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Isolation, Identification, Evaluation of *Purpureocillium Lilacinum* Egyptian Isolate Toxicity Test in Vitro and Analysis Its Bioactive Products

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ABSTRACT: *Purpureocillium* is an essential natural bio-control agent and a source of mycopesticides for pest management of several insect orders all over the world. In this study, *Purpureocillium lilacinum* Egyptian isolate bioactive constituents and its insect host range were estimated.

The tested isolate was identified morphologically and molecularly by amplification of internal transcribed spacer (ITS) region using the polymerase chain reaction technique (PCR). The ITS sequence was compared with other *Purpureocillium* published isolates in genbank data base and our isolate showed that *Purpureocillium lilacinum* (MYS) (MT102250) isolate has 98% homology with most published isolates. The pathogenicity of the tested isolate *P. lilacinum* (MYS) was evaluated by using five concentrations of fungal spore suspensions and fungal filtrates against four pests, *Bemisia tabaci, Tetranychus urticae, Thrips tabaci* and *Diuraphis noxia*. The fungal filtrate had a higher toxic effect on all tested pests than the spore suspension. The greater fungal filtrate concentration resulted in higher mortality rates seven days post treatment, reached 100% for *T. tabaci* and *T. urticae* and 97% for *B. tabaci* and *D. noxia*, respectively. LC_{50} values for spore suspension concentrations were $0.3x10^2$ spore/ml for *T. tabaci* and *T. urticae* and $0.2x\ 10^3$ and $0.2x10^2$ spore/ml for *B. tabaci* and *D. noxia* respectively and were 2% for *T. tabaci* and *B. tabaci* and 5 and 9% for *D. noxia* and *T. urticae* respectively with culture filtrate concentrations. GC-MS was used to estimate the bioactive components of *P. lilacinus* extracts, and about 31 secondary metabolites with different bioactivities were present, some of them identified as insecticides

Keywords: *P. lilacinus, host range, GC-MS, Bemisia tabaci, Tetranychus urticae, Thrips tabaci, Diuraphis noxia*

INTRODUCTION

Certain homopterous insect pests such as aphids, spider mite, whitefly and thrips are of great economic importance cause serious damage either directly by sucking plant juice or indirectly as vectors of plant pathogenic viruses **(Yokomi *et al.*, 1990 and Abdulsalam *et al.*, 1998).** Entomopathogenic fungi such as *Beauveria bassiana*, *Verticillium lecanii*, *Metarhizium anisopliae* and *Isaria fumosorosea* are appearing to be effective, environment-friendly and target specific bio-control tools against many sucking insect pest species. They are being utilized to induce fast mortality of target pests by inhibiting enzymatic detoxification mechanisms that successively dispose the target pest insects for fungal infection **(Majeed *et al.*, 2017 and Ambethgar 2018).** Today, biological control is an increasingly important component of integrated pest management (IPM) program for agriculture as well as for the urban environment **(Ali *et al.*, 2020).**

Purpureocillium is a prevailing filamentous fungus found in a broad range of habitats, including soils, forests, grassland, deserts, sediments, and even sewage sludge **(Mountfort and Rhodes 1991).** *Purpureocillium* belongs to the phylum Ascomycota and to the order Eurotiales, which has septate, branched hyphae, carrying long chains of conidia from the tips of the conidiophores, and flask to oval or subglobose phialides**.** Colonies of *Purpureocillium* are initially flaky and white, then become a distinct color. *Purpureocillium* strains are generally not harmful to health and are sometimes opportunistic in humans and mammals.

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Many species of *Purpureocillium* are important entomopathogenic fungi, refer to a class capable of infecting or parasitizing living host organisms and representing an ecologically highly specialized group of microorganisms. Entomopathogenic fungi are known for their ability to produce various bioactive compounds during infection and proliferation in insects and are considered potential sources of new bioactive compounds. Entomopathogenic fungi belonging to the Purpureocillium genus have been extensively studied as potential biological control agents against insects**.** (**Ze-Bao *et al*., 2022**).

The objectives of the current study were the isolation, identification of *P. lilacinus Egyption isolate*, estimation of its efficacy as a biocontrol agent against some sucking insects and analysis its bioactive products.

MATERIALS AND METHODS

The Microorganism:

Purpureocillium lilacinum isolate was isolated from soil by the soil dilution plating method with slight modification **(Desoky *et al.,* 2020)**

Identification of the fungal isolate

Morphological identification:

The fungal isolate was characterized morphologically based on some fungal structure including morphology of the colony, conidiophore diameter and shape of phialides and conidia. The isolate was identified in the Regional Center for Mycology and Biotechnology, Culture Collection and Identification Unit based on the identification key of **Samson, (1974).**

Molecular identification

The morphological characterization of the fungal isolate was confirmed molecularly as the following:

Fungal DNA purification

Sambrook *et al*. (1989) prescribed the extraction of the Genomic DNA from a fresh culture of fungal mycelium that was followed accordingly. A sterile mortar and pestle were utilized to thoroughly smash the mycelium sample after being frozen in liquid nitrogen. After that, 500 µl CTAB buffer {100mM Tris-HCl, pH 8.0, 1.4M NaCl, 20mM EDTA, 2% w/v hexadecyl-trimethyl-ammonium bromide (CTAB)} and 5 µl of 2-mercaptoethanol (0.1 %) were added and mixed thoroughly. For 30 minutes, the homogenate sample was incubated at 60°C. A homogenate sample was centrifuged at 14,000 xg for 5 minutes. A fresh tube was used to transfer the supernatant. Chloroform: isoamyl alcohol (24:1) was added in an equal volume. To separate the phases, the specimen was centrifuged for 60 seconds at 14,000 xg. A fresh tube was utilized to transfer the upper aqueous phase. 0.7 volumes of cold isopropanol were added to precipitate the DNA, after which it was incubated at -20°C for 15 minutes. For 10 minutes, the sample was centrifuged at 14,000 x g. 500 ml of ice-cold, 70% ethanol was used to rinse the DNA pellet. In 50 l of nuclease-free water, dried DNA was dissolved. On an agarose gel, DNA quantity and integrity were analyzed.

ITS Region Amplification of the Fungal Isolate:

The ITS region (ITS1 and two and the 5.8S gene) sequencing was implemented as molecular method for identifying the fungal isolate. Polymerase chain reaction (PCR) was applied to amplify the ITS region from the fungal isolate's extracted DNA. Vivantis Co. (Selangor Darul Ehsan, Malaysia) generated a pair of oligonucleotide primers, forward (ITS1): CTTGGTCATTTAGGAAGTAA and reverse (ITS4): TCCTCCGCTTATTGATATGC **(Gardes and Bruns, 1993).** 14.5 µL of deionized sterile water, 2.5 µL of buffer 10X, 1 µL of MgCl2 (50 mM), 0.5 µL of dNTP´s (20mM), 2 µL of each primer (10 pM), 0.5 µL of Taq polymerase (5U/mL) and 2 µL of sample DNA will be used in each reaction. The following conditions were used for PCR amplification: initial denaturation at 94°C for 5 min, then 35 cycles of denaturation at 94 °C for 1 min, annealing at 65 °C for 1 min, and elongation at 72 °C for 1 min, followed by a final extension at 72 °C for 7 min. The amplified products were electrophoretically separated on a 1.0% agarose gel stained with ethidium bromide (0.5 g/ml), and they were then seen on a UV gel documentation system (BioRad, USA).Following the manufacturer's recommendations, the QIAquick PCR Purification Kit (Qiagen) was used to separate the PCR product from unincorporated PCR primers and dNTPs.The laboratory of LGC, Germany, used the Big TriDye sequencing kit (ABI Applied Biosystems) to analyze the sequence of the purified PCR product of the ITS gene. Using the Basic Local Alignment Search Tool (BLAST) and the National Center for Biotechnology Information (NCBI) database, USA (http://www.ncbi.nlm.nih.gov), a homology search for the DNA sequence for the ITS region was performed. Using the BLASTN program **(Altschul et al., 1990),** the resulting sequences were compared to ITS sequences in the National Center for Biotechnology Information (NCBI) GeneBank database, and closely comparable sequences were retrieved. The sequence was manually imported into and aligned using the Clustal W function in the Molecular Evolutionary Genetics Analysis program, version 7.0 **(Kumar et al., 2016).** The DNA sequence obtained in this study was submitted to the NCBI database (accession No. MT102250).

Toxicity test

Conidial suspension preparation

The tested fungal isolate was cultivated on Czapek Dox agar medium and left to incubate for 15 days at 27ºC and 50–60% RH. After an incubation period the conidia were harvested from the surface of the fungal culture by scraping the sporulating colonies and suspended in distilled serialized water containing 0.02% Tween-80 (as a wetting agent) **(Hicks, *et al.*, 2001).** The resulting conidial suspension was firstly clarified from hyphal debris by filtration using sterilized piece of cloth, then the concentration of this stock solution was estimated by using a Neubauer hemocytometer **(Alves and Moraes, 1998).** Five concentrations (10^3, 10^4, 10^5, 10^6, 10^7 spore/ml) were prepared from the stock solution by serial dilution to be used in pathogenicity experiments.

Fungal culture filtrate preparation

A culture disc (2 cm) of the tested fungal isolate cultured for 15 days on Czapek Dox agar medium was inoculated into 250ml Czapek Dox broth medium in 500 ml Erlenmeyer flasks and cultivated at 27°C for 15 days, then the liquid media was pass through chess cloth and five concentrations (25, 50, 75, 100%) were prepared from the collected filtrate to be used in the bioassay experiment.

Tested insects

The pathogenicity of *P. lilacinum* isolate was estimated on four insects species belonging to four family: *Aleyrodidae, Tetranychidae, Thripidae* and *Aphididae* (Table 1). *Bemisia tabaci* (Aleyrodidae: Hemiptera) insects were collected from a tomato agriculture greenhouse belonging to the Agriculture Research Center. *Tetranychus urticae* (Tetranychidae: Trombidiformes) mites were collected from a cucumber agriculture greenhouse belonging to the Agriculture Research Center. *Thrips tabaci* (Thripidae: Thysanoptera) were collected from an onion field belonging to the Agriculture Research Center and *Diuraphis noxia* (Aphididae: Hemiptera) were provided from the rearing lab of the biological control department of Plant Protection Research Institute Agriculture Research Centre as pots of highly infested wheat plant.

Table 1: Details of the insects bioassayed with the tested fungal isolate (*P. lilacinum*) to check its host range.

Species	Common Name	Order	Family
Bemisia tabaci	Whitefly	Hemiptera	Aleyrodidae
Tetranychus urticae	Red spider mite	Trombidiformes	Tetranychidae
Thrips tabaci	Thrips	Thysanoptera	Thripidae
Diuraphis noxia	Wheat Aphid	Hemiptera	Aphididae

Bioassay experiment

***Bemisia tabaci*:** Adult of whiteflies *B. tabaci*, were collected from tomato greenhouse, transported to the laboratory, and placed in plastic cups containing tomato leaves for 24 hours to lay eggs, the whiteflies adult were then removed, and the newly infested tomato leaves were left for approximately 12 days until the nymphs reached the second instar, which were used in the bioassay test. The number of 2nd instar per each tomato leaf was first counted and recorded then every infested leaf was dipped into each fungal concentrations prepared previously for 30 sec, air dried then get back into the cup and incubated at 25°C and 70% RH. Sterilized water with 0.02% Tween-80 was used in the control treatment and the experiment was replicated three times.

***Tetranychus urticae*:** Cucumber leaves infested with *T.urticae* were collected from cucumber greenhouse. *T.urticae* nymph were transferred to fresh leaf discs of approximately 2 cm in diameter cut from fresh cucumber leaves and these discs were placed on moistened cotton pads which were placed on a piece of sponge in plastic box. Enough water was added to the box to keep the cotton moist. The nymph with the leaf discs were sprayed with each tested fungal concentration using a fine atomizer. Three replicates were used for one concentration each one contains 30 nymphs with three replicates for control, nymphs were treated with sterilized water containing 0.02% Tween-80.

***Thrips tabaci*:-** The onion plants that were highly infested with *T.tabaci* were collected from an onion field. Thirty individuals of the first larval instar were carefully transferred to fresh onion leaf discs in a plastic box. Tested fungal concentrations were sprayed directly onto the nymph. The control was sprayed with sterilized water containing 0.02% Tween-80. The boxes were covered with ventilated lids to permit airflow. Each treatment consisted of three replicates of 30 thrips per replicate.

***Diuraphis noxia*:** Thirty individuals of the third instar aphid were accurately transferred from the wheat plant pots to wheat leaves spread over filter paper placed on the bottom of plastic boxes with a wet cotton piece to provide moisturizing. A fine sprayer was used to spray the tested fungal concentrations directly to the tested nymph. Three replicates for each concentration plus three replicates for control contained nymph sprayed only with sterilized water including 0.02% Tween-80 were used.

Statistical Analysis

The mortality rate for each tested pest was recorded at 3, 5 and 7-days post treatment. Concentrations of fungal spore suspension and culture filtrate with the mortality data were computed to be analyzed and to determine the fifty percent lethal concentration (LC50) by using Ldp line software (**Bakr, 2000**).

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

The GC-MS system (Agilent Technologies) was equipped with a gas chromatograph (7890B) and a mass spectrometer detector (5977A) at the Central Laboratories Network, National Research Centre, Cairo, Egypt. The GC was equipped with an HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 µm film thickness). The Analysis was performed with hydrogen as the carrier gas at a flow rate of 1.0 ml/min at a split less, injection volume of 1 µl and the following temperature program: 50 °C for 1 minute; increase at 10 °C /min to 300 °C and hold for 20 min. The injector and detector were held at 250 °C. The mass spectra were obtained by electronic ionization (EI) at 70 eV; with a spectral range of m/z 30-700 and a solvent delay of 9 min. The mass temperature was 230°C and Quad 150 °C. The identification of various constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

RESULTS

Macroscopic and Microscopic Features Isolated Fungus

The isolated fungus was identified based on the morphology of its colony and microscopic structure. Firstly, the colonies shown in Fig (1) appear on Malt Extract Agar media (the most common media for fungal growth) flat, white, pink to violet in color with a 2-4 cm diameter.

The microscopic examination, Fig. (2), showed that, the conidiophores with 2.4µm diameter appear cylindrical and branched giving rise to clusters of phialides. The phialildes 7.0 x 2.5 µm have a broad base tapers to form a long and narrow neck takes the shape of flask. The conidia 2.5 x 3.0 µm arranged forming very long chains at the apical end of the phillides and are globose and elliposoidal in shape.

Fig (1): *P. lilacinum* colony features on Malt Extract Agar media

Fig (2): Microscopic examination of *P. lilacinum*

Molecular Identification of Fungal Isolate

The isolate was identified as *Purpureocillium lilacinum* by molecular sequences of the ITS region. To obtain the coding sequence of ITS (Internal Transcribed Spacer 1, 2, and 5.8S subunit), polymerase chain reaction (PCR) amplification was performed with forward universal primers (ITS1) and reverse universal primers (ITS4). The amplified band at ~750 bp was shown in Fig (3). The amplified fragment was sequenced and homology searches in the database. The amplified DNA sequence of the ITS region (Internal Transcribed Spacer 1, 2, and 5.8S subunit) of the purified isolate was submitted to NCBI Genbank under the accession number MT102250.

Phylogenetic Analysis

The phylogenetic relationship was derived from comparisons of the ITS gene sequences and a dendrogram was constructed with other *Purpureocillium* species (Figure 4). The result of BLASTn program

showed that our *Purpureocillium* (MT102250) MYS isolate has 98% homology with most published isolates such as *Purpureocillium lilacinum* (MN242828), *P. lilacinum* (MH137675), *P. lilacinum* (MT672601). The phylogenatic tree showed that the fungal isolate in this study was closely related to all other database isolates

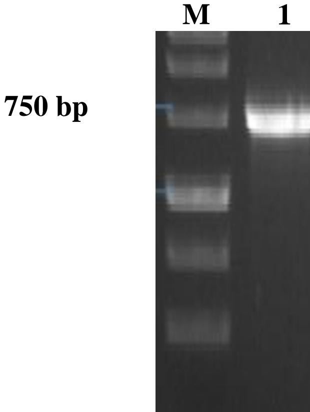
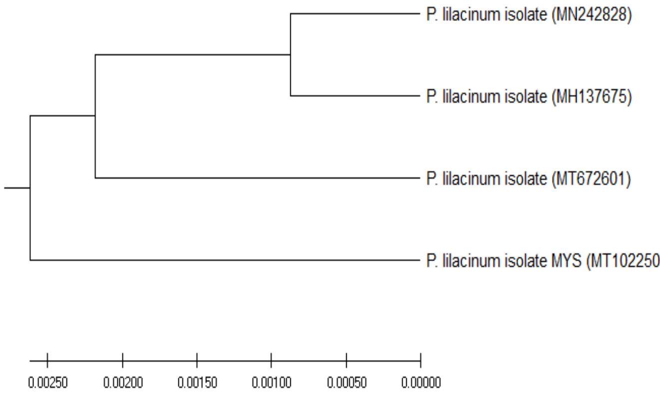


Fig (3): An ethidium bromide stained gel of PCR products for the ITS of fungal isolate. M: 1 kb plus marker BioHelix. Lane (1) shows ~ 750 bp ITS produced by PCR .



Fig(4): A phylogenetic tree of *P. lilacinum* MYS isolate (MT102079) of study with other species of *Purpureocillium* based on the nucleotide of ITS sequences constructed by the neighbor-joining method. Evolutionary analyses were conducted in MEGAX. Alignment of the sequences was done with CLUSTALW, bootstrap values (in percent) are calculated from 1000 resamplings. The scale bar shows the genetic distance. The number presented next to each node shows the percentage bootstrap value of 1000 replicates.

Toxicological Studies

For investigation the pathogenicity of the tested fungal isolate, five fungal spore suspension and five fungal culture filtrate concentrations were treated against four insect pests (*Thrips tabaci, Tetranychus urticae, Bemisia tabaci and Diuraphis noxia*). Bioassay results proved the pathogenicity of the tested isolate at all concentrations used and revealed that the fungal culture filtrate has higher toxic effect than the fungal spore suspension.

High pathogenicity was observed in *Thrips tabaci* whereas the mortality rate reached 100% at both the higher culture filtrate concentration (100%) and the higher spore suspension concentration (10^7 spore/ml) used, the remaining filtrate concentrations (75, 50, 25%) achieved 97, 97 and 87% mortality percentage while the rest of spore suspension concentrations (10^6, 10^5, 10^4, 10^3 spore/ml) attained mortality percentages ranged from 70 to 97% seven day post treatment Table (2). *Bemisia tabaci* and *Diuraphis noxia* showed the same response to filtrate and spore suspension with a mortality rate of 97% and 93% respectively achieved by the highest concentration used after the same day mentioned above Table (2). *Tetranychus urticae* was more susceptible to fungal filtrate with a mortality percentage reaching 100 and 97% at the two higher concentrations and 90 and 83% at the two lowest concentrations used. The mortality percentages of *T. urticae* treated with spore suspension were lower than those treated with the fungal filtrate ranged from 63 to 87% Table (2). The LC_{50} values

for the treated spore suspension calculated seven days after treatment were $0.3x10^2$ spore/ml for *T. tabaci* and *T. urticae* and $0.2x\ 10^3$ and $0.2x10^2$ spore/ml for *B. tabaci* and *D. noxia,* respectively, while it was 2% for the *T. tabaci* and *B. tabaci* treated with the fungal filtrate and were 5 and 9% for *D. noxia* and *T. urticae* after the same day post treatment.

Table (2) Efficacy of *P. Lilacinum* spore suspensions and culture filtrates against *Diuraphis noxia*, *Thrips tabaci* , *Bemisia tabaci* and *Tetranychus urticae* nymph after 3, 5, 7 days post treatment

Insect Nymph	*P. Lilacinum* spore suspensions				*P. Lilacinum* culture filtrates			
	Concentrations (Spore/ml)	Mortality Percentage (%)			Concentrations (%)	Mortality Percentage (%)		
		3 days	5 days	7 days		3 days	5 days	7 days
Diuraphis noxia	10^7	60	80	93	100	70	87	97
	10^6	57	73	90	75	65	80	93
	10^5	57	73	87	50	60	73	87
	10^4	50	63	77	25	53	67	77
	10^3	43	60	73	LC_{50}	21	11	9
	LC_{50}	$1.4x10^4$	$0.2x10^2$	$0.2x10^2$				
	Concentrations (Spore/ml)	Mortality Percentage (%)			Concentrations (%)	Mortality Percentage (%)		
		3 days	5 days	7 days		3 days	5 days	7 days
Thrips tabaci	10^7	60	83	100	100	77	93	100
	10^6	60	80	97	75	70	87	97
	10^5	53	73	93	50	65	83	97
	10^4	47	67	83	25	53	77	87
	10^3	40	57	70	LC_{50}	21	5	2
	LC_{50}	$0.4x10^5$	$0.8x10^2$	$0.3x10^2$				
	Concentrations (Spore/ml)	Mortality Percentage (%)			Concentrations (%)	Mortality Percentage (%)		
		3 days	5 days	7 days		3 days	5 days	7 days
Bemisia tabaci	10^7	53	70	93	100	70	83	97
	10^6	47	67	87	75	67	80	97
	10^5	40	60	87	50	60	77	87
	10^4	40	57	67	25	57	70	80
	10^3	33	47	53	LC_{50}	14	4	2
	LC_{50}	$0.3x10^7$	$0.1x10^4$	$0.2x10^3$				
	Concentrations (Spore/ml)	Mortality Percentage (%)			Concentrations (%)	Mortality Percentage (%)		
		3 days	5 days	7 days		3 days	5 days	7 days
Tetranychus urticae	10^7	70	73	87	100	80	90	100
	10^6	67	70	80	75	77	87	97
	10^5	60	67	80	50	70	80	90
	10^4	50	60	77	25	63	73	83
	10^3	43	50	63	LC_{50}	11	7	5
	LC_{50}	$0.7x10^4$	$0.3x10^3$	$0.3x10^2$				

Gas chromatography–mass spectrometry (GC-MS) analysis:

P. lilacinus filtrate consists of 31 compounds (Table 3). The total peak area of the detected compounds was 100 % and the major peak areas were 23.15 % for D-(-)- Fructofuranose, pentakis(trimethylsilyl) ether (isomer 1) ($C_{21}H_{52}O_6Si_5$) , 19.62 % for D-Fructose, 5TMS derivative ($C_{21}H_{52}O_6Si_5$),11.49 % for D-Psicofuranose, pentakis(trimethylsilyl) ether (isomer 1) ($C_{21}H_{52}O_6Si_5$), 10.5 % for L-(-)-Sorbose, 5TMS derivative ($C_{21}H_{52}O_6Si_5$) and 7.31 % for D-Mannitol, 6TMS derivative ($C_{24}H_{62}O_6Si_6$) they are Monosaccharides .

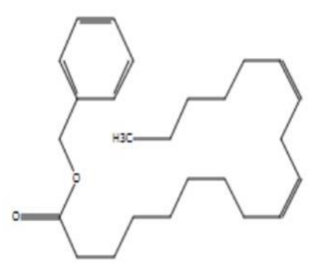
Peak No.	Metabolites Identified compounds	MF	MW	Area %	RT (min)	Spectrum Structure
1	Butylated Hydroxytoluene	C15H24O	220.35	1.39	14.045	
2	Tyrosol, 2TMS derivative	C14H26O2Si$_2$	282.5260	0.4	14.709	
3	Pentitol, 3-desoxytetrakis-O-(trimethylsilyl)-	C17H44O4Si$_4$	424.9	0.66	15.379	
4	Decane, 2,3,5,8tetramethyl	C14H30	198.39	0.41	16.148	
5	Xylitol, 5TMS derivative	C20H52O5Si$_5$	513.0514	1.3	16.51	
6	Tetradecane, 2,6,10trimethyl-	C17H36	240.5	0.28	16.607	
7	2-Deoxypentofurano se, 3TMS derivative	C14H34O4Si$_3$	350.6739	1.08	16.811	

8	D-(-)-Fructofuranose, pentakis(trimethylsil yl) ether (isomer 1)	$C_{21}H_{52}O_6Si_5$	541.0615	23.15	17.429	
9	D-Psicofuranose, pentakis(trimethylsil yl) ether (isomer 1)	$C_{21}H_{52}O_6Si_5$	541.0615	11.49	17.504	
10	D-Fructose, 5TMS derivative	$C_{21}H_{52}O_6Si_5$	541.0615	19.62	17.587	
11	D-(-)-Tagatofuranose, pentakis(trimethylsil yl) ether (isomer 1)	$C_{21}H_{52}O_6Si_5$	541.0615	2.45	17.956	
12	L-(-)-Sorbofuranose, pentakis(trimethylsil yl) ether	$C_{21}H_{52}O_6Si_5$	541.0615	1.6	18.069	

13	D-(-)-Fructofuranose, pentakis(trimethylsil yl) ether (isomer 2)	$C21H52O6Si_5$	541.0615	1.8	18.16	
14	L-(-)-Sorbose, 5TMS derivative	$C21H52O6Si_5$	541.0615	10.5	18.265	
15	Ribitol, 5TMS derivative	$C20H52O5Si_5$	513.0514	1.52	18.627	
16	D-Mannitol, 6TMS derivative	$C24H62O6Si_6$	615.2585	7.31	18.695	
17	3-Hexadecyloxycarbonyl-5-(2hydroxyethyl)-4methylimidazolium ion	$C_{24}H_{45}N_2O_3$	409.635	0.35	18.989	

18	beta.-D-Glucopyranose, 5TMS derivative	C21H52O6Si5	541.0615	2.61	19.109	
19	Palmitic Acid, TMS derivative	$C_{19}H_{40}O_2Si$	328.6052	0.93	19.471	
20	5-Tridecene, (Z)-	$C_{13}H_{26}$	182.3455	0.24	20.292	
21	E-10-Dodecen-1-ol propionate	$C_{15}H_{28}O_2$	240.38162	0.24	20.503	
22	Thiosulfuric acid (H2S2O3), S-(2aminoethyl) ester	$C_2H_7NO_3S_2$	157.22	0.56	20.564	
23	1,2-15,16-Diepoxyhexadecane	$C_{16}H_{30}O_2$	254.41	0.61	20.646	
24	1-Monopalmitin, 2TMS derivative	$C_{25}H_{54}O_4Si_2$	474.8649	3.31	23.985	

25	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	298.9	0.34	24.58	
26	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306.4828	0.41	25.115	
27	Glycerol monostearate, 2TMS derivative	$C_{27}H_{58}O_4Si_2$	502.9180	1.37	25.348	
28	5,8,11,14Eicosatetraenoic acid, methyl ester, (all-Z)-	$C_{21}H_{34}O_2$	318.4935	0.24	28.581	
29	2H-Indeno[1,2b]furan-2-one, 3,3a,4,5,6,7,8,8boctahydro-8,8dimethyl	$C_{13}H_{18}O_2$	206.28	1.62	31.467	
30	2-Methyl-4-(2,6,6trimethylcyclohex-1enyl)but-2-en-1-ol	$C_{14}H_{24}O$	208.34	1.96	34.677	

31	9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester	$C_{25}H_{38}O_2$	370.6	0.25	40.962	

Rt : Retention time; MW : Molecular weight; MF: Molecular formula

DISCUSSION

The piercing sucking pests are widely distributed and attack a wide range of crops, causing significant damage, either directly by inserting their mouthparts and sucking plant juice, or indirectly by inject toxic material to the plants through feeding or transmitting disease organisms (vectors of plant diseases) (**Coffin and Coutts, 1995 and Guzman *et al*., 1997**). Entomopathogenic fungi are presently considered the most important factor developed as ecofriendly biopesticides used for controlling pest populations **(Kumar and Riffat 2015)**. They showed great potential for use as biological control agents against a wide range of insect pests **(Majeed *et al*., 2017).** Among them, ***Purpureocillium*** that is mainly known for its nematophagous capacity, and also reported as an insect parasite and biological control agent of several phytopathogenic fungi and bacteria through different mechanisms of action (**Alejandro *et al.* 2020**). *P.lilacinus* is a very important natural control agent used for pest management worldwide **(Jamali and Ghasemi, 2016)**. It is a promising biological control agent used for controlling various piercing sucking insect pests **(Faria and Wraight, 2001; Fiedler and Sosnowska, 2007).** It has also been tested as a potential bioinsecticide to fight pests of great economic importance worldwide (**Sanjaya *et al*., 2016**) Morphological observation as a preliminary identification of this isolate revealed that it belongs to the genus *Purpureocillium* (**Qinglin *et al*., 2012**)The current study aimed to test both conidial suspension and filtrate of P. lilacinus against the most destructive sucking pests (*T. tabaci, T. urticae ,B. tabaci and D. noxia*,). Firstly, the fungal isolate was isolated from a soil sample and identified as *P. lilacinus* based on morphological and molecular studies. The macro and micro morphological features of our tested isolate is agreeing with what was reported by **Samson (1974),** also studies based on sequence analysis using the ITS region confirmed that the tested isolate was the species *P. lilacinus* (Accession no MT 102250).

The results of our study obviously indicated that *P.lilacinus* is very effective for the control of all tested insect pests. Several studies have proved the efficacy of *P. lilacinus* and other entomopathogenic fungi to control various insect pests. **Ayhan (2005)** is in accordance with the present findings as he emphasized the potential use of this fungus as a biocontrol agent against whitefly after examination the efficacy of seven *Purpureocillium* isolates against second instar whitefly and indicated that they caused over 70% mortality six days after inoculation. **Lopez *et al.* (2014)** confirmed the potential pathogenicity of *P. lilacinum* against herbivores and aphids of cotton under greenhouse and field conditions. Our obtained data showed that fungal filtrate was more toxic than fungal spore suspension and this agreed with **Desoky *et al.* 2020** who compared both *P. lilacinum* spore suspension and filtrate with their counterpart of Metarhizium anisopliae against *Aphis craccivora* nymph and revealed firstly the high toxicity of the filtrate than the spore suspension and asserted that *P.lilacinum* has a higher toxic effect than M. anisopliae with fungal spore suspensions LC_{50} (7×10^2, 1×10^5 spore/ml) and culture filtrates LC_{50} (3.2, 4.7 %) 7 days post-inoculation for the two fungi respectively. **Wakil *et al.* 2012** indicated that *P. lilacinus* is very effective for controlling *T. tabaci* when used alone and in combination with neem on cotton. The application of *P. lilacinus* and neem resulted in a great reduction in pest population in the laboratory and semi-natural conditions compared to untreated control. Parallel to our findings **(Kim and Kim, 2008)** who examined the pathogenicity of different isolates of *Purpureocillium* spp. *Lecanicillium attenuatum* and *Beauveria bassiana* against cotton aphid and reported that the mortality percentage reached 100% five day post inoculation either by conidia or blastospores of the tested fungi. Also **Wakil *et al.* (2012)** tested the pathogenicity of *P. lilacinus*, *Azadirachta indica* and the formulation of diatomaceous earth (PyriSec) for the control of cotton aphid, Aphis gossypii under laboratory and semi-natural conditions and revealed that all the tested treatments gave significant control of aphid, and confirmed that, *P. lilacinus* in combination with Neem showed the best control of aphids in detached leaf bioassay and semi-natural conditions. **Tae-Young Shin *et al.* (2011)** evaluated the virulence of *P. lilacinus* against *T.*

urticae and positive results were recorded at laboratory conditions with a mortality percentage reaching 73.3% at 6 day post inoculation and concluded that *P. lilacinus* is an effective pathogen has potential for management *T. urticae.*
The GC-MS chromatography showed 31 peaks corresponding to 31 biological compounds (Table 10), their structures can be described as polyketides, terpenoids, peptides, alkaloids, quinone, pyrones, sterols, and fatty acids which have various biological activities including antimicrobial,antitumor, insecticide,antiplasmodic , antimalarial, nematicide,herbicide and inhibitory enzyme, and could be used as pesticides ,nematicides , antifungal, antibacterial, antiviral, anticancer, antioxidants and anti-inflammatory agents, This results agree with **Ze-Bao *et al* (2020)** who reviewed that *Purpureocillium* produces 223 secondary metabolites and their biological activities from 13 known species and several unidentified strains of *Purpureocillium* . The major compounds in fungal extract were mentioned in several previous studies as larvicidal, nematicidal, pesticide, Insecticidal agents. (**Kumar *et al*. 2010, Krishnamoorth and Subramaniam 2014, Sivakumar *et al*. 2011, Tulika and Mala 2017, Yokeswari *et al*. 2018 , Michalina *et al* 2021, Hernández *et al* 2020 and Shaker *et al* 2019**). GC-MS analysis indicated the presence of some identified compounds as insecticides in fungal extract such as: Butylated Hydroxytoluene , 9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester, Tetradecane, 2,6,10trimethy, This agrees with the results of **Abdullah (2019)** who proved that GC-MS analysis indicated the presence of some identified compounds as insecticides in both extracts of *Beauveria bassiana* and *Trichoderma harzianum* such as: n-Hexadecanoic acid; Hexadecanoic acid, methyl ester; 9,12Octadecadienoic acid, methyl ester, (E,E)-; Tetradecanoic acid, 12- methyl-, methyl ester; 7,10-Octadecadienoic acid, methyl ester and trans-13-Octadecenoic acid and also The presence of high percentage of monosaccharides in *P. lilacinus* filtrate is evidence for their significant biological activities . This is in agreement with **(Muhammad et al 2015)** results which showing that phytochemical and pharmacological studies have revealed that polysaccharides are one of the main active ingredients in several types of edible and medicinal fungi and show significant biological activities, including immunomodulation, antitumor, antioxidant and antiviral activities

CONCLUSION

P. lilacinum has been proven to be an insect pathogen. Its metabolites have several properties which are considered more effective, non-toxic and eco-friendly enabling them to be used in the management of target insect pests as insecticidal formulations. So they play an important role in pest control and also have bioactive products used in several biocontrol fields and medicine.

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الملخص العربي

عزل – تعريف – تقييم فاعلية فطر الباربيورسيليم ليلسينم (عزلة مصرية) معمليا وتحليل منتجاته الأيضية

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فطر البابيورسيليم يعتبر من أهم العوامل الحيوية ومصدر رئيسي للمبيدات الفطرية المستخدمة في مجال المكافحة الحيوية للتحكم في الافات الحشرية الضارة للمحاصيل النباتية الهامة في جميع انحاء العالم ومن خلال هذه الدراسة تم عزل الفطر من التربة المصريه وتعريفه من خلال الشكل الظاهري والتركيب الجيني باستخدام الشفرة الجينية لمنطقة ITS وهي المنطقة المعتمدة عالميا للتشخيص الجزيئي المتقدم وقد تم اختبار المدي العوائلي له باستخدام كلا من الراشح الفطري والمعلق الجرثومي علي أربعة من أخطر الافات الاقتصادية التي تنتمي للافات الثاقبة الماصة وهم ذبابة القطن البيضاء – أكاروس العنكبوت الأحمر – التربس – من القمح وايضا تحديد المنتجات الحيوية النشطة من خلال تحليل الراشح الفطري بواسطة التحليل الكروموتوجرافي وقد أظهرت النتائج ان العزلة الفطرية تتطابق بنسبة 98% مع معظم عزلات الباربيويسليم ليلسينم الاخري المسجلة في قاعدة بيانات بنك الجينات لذلك تم تسجيلها كعزلة مصرية جديدة في بنك الجينات برقم (MT102250). كما أظهرت النتائج أن الراشح الفطري له التأثير الأعلي سمية من المعلق الجرثومي حيث أحدث نسبة موت 100% للتربس و أكاروس العنكبوت الأحمر و 97% لذبابة القطن البيضاء ومن القمح كما كان التركيز نصف المميت للراشح الفطري 2% للتربس وذبابة القطن البيضاء و 5 و9 % لمن القمح و أكاروس العنكبوت الأحمر علي التوالي بينما كان التركيز النصف مميت للمعلق الجرثومي 0.3×10^2 جرثومة / مل للتربس و أكاروس العنكبوت الأحمر و 0.2×10^3 و0.2×10^2 جرثومة / مل لذبابة القطن البيضاء ومن القمح علي التوالي. وبالتحليل الكروماتوجرافي للراشح الفطري تم معرفة وتحديد 31 مركب ذو خصائص بيولوجية متنوعة لها أهمية كبيرة في مجال مكافحة الافات وأيضا العديد من المجالات البيولوجية الأخري.