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EFFECTIVENESS OF LOCALLY ISOLATED BIOLOGICAL CONTROL AGENTS AGAINST BLACK BLIGHT DISEASE OF DATE PALMS

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ABSTRACT

This study was aimed to isolate the aim of isolating the *Trichoderma longibrachiatum* and the *Bacillus subtilis* from healthy date palm root samples and evaluating their antagonistic effectiveness against the two pathogenic fungi *Thielaviopsis paradoxa* and *Thielaviopsis punctulata* that cause black scorch in date palm *Phoenix dactylifera*. The biological control agents *T. longibrachiatum* and *B. subtilis* showed efficiency in inhibiting the growth of both pathogenic fungi *T. paradoxa* and *T. punctulata* when double cultured in PDA medium. In specific, the *T. longibrachiatum* inhibited the growth of both pathogenic fungi *T. paradoxa* and *T. punctulata* by 50.58 and 60.17%, respectively. While *B. subtilis* gave an inhibition rate of 39.99 and 38.09%, respectively. The results of detecting secondary metabolite compounds in cultures of the *T. longibrachiatum* and the *B. subtilis* using a GC-MS instrument showed that each of them can produce many bioactive compounds. Among these compounds are Hexanoic acid, 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester, Terephthalic acid, 2-ethylhexyl octyl ester, Heptane, 1-(ethenylthio)- and 4(1H)-Pyrimidinone, 2-(ethylthio).

Key words: **Trichoderma, Bacillus, bioactive metabolite, antifungal, natural products.**
***Part of Ph.D. dissertation of the first author.**

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INTRODUCTION

The date palm *Phoenix dactylifera* L. is one of the most planted fruit trees in arid and semi-arid areas due to its high ability to produce under various stresses. Plant pathogens are one of the most important biological stresses to which palm trees are exposed, negatively affecting production quantitatively and qualitatively (Matloob et al., 2017). The two soil-borne fungi *Thielaviopsis paradoxa* (De Seyeres) Hohn and *T. punctulata*, which cause black blight disease in date palms (*P. dactylifera*), which attacks date palms in various growing areas in the world and at all stages of growth, are one of the most important of these pathogens (Hantoosh & Hussein, 2023a, Hantoosh & Hussein, 2023b). The use of chemical fungicides to control plant diseases is one of the most important, quickest, and easiest applications, especially in cases of severe infection, but their application leads to the development of resistance in fungal pathogens (Zhang et al., 2021). Excessive use of these pesticides also eliminates many beneficial soil organisms as a result of the spread of these pesticides from plant roots to the soil. Therefore, they pose danger to and environment (Adhab et al., 2021). Given these serious problems with fungicides, many recent researchers have focused on environmentally friendly programs to control plant diseases using fungal and bacterial species with anti-pathogen potential, especially endophytic organisms, which have been called endophytes, with the ability of biological control against various types of pathogens (Al-Ani et al., 2011; Al-Ani et al., 2012; Qasem et al., 2014; Al-Ani et al., 2022).The introduction

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of beneficial microbiology technology in the agricultural field to control diseases is the most important factor in achieving a sustainable agricultural program. Moreover, to increase the area of cultivated plants in the environment of production systems (Adhab, 2021; Swain et al., 2019). Among the root-endemic microbes that have been used in the field of biological control is the *Trichoderma longibrachiatum* fungi, which is one of the most important agents of fungal biological control that has been studied recently and can produce a variety of volatile and non-volatile secondary metabolites, which has made the *T. longibrachiatum* fungi a source of unique antibiotics and their effectiveness as a biological control agent against plant pathogens (Ngo et al., 2021). In the same context, many strains of *T. longibrachiatum* have been revealed as promising biocontrol agents against plant-pathogenic fungi, bacteria, and nematodes (Degani & Dor, 2021). However, *T. longibrachiatum* colonizes root tissues, and this colonization is an essential factor in the success of biological control (Liu et al., 2023). Moreover, it also increases the plant's ability to tolerate salt stress, as well as stimulating plant growth (Liu et al., 2023). In addition, many researchers have also pointed out the ability of bacteria that inhabit plant roots to resist many plant pathogens (Al-Ani et al., 2013; Kumari et al., 2019). Furthermore, it is possible to control plant-pathogenic fungi by using several bacteria strains, as they possess several biological control mechanisms, including their antagonistic ability against plant pathogens through their production of siderophore compounds, the production of antibiotics, the production of hydrolytic enzymes, and the ability to stimulate systemic resistance in host plants against plant pathogens (Gouda et al., 2016). Among these bacteria is Bacillus sp., which is distinguished by its ability to protect plants from devastating diseases that affect both crops and perennial woody plants (Swain et al., 2019). Since antibiotics were more abundant in the environment of root-inhabiting bacteria that belong to the Bacillus sp., they also help in the production of many different secondary metabolite compounds that belong to various groups such as fengycins, iturins, and surfactins (Hardoim, 2019). In addition, it is also characterized by its ability to stimulate plant growth, among these bacterial species is *Bacillus subtilis* (Kumari et al., 2019). Hence, the study is aimed to isolate the *Trichoderma longibrachiatum* and the *Bacillus subtilis* from the roots of healthy date palms and test their efficiency in inhibiting the growth of the *Thielaviopsis paradoxa* (De Seyeres) Hohn fungi and *T. punctulata*, which cause black blight in date palms in Iraq.

MATERIALS AND METHODS

Isolation of the two pathogenic fungi and biocontrol agents: Small pieces (5×5 cm) of frond bases that were taken from palm trees infected with black blight were surface sterilized with 10% sodium hypochlorite (commercial preparation) for three minutes, then washed with sterile distilled water for one minute and dried with filter paper. Then these pieces were planted in Petri dishes with a diameter of 9 cm containing the culture medium PDA (Potato Dextrose Agar), sterilized in an autoclave at 121°C and a pressure of 1.5 bar for 15 minutes, to which the antibiotic tetracycline was added at 45°C and incubated at a temperature of 25 + 2°C for three days. The dishes were examined after that, and the fungi were purified on new dishes containing the PDA medium to grow the two fungi. The diagnosis was made morphologically according to approved taxonomic sources (Hantoosh & Hussein, 2023a). Superficial root samples 20 cm deep and active leaf samples were taken from healthy date palm trees approximately 50 years old and with an average height of 7-8 meters. The samples were washed with tap water for half an hour, after which small pieces (0.5 - 1 cm) of roots and leaves were superficially sterilized with 70% ethanol for one minute, then immersed for three minutes in 2% sodium hypochlorite, then sterilized again with 70% ethanol for 30 minutes then washed for three times with sterile distilled water and dried on sterile filter paper. Pieces of roots and leaves were planted at a rate of 4 pieces in Petri dishes prepared with sterile agricultural medium PDA supplemented with the antibiotic tetracycline and incubated at a temperature of

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25 ± 2°C to isolate the fungi (López-López et al., 2022). To isolate the bacteria, 10 g of roots and leaves were ground separately mashed in a ceramic mortar, after adding 90 ml of sterile distilled water to each one. The suspension was heated at 80°C for 20 minutes, and 100 microliters of each were taken for each replicate, spread on nutrient agar medium, and incubated at 28 ± 2°C (Khaledi & Assareh, 2023). All dishes were monitored daily after the incubation process to ensure the growth of fungal and bacterial isolates to purify the colonies growing in new dishes and diagnose them according to cultural and microscopic characteristics, and then they were preserved (Boulahouat et al., 2022; Hantoosh & Hussein, 2023a).

Testing the antagonistic potential of biological agents against the fungi *Thielaviopsis paradoxa* and *Thielaviopsis punctulata:* The double culture method was used by placing a 3 mm diameter disk of one of the two pathogenic fungi from a three-day-old culture in the center of one half of the dish and placing another similar disk from a seven-day-old *T. longibrachiatum* colony in the center of the other half of the dish containing the PDA culture medium, with a ratio of three replicates. After that, the plates were incubated at a temperature of 25 ± 2 °C, and after five days of incubation, the results were taken and the degree of contrast was calculated according to the gradation (Hantoosh & Hussein, 2023a), consisting of 5 degrees:

1 - The antagonistic fungi cover the entire dish.

2- The antagonistic fungi cover 3/4 of the dish.

3 - The antagonistic fungi and the pathogenic fungi each cover half the area of the plate.

4 - The pathogenic fungus covers 4/3 of the dish.

5 - The pathogenic fungi cover the entire plate.

A biological agent is considered effective when it shows a second or lower degree of opposition. The percentage of inhibition of radial growth (PIRG) was calculated according to the following equation:

$$\mathbf{PIRG\% = (R1- R2)/R1\times100}$$

Where PIRG is the percentage of inhibition of radial growth of the pathogenic fungus, R1 is the radial growth of the pathogenic fungi in the control treatment, R2 is the radial growth of the pathogenic fungi in the double culture treatment (Liu et al., 2023). In another experiment, a nutrient broth culture medium of Bacillus bacteria was grown at a temperature of 28 + 2°C for 48 hours, and a test was conducted for the antagonistic activity of these strains against the pathogenic fungi *Thielaviopsis paradoxa* and *Thielaviopsis punctulata* by the adjacent culture method, as it is one of the best methods for testing the efficacy of bacteria against pathogens (Boulahouat et al., 2022). Petri dishes containing sterilized PDA culture media were prepared in an autoclave at a temperature of 121°C and a pressure of 1.5 kg/cm2 for 15 minutes. A line of bacteria was created at 2 cm from one edge of the dish by taking many bacteria grown on nutrient broth (NB) culture medium at 48 hours of age using an inoculation needle, then, a 3 mm diameter disk was placed separately, taken from each of the two pathogenic fungi cultures, *T. paradoxa* and *T. punctulata*, grown on PDA medium at three days old. The results were calculated by calculating the percentage of fungal growth inhibition after the fungal hyphae in the control treatment reached the edge of the dish, according to the following equation:

Inhibition rate % = 1 – (B/A) x 100, where A represents the distance of fungi growth in the Petri dish inoculated with the bacterial strain, and B represents the distance of fungi growth in the control treatment dish without bacteria (Boulahouat et al., 2022).

Molecular diagnosis of the pathogenic fungi *T. paradoxa* and *T. punctulata* and the biogenic fungi *Trichoderma longibrachiatum:* Molecular diagnosis by using the international molecular code for the transcribed spacer (ITS1-ITS2) internal regions in the polymerase chain reaction (PCR) to diagnose the two fungi. DNA was extracted from the two pure fungi samples using an extraction and purification kit prepared by the Korean company Geneaid. The quantity and purity of DNA (nanograms/micrograms) were measured using a Nanodrop device. Master mix from Bioneer (Korea) with TCCGTAGGTGAACCTGCGG (forward primer), TCCTCCGCTTATTGAT

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ATGC (reverse primer) and extracted DNA were collected in 200 microliter Eppendorf tubes according to manufacturer guide. The amplification of DNA was done using 35 cycles of 95C for 30 sec (denaturation step) followed by 55C for 30 sec (Annealing step) and 72C for 60 sec (Extension step) with single cycle of 72C for 5 minutes (final extension step) in the thermal cycler.

Diagnosis of *Bacillus subtilis*

The method of amplifying the 16S rRNA gene and determining its sequences was adopted to confirm the diagnosis of the isolate under study. DNA was extracted from the bacterial sample using the Presto Mini gDNA Bacteria Kit prepared by the Korean company Geneaid. The quantity and purity of DNA (nanograms/micrograms) were measured using a Nanodrop device. Bacteria-specific primers (FWD: AGAGTTTGATCCTGGCTCAG and REV:TACCTTGTTACGACTT) were used in to amplify 16S rRNA using the same PCR program used for fungi. After the end of the program period, the samples were electrophoresed on a 1% agarose gel to which 2 microliters of ethidium bromide were added. Electrophoresis was performed at a voltage of 70 volts for 60 minutes, and after ensuring that the target region was amplified. The samples were sent to the Korean company Biogene to obtain the sequence of the nitrogenous bases. After obtaining the sequence, the sequences were entered into the NCBI BLAST program for each sample separately to determine that the registered sequences present in GenBank were similar to the sample under study.

Diagnosis of secondary metabolites of the *Trichoderma longibrachiatum*, and the *Bacillus subtilis* cultures using GC/MS: Sterile PSB liquid culture medium was prepared in 500 ml beakers, each containing 300 ml of the medium. Flasks were inoculated with the fungus *T. longibrachiatum* after adding the antibiotic tetracycline (250 mg/l) and incubated at a temperature of 25 + 2°C for fourteen days. Other flasks were inoculated with the bacteria *B. subtilis* and incubated at a temperature of 28 + 2°C for 72 hours and left. Beakers without inoculation for comparison. Liquid cultures of *T. longibrachiatum* were passed through Whatman No.1 filter paper, centrifuged at 10,000 rpm to remove mycelium and sporulation, and the filtrate was boiled and sterilized using a 0.2 µm Millipore filter. As for the liquid cultures of *B. subtilis* bacteria, they were subjected to a centrifugation process at 10,000 rpm to get rid of the vegetative cells and spores, and then the filtrate was boiled after sterilizing it using a Millipore filter with a diameter of 0.2 micrometers. The boiled filtrate of both the PSB culture medium and the filtrate of the biological agents, the fungus *T. longibrachiatum* and the bacterium *B. subtilis* growing on the PSB culture medium, was concentrated by a rotary evaporator under low pressure, and the compounds were characterized by a Gas chromatography–mass spectrometry (GC–MS).

RESULTS AND DISCUSSION

Isolation and diagnosis of the two pathogenic fungi *T. paradoxa* and *T. punctulata*: Isolation results from palm trees infected with black blight showed the presence of both fungi. *T. paradoxa* and *T. punctulata* in the two samples under study. The cultures of both fungi were white at first and then turned black centrally with the formation of conidia. Each of them produced two types of spores. The first, Phialoconidia (endo-conidia), was transparent to brown, and it was cylindrical to rectangular in shape and formed longitudinally in chains. As for the second type of spores, they were aleuroconidia or chlamydospores, which have thick walls and are transparent at first, then they become dark in color and oval in shape. They are carried in chains on top of short fungal hyphae in the first fungi, while they are carried singly in the second fungi. This result agrees with previously published work describing the same pathogens (Hantoosh & Hussein, 2023a).

Morphological diagnosis of *Trichoderma longibrachiatum* and *Bacillus subtilis:* Isolation results from date palm root samples showed the presence of the fungi *Trichoderma* sp. and *B. subtilis* bacteria in the samples under study. The fungus was distinguished morphologically by the presence of rapidly growing white colonies in the form of concentric rings on the PDA culture medium, and the color of the colony turned yellow with

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the formation of conidial spores and the production of secondary metabolic compounds (Mulatu et al., 2022). The *B. subtilis* was distinguished morphologically through its growth on the culture medium by the formation of colonies that were creamy white in color at first, rod-shaped, gram-positive, and had several flagella in a circumferential location (Vos et al., 2011).

Molecular diagnosis of the pathogenic fungi *T. paradoxa* and *T. punctulata*, the fungus *T. longibrachiatum*, and the bacterial fungus *B. subtilis*: Molecular diagnosis of pure cultures of *T. paradoxa*, *T. punctulata* and *T. longibrachiatum* was performed by relying on the general molecular code type ITS, while bacteria were diagnosed based on amplification of the 16S rRNA gene (Figure 1). After determining the sequences of the nitrogenous bases and matching the results using nucleotide BLAST, a high percentage of matches were found between the two species under study with their species recorded in GenBank. The matching results for the first fungi were 98% with *T. paradoxa* registered in GenBank with the genetic access code OQ448884. As for the second fungi a 100% match rate was recorded with *T. punctulata* registered in GenBank with the genetic access code OQ427358, and the genetic access code for the fungi *T. longibrachiatum* is LC770387 and for the *B. subtilis* is LC770388. The antagonistic ability of the fungus *T. longibrachiatum* and the bacteria *B. subtilis* against the two pathogenic fungi *T. paradoxa* and *T. punctulata* Figure (2) showed the efficient antagonistic ability of *T. longibrachiatum* against the two pathogenic fungi. The degree of antagonism reached 2 according to the standardization scale (Hantoosh & Hussein, 2023a) after four days of incubation on the PDA culture medium, and it led to the inhibition of the growth of the two pathogenic fungi *T. paradoxa* and *T. punctulata* by 50.58 and 61.17%, respectively (table 1). The antagonistic potential of *T.longibrachiatum* may be attributed to its ability to produce several volatile and nonvolatile secondary metabolites (Sridharan et al., 2021). Several strains of *T. longibrachiatum* have been detected as promising biocontrol agents against many plant pathogens (Degani & Dor, 2021). The bacteria *B. subtilis* gave an inhibition rate of 39.99 and 38.09% for the *T. paradoxa* and *T. punctulata*, respectively (table 1). A separating area was formed between it and each of the two pathogenic fungi on the PDA medium, and this indicates its ability to produce secondary metabolic compounds with activity against both pathogenic fungi, as it can produce siderophore compounds, antibiotics and produce hydrolytic enzymes with antimicrobial activity (Nihorimbere et al., 2024). *B. subtilis* has a high antagonistic activity that enables it to affect a wide range of plant pathogens through various mechanisms, as it can work to distort the growing tips of fungal hyphae or work to degrade the cytoplasm (Hardoim, 2019; Swain et al., 2019).

Table 1. Antagonistic activity of the *Trichoderma longibrachiatum* fungi and the *Bacillus subtilis* bacteria in inhibiting the growth of the two pathogenic fungi *Thielaviopsis paradoxa* and *Thielaviopsis punctulata*

Biological agent	%growth inhibition *T. paradoxa*	%growth inhibition *T. punctulata*	Means
T.longibrachiatum	50.58	61.17	55.88
B. subtilis	39.99	38.09	39.05
Control	0	0	00.00
L.S.D. 0.05	6.65		L.S.D. 0.05 4.7
Means	30.19	33.09	
L.S.D. 0.05	3.84		

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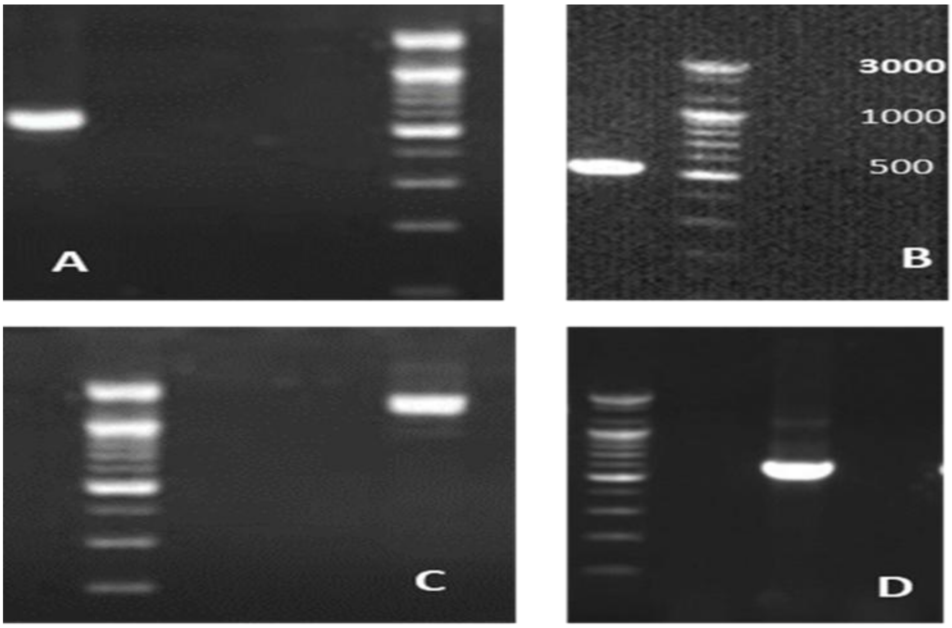

Figure 1. The agarose gel electrophoresis of PCR-amplified DNA segments of the *T. punctulata* ITS region (A), *T. paradoxa* ITS region (B), *Bacillus subtillis* 16 rRNA (C) and *T. longibrachiatum* ITS resgion (D).

Detection of active compounds in cultures of the *T. longibrachiatum* and *B. subtilis* using gas chromatography-mass spectrometry GC/MS strains Different peaks appeared in the results of detecting active compounds in cultures of *T. longibrachiatum* and *B. subtilis*, indicating the presence of different chemical compounds compared to the compounds in the culture medium only (tables 2, 3, 4). In specific, peak number 7 appeared in the results of detecting compounds of *T. longibrachiatum*, which contained Hexanoic acid compound in the highest percentage, followed by Peak number 20, which contained the 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester, 1,4-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester and Terephthalic acid, 2-ethylhexyl octyl ester compounds.Moreover, peak number 14 appeared at the highest level in the results of detecting compounds in *B.subtilis* (table 3), which contained Heptane, 1-(ethynylthio)- and Urea, followed by peak number 16, which contained 4(1H)-Pyrimidinone, 2-(ethylthio)– compounds. The results of the analysis also showed the ability of *T. longibrachiatum* fungi and the *B. subtilis* bacteria to produce many biological compounds such as aldehydes, alcohols, ketones, terpenoids, and pyrrone (lactones), which are considered biologically active compounds with wide applications. Fortunately, these materials have no negative effect on consumers and the environment (López-López et al., 2022). The results also showed that peak number 10 appeared at the highest level in the results of detecting vital compounds in the PSB culture medium (table 4), which contained compounds Heptanedioic acid, dimethyl ester, ethanol, 2-nitro-, propionate (ester), and Propanoic acid, 2-bromo-, methyl ester, followed by Peak 5 contained the Heptane, 1-(ethenylthio) – compound.The Hexanoic acid compound produced by *T. longibrachiatum* is one of the antibiotics produced by many fungi. Peaks number 1, 2, and 3 in the results of the analysis of *T. longibrachiatum* and *B. subtilis* were distinguished, respectively, by the presence of the biological compound 2-Hydroxy-gamma-butyrolactone, which is one of the important biological compounds that is characterized by antibiotic properties as well as being an antioxidant (Włoch et al., 2020).In the same context, the results also showed the ability of the *T. longibrachiatum* and *B. subtilis* to produce the biological Hexadecanoic acid and methyl ester compounds, which is one of the biologically active fatty acids produced by many fungi, and it's considered an antibiotic and an inhibitor of microbes (Octarya et al.,

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2021; Iqbal et al., 2024). Furthermore, it has been observed through several studies that species belonging to the genus Trichoderma are superior to other fungi in producing many secondary metabolic compounds that exhibit various vital activities such as controlling and stimulating plant growth (Phoka et al., 2020; Sridharan et al., 2021). The power of Trichoderma species can be attributed to the synergy of all these compounds in influencing plant pathogens (Sridharan et al., 2021). Studies have also indicated the ability of the *T. longibrachiatum* fungi to produce many biologically active compounds that control pathogens and stimulate plant growth (Sridharan et al., 2021). Phthalic acid esters (Diethyl Phthalate, Phthalic acid, di(2-propylpentyl)ester, Bis(2-ethylhexyl) phthalate, Di-n-octyl phthalate, Diisooctyl phthalate, 1,3-Benzenedicarboxylic acid and bis(2-ethylhexyl) ester) which was found in the results of the analysis of the detection of biological compounds in cultures of *T. longibrachiatum* and *B. subtilis*. It was also found in many natural sources such as plant extracts and essential oils of a large number of different plants, as well as in root secretions, algae, bacteria, and various fungi. It is also characterized by its antimicrobial, antibiotic, and other biological activities that enhance the competitive ability of plants, algae, and fungi to withstand biotic and abiotic stresses (Huang et al., 2021; Khaledi & Assareh, 2023). It has also been shown to inhibit the ability of plant pathogenic bacteria *Pectobacterium carotovorum* ssp. *carotovorum* on the formation of biofilms as well as its effect on biological processes such as growth, energy production, and breakdown of the bacterial cell membrane (Wang et al., 2019). Another study indicated that the compound di-(2-ethylhexyl) phthalate led to the inhibition of cellular metabolism of the plant pathogenic bacterium *Acidovorax citrulli*, as well as a decrease in its pathogenicity (Swain et al., 2019). Other studies have also indicated the toxicity of these compounds against many different fungal and bacterial pathogens (Rashiya et al., 2021).

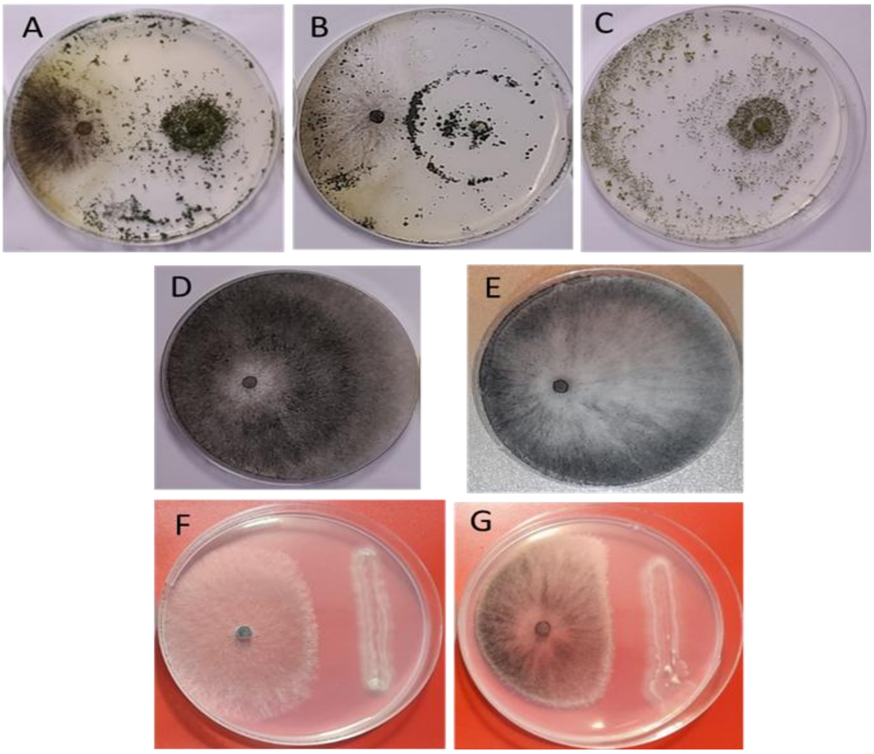

Figure 2. Antagonistic activity of the fungi *T.longibrachiatum* and the *B. subtilis* against the two pathogenic fungi *T.paradoxa* and *T.punctulata*. (A) *T. longibrachiatum* against the pathogenic fungi *T. punctulata*. (B) *T. longibrachiatum* against the fungi *T. paradoxa*. (C) Fungus *T. longibrachiatum* only. (D) The pathogenic fungi *T. punctulata* only (Control). (E) The pathogenic fungi *T. paradoxa* only (Control). (F) *B. subtilis* against the pathogenic fungi *T. punctulata* (G) *B. subtilis* against the pathogenic fungi *T. paradoxa*

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Table 2. Chemical compounds in *T. longibrachiatum* detected by GC-MS. Peak# refers to the number of peak detected. RT refers to the rotation time for each compound. %Arear refers to the percentage area of each peak

Peak#	RT	%Area	ID/Library	Ref
1	4.911	9.39	2-Hydroxy-gamma-butyrolactone 3-Furanol, tetrahydro- Formic acid, 2-propenyl ester	4232 2041 1687
2	5.309	1.39	Succindialdehyde Carbamic acid, (2-chloroethylidene) bis-, diethyl ester 1,2-Hydrazinedicarboxylic acid, diethylester	1664 92678 42712
3	5.595	2.62	Ethyl 2-(acetylamino)-3,3,3-triflu oro-2-hydroxypropanoate Piperazine, 1,4-dimethyl-Piperazine, 1,4-dimethyl-	85337 7354 7359
4	5.846	3.33	N-(9,10-Dimethyl-9,10-dihydroanthr acen-9-yl)-2,2-dimethylpropionamid 2,5-Dimethyl-4-hydroxy-3(2H)-furan one Pivalic acid, 2-chlorophenyl ester	150620 12136 70717
5	6.218	4.33	Molinate Thymine Thymine	51276 11025 11024
6	6.460	4.19	Pentanal 1,3-Propanediamine, N-methyl- Butanoic acid, 2-oxo-	1704 1981 4230
7	6.911	20.26	Hexanoic acid Hexanoic acid Hexanoic acid	20862 20867 20866
8	8.192	1.53	Acetic acid, (amino(nitroamino)methy lene)hydrazide .alpha.-D-Galactopyranose, 2-(acet ylamino)-2-deoxy- 7-[.beta.-d-Ribofuranosyl]imidazo[4,5-d][1,2,3]-triazin-4-one (2-azainosine)	31627 78436 118440
9	8.728	1.38	No matches found	
10	11.212	2.20	L-Glutamic acid 1H-Pyrrole-1-ethanamine, tetrahydr o-.alpha.-methyl- 2-(2-Hydroxyethyl)piperidine	22359 12393 12963
11	12.848	8.30	Butanoic acid, 2-oxo- 1,4-Dioxane-2,6-dimethanol t-Butyl 1-thio-1-desoxy-.beta.-D-glucopyranoside	4230 22986 103378
12	14.380	1.24	Diethyl Phthalate Diethyl Phthalate Diethyl Phthalate	78784 78782 78786
13	19.166	1.65	Hexadecanoic acid, methyl ester Hexadecanoic acid, methyl ester Hexadecanoic acid, methyl ester	119400 119408 119407
14	19.720	2.50	Heptane, 1-(ethenylthio)- Urea Urea	30355 299 298
15	21.486	1.93	1H-Pyrrole-2,5-dione 1H-Pyrrole-2,5-dione 1H-Pyrrole-2,5-dione	2943 2942 2941
16	22.023	3.93	4(1H)-Pyrimidinone, 2-(butylthio)- 4(1H)-Pyrimidinone, 2-(propylthio) 4(1H)-Pyrimidinone, 2-(ethylthio)-	49147 38559 28670
17	24.792	5.86	9-Octadecenamide, (Z)- 9-Octadecenamide, (Z)- 9-Octadecenamide, (Z)-	128446 128447 128445
18	26.671	7.34	Bis(2-ethylhexyl) phthalate Bis(2-ethylhexyl) phthalate Phthalic acid, di(2-propylpentyl) ester	207665 207664 207709
19	27.631	2.94	No matches found	
20	28.947	13.69	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester Terephthalic acid, 2-ethylhexyl octyl ester	207811 207809 207754

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Table 3. Chemical compounds in *B. subtilis* detected by GC-MS. Peak# refers to the number of peak detected. RT refers to the rotation time for each compound. %Arear refers to the percentage area of each peak

Peak#	RT	%Area	ID/Library	Ref
1	3.976	6.16	1-Pyridineacetic acid, hexahydro- Phenol, o-[4-[1-cycloazapropyl]-n-butyl]-2,6-dimethyl- Ethanone, 1-phenyl-2-(1-piperidinyl)-	20255 76583 63400
2	4.695	2.82	No matches found	
3	4.954	7.49	2-Hydroxy-gamma-butyrolactone Oxirane, 2,2'-[oxybis(methylene)]bis- Cyclobutanemethanol	4232 13279 1751
4	5.344	3.00	cis-Aconitic anhydride	28618
5	5.621	4.32	Ethanone, 1-(5-methyl-1,2,3-thiadiazol-4-yl)- 2-Vinyl-9-[.beta.-d-ribofuranosyl] hypoxanthine (Trimethylsilyl)diazomethane	19361 138978 7060
6	5.872	4.34	2,5-Dimethyl-4-hydroxy-3(2H)-furan one 2,4,5-Trihydroxypyrimidine Carbamothioic acid, dipropyl-, S-ethyl ester	12136 11988 52659
7	6.218	5.69	2H-Pyran-2-one, 4-hydroxy-6-(2-oxopropyl)- Acetic acid, 1-(2-methyltetrazol-5-yl)ethenylester Glycine, N-(trifluoroacetyl)-, 1-m ethylbutylester	37253 37047 95086
8	6.478	5.69	1,3-Propanediamine, N-methyl- Glutaraldehyde Butanoic acid, 2-oxo-	1981 3668 4230
9	7.326	3.62	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	20640 20638 20639
10	8.200	2.63	Oxalic acid, hexyl propyl ester Heptane, 2,3-dimethyl- Octane, 4-methyl-	73855 12693 12674
11	10.234	2.32	No matches found	
12	12.640	5.48	No matches found	
13	19.175	4.88	Hexadecanoic acid, methyl ester Hexadecanoic acid, methyl ester Hexadecanoic acid, methyl ester	119400 119408 119407
14	19.755	12.26	Heptane, 1-(ethenylthio)- Urea Urea	30355 298 299
15	21.512	3.05	Ethanol, 2-nitro-, propionate (ester)	22373
16	22.100	11.07	4(1H)-Pyrimidinone, 2-(ethylthio)- 4(1H)-Pyrimidinone, 2-(butylthio)- 4(1H)-Pyrimidinone, 2-(propylthio)	28670 49147 38559
17	22.395	3.43	2-Pyrrolidinethione 1,2,2,3,4-Butanepentacarbonitrile Heptane, 1-(ethenylthio)-	4048 48291 30355
18	22.715	2.66	No matches found	
19	26.679	4.67	Phthalic acid, di(2-propylpentyl)ester Bis(2-ethylhexyl) phthalate Diisooctyl phthalate	207709 207664 207658
20	28.955	4.41	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester Fumaric acid, 2-ethylhexyl undecylester 2-Propenoic acid, 2-methyl-, octylester	207811 203492 59597

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Table 4. Chemical compounds in Potato Sucrose Broth detected by GC-MS (Control). Peak# refers to the number of peak detected. RT refers to the rotation time for each compound. %Arear refers to the percentage area of each peak.

Peak#	RT	%Area	ID/Library	Ref
			3-Butyn-1-ol	514
1	4.920	4.80	3-Butyn-1-ol	510
			3-Butyn-1-ol	516
			3-Butyn-1-ol	514
2	5.318	5.17	3-Butyn-1-ol	510
			3-Butyn-1-ol	516
			3-Butyn-1-ol	514
3	5.604	7.31	3-Butyn-1-ol	516
			3-Butyn-1-ol	515
			cis-Aconitic anhydride	28618
4	6.452	7.53	3-Butyn-1-ol	514
			Pyrimidine, 4,6-dimethoxy-5-nitro-	49604
5	6.746	8.37	Heptane, 1-(ethenylthio)-	30355
6	8.088	4.63	No matches found	
			3-Chloro-N-[2-methyl-4(3H)-oxo-3-q uinazolinyl]-2-thianaphthenecarbox amide	196002
7	8.815	5.20	Pyrimidine, 4,6-dimethoxy-5-nitro-	
			2,2'-(1,4-Piperazinediyl)bis[N-(4-methoxyphenyl)succinimide]	49604 234311
8	10.961	3.64	No matches found	
			3-Butyn-1-ol	514
9	12.337	13.62	2-(N-Morpholino)ethanesulfonic aci	57336
			Urea	298
			Heptanedioic acid, dimethyl ester	52079
10	19.192	13.72	Ethanol, 2-nitro-, propionate (ester)	22373
			Propanoic acid, 2-bromo-, methyl ester	35282
11	21.477	4.39	No matches found	
			Heptanedioic acid, dimethyl ester	52079
12	21.858	6.92	Ethanol, 2-nitro-, propionate (ester)	22373
			Propanoic acid, 2-bromo-, methyl ester	35282
13	22.118	4.07	No matches found	
14	26.696	5.65	2,2'-(1,4-Piperazinediyl)bis[N-(4-methoxyphenyl)succinimide]	234311
15	26.939	4.98	No matches found	

CONFLICT OF INTEREST

The author declares that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

REFERENCES

Adhab, M. (2021). Be smart to survive: Virus-host relationships in nature. Journal of Microbiology, Biotechnology and Food Sciences, 10(6), e3422. https://doi.org/10.15414/jmbfs.3422

Adhab, M., Al-Kuwaiti, N., & Al-Ani, R. (2021). Biodiversity and occurrence of plant viruses over four decades: Case study for Iraq. In 2021 Third International Sustainability and Resilience Conference: Climate Change (pp. 159–163). IEEE. https://doi.org/10.1109/IEEECONF53624.2021.9668128

Al-Ani, R. A., Athab, M. A., & Matny, O. N. (2013). Management of potato virus Y (PVY) in potato by some biocontrol agents under field conditions. Advances in Environmental Biology, 7(3), 441–444.

Al-Ani, R. A., Adhab, M., & Diwan, S. (2011). Systemic resistance induced in potato plants against Potato virus Y common strain (PVYO) by plant extracts in Iraq. Advances in Environmental Biology, 375–381.

Al-Ani, L. K. T., Soares, F. E. D. F., Sharma, A., de los Santos-Villalobos, S., Valdivia-Padilla, A. V., & Aguilar-Marcelino, L. (2022). Strategy of nematophagous fungi in determining the activity of plant parasitic nematodes and their prospective role in sustainable agriculture. Frontiers in Fungal Biology, 3, 863198. https://doi.org/10.3389/ffunb.2022.863198

Al-Ani, R. A., Adhab, M. A., Mahdi, M. H., & Abood, H. M. (2012). Rhizobium japonicum as a biocontrol agent of soybean root rot disease caused by Fusarium solani and Macrophomina phaseolina. Plant Protection Science, 48. https://doi.org/10.17221/16/2012-PPS

Boulahouat, S., Cherif-Silini, H., Silini, A., Chenari Bouket, A., Luptakova, L., Saadaoui, N., Alenezi, F. N., & Belbahri, L. (2022). Critical evaluation of biocontrol ability of Bayoud infected date palm phyllospheric Bacillus spp. Agronomy, 12(10), 2403. https://doi.org/10.3390/agronomy12102403

Degani, O., & Dor, S. (2021). Trichoderma biological control to protect sensitive maize hybrids against late wilt disease in the field. Journal of Fungi, 7(4), 315. https://doi.org/10.3390/jof7040315

George, P., Gupta, A., Gopal, M., Mohanan, R. C., Thomas, L., & Thomas, G. V. (2011). Antagonistic activity of coconut rhizospheric and endophytic Bacillus spp. Journal of Plantation Crops, 39, 278–284.

Gouda, S., Das, G., Sen, S. K., Shin, H. S., & Patra, J. K. (2016). Endophytes: A treasure house of bioactive compounds of medicinal importance. Frontiers in Microbiology, 7, 1538. https://doi.org/10.3389/fmicb.2016.01538

Hantoosh, M. N., & Hussein, H. Z. (2023). Bioactivity of magnesium oxide nanoparticles synthesized by alcoholic extract of walnut tree bark (Juglans regia) against Thielaviopsis paradoxa and T. punctulata in *vitro*. In IOP Conference Series: Earth and Environmental Science (Vol. 1252, No. 1, p. 012021). https://doi.org/10.1088/1755-1315/1252/1/012021

Hantoosh, M. N., & Hussein, H. Z. (2023). First molecular record of Thielaviopsis punctulata causing black scorch disease on date palms in Iraq. In IOP Conference Series: Earth and Environmental Science (Vol. 1252, No. 1, p. 012020). https://doi.org/10.1088/1755-1315/1252/1/012020

Hardoim, P. R. (2019). Biologically active compounds from bacterial endophytes. In Endophytes and Secondary Metabolites (pp. 3–31). Springer.

Huang, L., Zhu, X., Zhou, S., Cheng, Z., Shi, K., Zhang, C., & Shao, H. (2021). Phthalic acid esters: Natural sources and biological activities. Toxins, 13(7), 495. https://doi.org/10.3390/toxins13070495

Iqbal, A., Imran, M., Badshah, S. L., Shami, A., Ali, B., Shah, Z., Ayaz, M., Alasmari, A., Albalawi, M. A., Al-Doaiss, A. A., & Bajaber, M. A. (2024). Biochemical profile of Vaucheria karachiensis. Algal Research, 77, 103346. https://doi.org/10.1016/j.algal.2023.103346

Khaledi, N., & Assareh, M. H. (2023). Evaluation of antifungal activity of Trichoderma harzianum. Journal of Crop Protection, 12(3), 265–282.

Kumari, B., Mallick, M. A., Solanki, M. K., Solanki, A. C., Hora, A., & Guo, W. (2019). Plant growth promoting rhizobacteria. In Plant Health Under Biotic Stress (pp. 109–127).

Liu, Z., Xu, N., Pang, Q., Khan, R. A. A., Xu, Q., Wu, C., & Liu, T. (2023). Salt-tolerant Trichoderma longibrachiatum. Journal of Fungi, 9(3), 304. https://doi.org/10.3390/jof9030304

López-López, M., et al. (2022). Isolation of Trichoderma spp. Horticulturae, 8(8), 714.

Mulatu, A., et al. (2022). Biodiversity of Trichoderma. Crops, 2(2), 120–141.

Vos, P., et al. (Eds.). (2011). Bergey's manual of systematic bacteriology (Vol. 3). Springer.

Ngo, M. T., et al. (2021). Antifungal activity of sorbicillinoids. Journal of Fungi, 7(6), 428.

Nihorimbere, G., et al. (2024). Bacillus-based biocontrol. Frontiers in Plant Science, 15, 1349357.

Octarya, Z., et al. (2021). Antimicrobial activity of Aspergillus fumigatus. Biodiversitas, 22(4).

Phoka, N., et al. (2020). Role of volatiles from Trichoderma. Journal of Fungi, 6(4), 341.

Qasem, M., Smair, S. H., & Ahmed, A. S. (2014). Control of Macrophomina phaseolina. Iraqi Journal of Agricultural Science, 45(2), 185–195.

Rashiya, N., et al. (2021). Inhibition of biofilm formation. Microbial Pathogenesis, 150, 104693.

Sridharan, A. P., et al. (2021). Metabolites of Trichoderma. Microbial Pathogenesis, 150, 104714.

Swain, H., Naik, S. K., & Mukherjee, A. K. (2019). Growth promotion in rice. Biological Control, 133, 123–133.

Wang, Z., et al. (2019). Response of Pseudomonas fluorescens. Ecotoxicology and Environmental Safety, 167, 36–43.

Włoch, A., et al. (2020). Antimicrobial activity of lactones. Biomolecules, 10(12), 1594.

Zhang, C., et al. (2021). Difenoconazole resistance. Plant Disease, 105(2), 400–407.

Zhang, S., Xu, B., & Gan, Y. (2019). Seed treatment with Trichoderma. International Journal of Molecular Sciences, 20(15), 3729.

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فعالية العزلات المحلية لعوامل السيطرة الحيوية ضد مرض اللفحة السوداء على نخيل التمر

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المستخلص

اجريت هذه الدراسة بهدف عزل الفطر *Trichoderma longibrachiatum* والبكتريا *Bacillus subtilis* من عينات جذور نخيل التمر السليمة وتقويم فعاليتها التضادية ضد الفطرين الممرضين *Thielaviopsis paradoxa* و *Thielaviopsis punctulata* المسببين لمرض اللفحة السوداء في نخيل التمر *Phoenix dactylifera*. واظهر كل من الفطر الاحيائي *T. longibrachiatum* والبكتريا *B. subtilis* الكفاءة في تثبيط نمو كل من الفطرين الممرضين *T. paradoxa* و *T. punctulata* عند الزرع المزدوج على وسط دكستروز البطاطا الصلب (PDA). اذ ادى الفطر *T. longibrachiatum* الى تثبيط نمو كل من الفطرين الممرضين *T. paradoxa* و *T. punctulata* بنسبة 50.58 و 60.17% بالتتابع واعطت البكتريا *B. subtilis* نسبة تثبيط 39.99 و 38.09% بالتتابع. كما أظهرت نتائج الكشف عن مركبات الايض الثانوي في مزارع كل من الفطر *T. longibrachiatum* و البكتريا *B. subtilis* باستخدام جهاز GC–MS ان لكل منهما القابلية على انتاج العديد من المركبات الحيوية النشطة. ومن بين تلك المركبات Hexanoic acid و 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester و Terephthalic acid, 2-ethylhexyl octyl ester و Heptane, 1-(ethenylthio)- و 4(1H)-Pyrimidinone, 2-(ethylthio)-.

الكلمات المفتاحية: Trichoderma، Bacillus، مركبات الايض الفعالة، مضادات الفطريات، المنتجات الطبيعية.

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