6): 1886-1893.

26. Karim, A. A. and S. A. Shams-Allah. 2020. Performance of endothelial fungi and bacteria in resisting cucumber root rot disease caused by the pathogens *Pythium aphanidermatum* and *Rhizoctonia solani*. Plant Arch. 20(2): 4701-4715.

27. Kazemipoor, M., P. Fadaei Tehrani, H. Zandi, H. and R. Golvardi Yazdi. 2021. Chemical composition and antibacterial activity of *Berberis vulgaris* (barberry) against bacteria associated with caries. Clin. Exp. Dent. Res. 7(4): 601-608.

28. Kharwar, R. N., A. L. Maurya, V. C. Verma, A. Kumar, S. K. Gond, S. K. and A. Mishra. 2012. Diversity and antimicrobial activity of endophytic fungal community isolated from medicinal plant *Cinnamomum camphora*. Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci. 82(4): 557-565.

29. Kumari, N., E. Menghani and R. Mithal. 2020. GCMS analysis & assessment of antimicrobial potential of rhizospheric Actinomycetes of AIA3 isolate. Indian J. Tradit. Knowl. 19(1): 111-119.

30. Leiva, S., K. Rubio, J. R. Díaz-Valderrama, M. Granda-Santos and L. Mattos. 2022. Phylogenetic Affinity in the Potential Antagonism of *Trichoderma* spp. against *Moniliophthora roreri*. Agronomy, 12(9): 2052.

31. López López, H., M. Beltrán Beache, Y. M. Ochoa Fuentes, E. Castro del Ángel, E. Cerna Chávez and J. Delgado. 2022. Methanolic extract of *Crotalaria longirostrata:* Identification of secondary metabolites and insecticidal effect. Scientia Agropecuaria,13(1): 71-78.

32. Lu, J., A. Muhmood, H. Liu, R. Dong, S. Pang and S. Wu. 2020. Exploring bioactive compounds in anaerobically digested slurry: extraction, characterization, and assessment of antifungal activity. Waste Biomass Valorization, 11: 1863-1872.

33. Mahadevi, M. and A. S. Sivalingam. 2020. Pharmacognostical and Phytochemical Screening of Physico-Chemical Parameters and Fluorescence Analysis on Ethanolic Leaves Extract of *Ipomoea Sepiaria* Koenig Ex. Roxb. Waffen-und Kostumkunde J. 11(8): 69-87.

34. Matloob, A. A. H., A.Y. Abid and K.Z. Khadhair. 2017. Efficiency of arbuscular mycorrhizal fungi and some species of plant growth promoting Rhizobacteria to control fungi *Fusarium chlamydosporum* causing agent of decline date palm off shoots. Iraqi Journal of Agricultural Sciences. 48(2): 507-519. https://doi.org/10.36103/ijas.v48i2.418

35. Mboyazi, S. N., M. I. Nqotheni, T. S. Maliehe, and J. S. Shandu. 2020. In vitro antibacterial and in silico toxicity properties of

phytocompounds from *Ricinus communis* leaf extract. Pharmacogn. 12(5): 977-983.
36. Mishra, V. K., A. K. Passari, P. Chandra, V. V. Leo, B. Kumar, S. Uthandi and B. P. Singh. 2017. Determination and production of antimicrobial compounds by *Aspergillus clavatonanicus* strain MJ31, an endophytic fungus from *Mirabilis jalapa* L. using UPLC-ESI-MS/MS and TD-GC-MS analysis. PloS one, 12(10): e0186234.
37. Mohammed, G. M. and S. N. Hawar. 2022. Green biosynthesis of silver nanoparticles from moringa oleifera leaves and Its antimicrobial and cytotoxicity activities. Int. J. Biomaterials, 2022(ID 4136641):1-10.
38. Nguyen, H. T. and D. N. Cao. 2022. Antimicrobial compounds of one *Streptomyces celluloflavus* strain isolated from Can Gio mangrove soil, Vietnam. GSC Biol. Pharm. Sci. 19(3): 120-126.
39. Othman, B. A. and E. S. Kakey. 2021. Pesticides bioaccumulation and their soil pollutant effect. Iraqi Journal of Agricultural Sciences. 52(1): 36-47.
https://doi.org/10.36103/ijas.v52i1.1234
40. Rai, N., P. Kumari Keshri, A. Verma, S. C. Kamble, P. Mishra, S. Barik and V. Gautam. 2021. Plant associated fungal endophytes as a source of natural bioactive compounds. Mycology, 12(3): 139-159
41. Safdar, M., S. A. Naqvi, F. Anjum, I. Pasha, M. Shahid, M. J. Jaskani and R. M. Aadil. 2021. Microbial biofilm inhibition, antioxidants, and chemical fingerprints of Afghani pomegranate peel extract documented by gas chromatography–mass spectrometry and Fourier transformation infrared. J. Food Process. Preserv. 45(7): e15657.
42. Sánchez, M., E. González-Burgos, I. Iglesias. and M. P. Gómez-Serranillos. 2020. Pharmacological update properties of *Aloe vera* and its major active constitutnts. Molecules, 25 (6):1324.
43. Segaran, G. and M. Sathiavelu. 2019. Fungal endophytes: A potent biocontrol agent and a bioactive metabolites reservoir. Biocatal. Agric. Biotechnol. 21: 101284.
44. Singh, H. B. 2014. Management of plant pathogens with microorganisms. Proc. Indian Natl. Sci. Acad. 80(2): 443–454.
45. Singh, N., A. Mansoori, G. Jiwani, A. U. Solanke, T. K. Thakur, R. Kumar, R. and A. Kumar. 2021. Antioxidant and antimicrobial study of *Schefflera vinosa* leaves crude extracts against rice pathogens. Arab. J. Chem. 14(7): 103243.
46. Sornakili, A., S. Thankappan, A. P. Sridharan, P. Nithya and S. Uthandi. 2020. Antagonistic fungal endophytes and their metabolite-mediated interactions against phytopathogens in rice. Physiol. Mol. Plant Pathol. 112:101525.
47. Sulaiman, M. M., S. T. A. Yass, A. A. Aish, L. Basheer, S. J. A. Yasir and S. A. Youssef. 2020. Activity of *Trichoderma* spp. against *Erwinia carotovora* causal agent of potato tuber soft rot. Plant Arch. 20:115-118.
48. Supaphon, P., S. Phongpaichit, V. Rukachaisirikul and J. Sakayaroj. 2013. Antimicrobial potential of endophytic fungi derived from three seagrass species: *Cymodocea serrulata*, *Halophila ovalis* and *Thalassia hemprichii*. PloS one, 8(8): e72520.
49. Syaifudin, A. and R. S. Kasiamdari. 2022. The inhibition of Fusarium wilt in Chili by Endophytic Fungi isolated from Green Betel (*Piper betle* L.) Leaf. J. Nat. Sci. Math. Res. 8(2): 84-93.
50. Tedsree, N., K. Likhitwitayawuid, B. Sritularak, K. Tedsree and S. Tanasupawat. 2022. Antifungal activity of endophytic *Streptomyces* strains from *Dendrobium* orchids and the secondary metabolites of strain DR7-3 with its genome analysis. J. Appl. Pharm. Sci.12(12): 31-41.
51. Thambugala, K. M., D. A. Daranagama, A. J. Phillips, S. D. Kannangara and I. Promputtha. 2020. Fungi vs. fungi in biocontrol: An overview of fungal antagonists applied against fungal plant pathogens. Front. Cell. Infect. Microbiol. 10: 604923.
52. To, N. B., Y. T. K. Nguyen, J. Y. Moon, M. K. Ediriweera and S. K. Cho. 2020. Pentadecanoic acid, an odd-chain fatty acid, suppresses the stemness of MCF-7/SC human breast cancer stem-like cells through JAK2/STAT3 signaling. Nutrients, 12(6):1663.
53. Yasin, B., O. O. Ali and T. S. Rashid. 2021. Antagonistic activity and plant growth promoting rhizobacteria isolated from plant rhizosphere against Fusarium solani Thuja seedlings. Iraqi Journal of Agricultural

Sciences. 52(6): 1508-1515. https://doi.org/10.36103/ijas.v52i6.1492

54. Yousif, A. A. and W. A. Hassan. 2023. HPLC analysis and antifungal activity of some plant extracts against decay apple fruits. Iraqi Journal of Agricultural Sciences. 54(1): 291-302. https://doi.org/10.36103/ijas.v54i1.1702.

55. Zivkovic, S., S. Stojanovic, Z. Ivanovic, V. Gavrilovic, T. Popovic, and J. Balaz.2010. Screening of antagonistic activity of microorganisms against *Colletotrichum acutatum* and *Colletotrichum gloeosporioides*. Arch. of Biol. Sci. 62(3): 611-623.