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Utilizing Chitinolytic Fungi for the Biocontrol of Root-Knot Nematode Infections

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Abstract

Chitinolytic soil organisms are considered as valuable sources of secondary metabolites. This research identified promising chitinolytic fungal strains isolated from strawberry-growing soils for use as bionematicides against root-knot nematodes, *Meloidogyne* spp. Out of eleven isolates, five potent fungi namely *Aspergillus niveus*, *A. oryzae*, *Fusarium incarnatum*, *Purpureocillium lilacinum*, and *Trichoderma yunnanense* exhibited noticeable chitinolytic activity. *A. oryzae* had the highest chitinolytic activity at 0.8427. Gas chromatography-mass spectrometry (GC-MS) revealed beneficial volatile metabolites, and toxicological assessments showed that all isolates were safe at a 1 ml/rat dose. In lab trials, *A. niveus* and *A. oryzae* effectively prevented *M. javanica* egg hatching, while *T. yunnanense* and *A. niveus* had the highest juvenile mortality rates. Greenhouse tests showed that *A. niveus* reduced root-knot nematode populations by 70%, and field trials indicated that *P. lilacinum* and *A. oryzae* enhanced strawberry growth. All fungal treatments decreased nematode populations by 25.3 to 63.1% and improved strawberries' total soluble solids, sugar content, firmness, and vitamin C levels. *A. oryzae* increased polyphenol oxidase (PPO) and peroxidase (PO) activities, while *T. yunnanense* produced high phenolic compounds. *A. niveus* also raised proline content, enhancing plant resilience. This study suggests that these chitinolytic fungi can provide sustainable solutions for nematode management and improve agricultural practices.

Keywords: Chitinolytic fungi, *Trichoderma, Aspergillus, Purpureocillium, Meloidogyne*

1. Introduction:

According to FAO statistics from 2023, Egypt cultivated 9.408 ha of land for the production of 638.000 tonnes, making strawberries one of the country's most significant

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economic crops **[1]**. Strawberry plants, just like other commercial crops, are vulnerable to damage from biological, genetic, and environmental factors as well as from the interactions among these factors, **[2]**. These health problems with strawberries cause huge financial losses for the countries that grow and produce them. Strawberry plants are harmed by bacteria, viruses, fungi, nematodes, and arthropods. Every year, they cause enormous financial losses that amount to hundreds of millions of dollars or euros **[1]**.

Globally, the agricultural sector is significantly impacted by plant-parasitic nematodes (PPNs), which are estimated to cause losses of $80 billion to $157 billion annually **[3,4,5]**. Root knot nematodes (RKNs), *Meloidogyne* spp. are among the PPNs that have been widely studied because of their economic importance and extensive dispersion. Plant endoparasites known as RKN are required to stay immobile. Specific below-ground symptoms are the formation of root galling, although RKN may produce more general above-ground symptoms. According to **[6]**, root knot nematodes may parasitise a wide range of plants and live in a wide range of temperature zones, from temperate to tropical climates.

Nematicides, which are synthetic compounds with active ingredients like methyl bromide and carbofuran, were widely utilised in the past to manage PPNs. Due to their harmful to both human and environmental health, many of these agents have been removed from the market. Chemical nematicides often pose a serious risk to mammals and other non-target animals **[7]**. Several government agencies are fighting against the use of chemical nematicides in international trade. As a result, many are considering integrated pest management strategies and searching for "natural" nematicides **[8,9]**. This is important since increasing soil temperatures encourage the rapid growth of many PPNs, population expansions, and increased plant damage **[10]**. Furthermore, PPNs may become increasingly widespread worldwide, with tropical PPNs mostly migrating to warming temperate regions **[11]**. Using bioagents such as bacteria and fungus to control PPN populations (especially RKN populations) is a sustainable and environmentally acceptable approach. They can interact directly or indirectly with the pathogen through the host-plant, by inducing plant resistance **[12,13,14]**. These agents have lately drawn attention **[15,16,17]**.

Numerous studies on the topic suggest that our understanding of the application of bioagents derived from chitinolytic fungi is growing **[18]**. The two primary direct contact mechanisms that are described are competition and the generation of lytic enzymes and/or secondary metabolites (antibiosis). The production of nematicidal compounds is one of the many antagonistic activities that many fungi participate in **[19, 20]**. This facilitates the development of novel nematodes or novel mechanisms of action **[21]**.

Because of its biotechnological potential, researchers have lately experimented with extracellular chitinase generated by microorganisms **[22, 23, 24]**. Producing biopesticides is among the various applications for fungal chitinase. Fungal chitinase plays a crucial role in preventing the hatching of *M. javanica* eggs, according to **[25]**. Chitinases are useful for biological control of agricultural pests and the creation of biopesticides with an enzymatic base **[26, 27]**.

Strong activity against the root knot nematode was demonstrated by a fungal strain that was found while searching for strains with nematocidal activity **[28]**. Thus, the goals of this study were to: 1) isolate and molecularly identify a fungal strain antagonistic towards the root knot nematode; 2) measure the fungal isolates' chitinase activity; 3) define the active substance produced by the fungal isolates; 4) perform toxicological studies for the most prominent fungal isolates that produce chitinase; 5) investigate the effects of culture filtrate (CF) and fungal culture filtrate (FCF) on *M. javanica* juveniles and eggs *in vitro*; and 6) evaluate its nematicidal action on strawberries infected with *Meloidogyne* spp. *in vivo*.

1. Materials and methods

1.1.1. Isolation of fungal:

Samples of strawberry-growing soil (250g) were collected from the El-Behera governorate's Markaz Badr. Samples were shipped in an ice box and examined in a microbiological lab as soon as they arrived. A range of media were used, such as yeast extract sucrose, czapek's dox, malt extract, potato dextrose, and yeast malt extract agar. The repeated dilutions procedure was the isolation strategy. The 10^5 dilutions were evenly distributed across the medium's surface to create the infected plates. Five to seven days were spent incubating the plates at 25 °C. A colony of fungus growing on agar plates was purified using the single spore and hyphal-tip procedures. It was then put on malt extract slants and maintained as a stock culture.

1.1.2. Fungal identification :

The visual characteristics and microscopic examination of the isolated fungi led to their classification at the genus and species level. **[29, 30, 31, 32, 33]**.

1.1.3. The initial assessment of chitinase activity:

A new culture plug of each isolate was inoculated onto solid chitinase assay media supplemented with bromothymol blue as a pH indicator. After five days of incubation on chitinase assay medium, the chitinase activity exhibited by fungal isolates was classified into four groups according to the diameter of the colored zone, **[34]**.

Diameter of colored zone (mm)	Chitinase activity ratio
75-100	High
51-75	Moderate
26-50	Low
1-25	Weak

1.1.4. Molecular identification of isolated fungi:

To identify fungal isolates by molecular techniques, genomic DNA was extracted from the selected fungal pure cultures using the microbial DNA extraction kit (Quick-DNATM Fungal/Bacterial Microprep Kit (Zymo research #D6007)). In 18S rRNA sequencing, the universal forward and reverse primers are used to achieve molecular identification. The Maxima Hot Start PCR Master Mix (Thermo K1051) was then used for PCR. After that, the GeneJETTM PCR Purification Kit (Thermo K0701) was used to clean up the PCR product. All sequenced PCR findings were confirmed using the National Centre for Biotechnology Information's (NCBI) mega blast for species identification. The percentage of our isolates that are homogeneous with those that are recognized was recorded. Both the genus and the species could be identified.

1.1.5. Evolutionary relationships of taxa

The evolutionary history was inferred using the Neighbor-Joining technique **[35]**. The bootstrap consensus tree created from 1000 replicates is thought to represent the evolutionary history of the taxa being studied **[36]**. Collapsed branches belong to partitions that were replicated in less than half of the bootstrap replicates. The percentage of duplicate trees that the related taxa clustered in during the bootstrap test (1000 repetitions) is displayed beside the branches. The Kimura 2-parameter method was used to compute the evolutionary distances, which are expressed in base substitutions per site **[37]**. Twenty-eight nucleotide sequences were analyzed in this investigation. The final dataset contained 303 places in total. In MEGA11, evolutionary research was carried out **[38]**.

1.1.6. Chitinase activity induction and measurement:

To initiate the synthesis of chitinase, a variety of inducers were employed as chitin sources. The chitinase induction experiment required. 1.0 g of peptone, 1.4 g of (NH4)2 SO4, 0.2 g of KH2 PO4, 6.9 g of NaH2 PO4.H2 O, 0.3 g of MgSO4.7H2 O, and a few carefully selected chitinase inducers comprise the basic liquid medium. Chitinase activity of the culture supernatant was measured using a modified version of **[39]** approach. In summary, the reaction

mixture consisted 150 µl of 0.1M phosphate buffer (pH 7.0), 300 µl of 0.1% colloidal chitin, and 150 µl of the culture supernatant. After 10 minutes of incubation at 55 °C, the reaction mixture was centrifuged for 5 minutes at 10,000 rpm. About 200 µl of the resulting supernatant was mixed with 500 ml of distilled water and 1000 ml of Schales reagent. After ten minutes of simmering, the previously indicated mixture was promptly chilled. The absorbance of the combination was measured spectrophotometrically at 420 nm. The amount of enzyme needed to release one micromole of reducing sugar in the form of N-acetyl-D-glucosamine (GlcNAc) equivalent in a minute is known as one unit of chitinase activity.

1.1.7. Preparation of fungal cultures and free cell culture filtrates

A method developed by **[34]** involved using modified Gliotoxin Fermentation Medium (GFM) to encourage different fungal strains to produce chitinase. GFM, the enrichment medium for fungal growth, was supplemented with 0.45% colloidal chitin as the sole carbon source. Crab shell chitin was used to make colloidal chitin, and the pH was ultimately brought down to 4.8 **[40]**. Fresh discs from each fungal strain were grown separately on 250 ml of modified GFM for seven days at 28ºC and cultural filtrates were obtained by centrifugation at 120 rpm for ten minutes in preparation for use in ensuing *in vitro* and *in vivo* studies. On the other hand, the cell free culture filtrate was prepared by centrifuging fungal biomass, and the resulting supernatant was filtered through a sterile 0.45µm filter membrane.

1.1.8. Gas chromatography–mass spectrometry analysis (GC-MS)

The most well-known fungal isolate was examined at the Central Laboratories Network, National Research Centre (NRC), Giza, Egypt, using gas chromatography-mass spectrometry (GC-MS).

1.1.9. Sample derivatization :

Prior to GC analysis, the sample was extracted, dried, and reconstituted in 50 µL of bis(trimethylsilyl) trifluoroacetamide (BSTFA)+trimethylchloro-silane (TMCS) 99:1 silylation reagent to derivate sample functional groups to trimethylsilyl groups (abbreviated TMS). It was then incubated for 90 minutes for the oximation reaction.

1.1.10. GC-MS analysis

The GC-MS system from Agilent Technologies comprised a mass spectrometer detector (5977A) and a gas chromatograph (7890B). An HP-5MS column with dimensions of 30 meters by 0.25 mm internal diameter and 0.25 µm film thickness was installed in the GC. The study was conducted using the following temperature program, using hydrogen as the carrier gas at a split-less injection volume of 2 µl and a flow rate of 2.0 ml/min. 5 °C/min to reach 100 °C and

hold it for 0 minutes; 5 °C/min to reach 320 °C and hold it for 10 minutes; and 50 °C for 5 minutes. The injector and detector were kept at 280 °C and 320 °C, respectively. Mass spectra with a spectrum range of m/z 25–700 and a solvent delay of 6 min were produced using electron ionisation (EI) at 70 eV. Quad hit 150 °C, while the mass reached 230 °C. By contrasting the spectrum fragmentation pattern with those found in the Wiley and NIST Mass Spectral Library data, a number of components were found.

1.1.11. Toxicology studies:

The acute oral toxicity study was conducted in the Animal Breeding House (ABH) of the Environmental Toxicology Research Unit (ETRU), Pesticide Chemistry Department, National Research Centre (NRC), Giza, Egypt. Male rats that weighed 115±5 g were used in this study. The animals were housed at 22± 3 °C and 42% humidity in cages with a 12-hour day and 12-hour night cycle. They received free access to water and a standard pellet diet. The care and use of laboratory animals at the ABH was approved by the Local Ethical Review Committee of the National Research Council, and those procedures were followed in accordance with the guidelines for the care and use of laboratory animals **[41]**. Six groups of rats were created: group I was the control group, group II was given *P. lilacinum*, group III was given *T. yunnanense*, group IV was given *A. oryzae*, group V was given *A. niveus*, and group VI was given *F. incarnatum*. Each rat (groups II through VI) received one millilitre of extract orally. The mortality and toxicity indicators were tracked for 14 days.

1.2. Evaluation of fungal isolates against *M. javanica in vitro*:

1.2.1. Pure Nematode Culture

According to **[42]**, *M. javanica* eggs were extracted from injured tomato (cv. Castle Rock) roots using a sodium hypochlorite solution. Nematode juveniles (J2s) in their second stage were collected daily from the hatching eggs and stored at 25°C. Juveniles just five days old were used for the tests.

1.2.2. *In vitro* test:

The fungal cultures (FCs) and fungal culture filtrates (FCFs) of five chitinolytic isolates were evaluated against the root-knot nematode *M. javanica*. In vials containing 100 eggs and newly hatched *M. javanica* juveniles (J2s), one millilitre (ml) of each isolate ($1x10^6$ cfu/ml) was introduced separately. Each treatment was replicated five times. Five vials of eggs and juveniles were served as a control at 25±2°C. Percentages of egg hatching inhibition were calculated after 3, 5, and 7 days of exposure, while percentages of J_2s mortality, were calculated after 72 hours of treatment according to formulae proposed by **[43]**:

Percentage of egg hatch inhibition= (Number of hatched eggs / Total eggs) × 100

Percentage of J_2s mortality = (Number of deceased J2s/Total J2s) × 100

1.3. *In vivo* evaluation of fungal isolates against *M. javanica*:

1.3.1. Greenhouse test:

Forty plastic pots (25 cm-d) were filled with 3 kg of sandy loam soil and kept moist before transplanting strawberry seedlings. Certain chitinolytic fungi, including *P. lilacinum, T. yunnanense, A. niveus, F. incarnatum,* and *A. oryzae*, were treated independently as soil drenches at a rate of 20 ml/pot @ $1x10^6$ cfu/ml. Ten days after planting, plants were inoculated with two thousand *M. javanica* eggs. Four pots were treated with the common nematicide Fenamiphos 40% EC (0.3 ml/pot) two days after the nematode inoculation. Additionally, four pots injected with root-knot nematodes were left without any extra material (chitinolytic fungus). The trial was conducted using a randomised complete block design, with four replicates of each treatment. Pots received as much water as they need and were kept in a greenhouse at a temperature of 25 ±5 °C.

The nematode-inoculated plants were collected sixty days later. Juveniles of *M. javanica* were isolated from 250g of soil per pot using sieving and modified Baermann procedures **[44]**. Roots were stained with acid fuchsine in lactic acid and examined under a microscope to count the stages of nematode growth, galls, and egg masses **[45]**. Furthermore, the root gall and egg mass index scales for 0–5 were used to record the root galls and egg masses. For example, 0 denoted no galls, or egg masses, 1 = 1-2, 2= 3-10, 3=11-30, 4=31-100, and 5 more than 100 galls or egg masses/root system. **[46]**.

1.3.2. Field experiment:

A field experiment was carried out in a farm in Markaz Badr City, El Beheira Governorate, to evaluate the nematicidal capability of chitinolytic fungal isolates against strawberries infected with root knot nematodes, *Meloidogyne* spp. Soil samples were collected at a depth of 30 cm to ascertain the initial population of nematodes, which was 500 J_2s/250 g soil. The transplants were placed 0.25 meters (interrow) apart in rows 0.75 meters apart on each side of the plots that made up the testing area. Four ridges, each 8 meters long and 0.75 meters wide, were present in each plot. The randomised complete block (RCB) experimental design included four replicates and seven treatments, each with twelve plants. After seven days, the plants were treated with a soil drenched with 100 ml of each of the fungal culture isolates (*P. lilacinum, T. yunnanense, A. niveus, F. incarnatum,* and *A. oryzae*) and the chemical nematicide,

Fenamiphos (40% EC) at the recommended dosage of 3 L/feddan. The same treatments were given again a month after the initial ones started.

Five plants were randomly selected from each replication, 90 days following the transplant date, in order to evaluate the growth parameters. A 250g composite sample, from each plot, was used for nematode J2s extraction through sieving and modified Baermann method **[44]**. One gram of each plant's root was stained **[45]** to determine the number of nematodes in the root, galls, and egg masses.

2.3.3. Fruits' qualitative attributes

a) Total soluble solids (T.S.S.) in fruits were measured using a handheld refractometer **[47]**.

b) The fruit firmness of completely mature fruits was measured using a Chatillon penetrometer (NY, USA) R gauge.

c- The **[48]** method was used to assay vitamin C.

d- The Lane and Eynon method was used to measure the total sugar in fresh strawberry fruits **[47]**.

2.3.4. Analysis of biochemistry

a) Peroxidase activity (PO) in fresh strawberry leaves was determined by measuring the oxidation of pyrogallol to pyrogallin at 425 nm in the presence of H2O2, **[49]**.

b) According to **[50]**, the activity of polyphenol oxidase (PPO) was measured. The activity of PPO was determined by measuring the change in absorbency of 1.0 mL of extract per minute at 420 nm using a UV spectrophotometer.

c- The instructions provided by **[51]** were followed in measuring the total phenols.

d- The modified **[52]** approach for strawberry plants was used to determine the proline content **[53]**.

2.3.5. Data analysis:

The data were analysed using ANOVA, and the means were compared using Duncan's multiple range test **[54]**.

2. Results

2.1. Primary screening of chitinase activity and fungal isolates:

Five of the eleven distinct fungal isolates extracted from the representative rhizospheric soil sample exhibited notable chitinase activity upon screening on the chitinase detection medium (Table 1). The augmentation of chitinase detection medium with colloidal chitin and bromothymol blue resulted in a substrate medium with clear yellow color at pH 4.7. The chitin as a substrate was broken down into N-acetyl glucosamine when chitinolytic fungal isolates were added to this medium. This caused the pH to shift towards alkalinity and the color of the pH indicator dye (BTB) to change from yellow to blue/blue green around the injected fresh fungal plugs in the chitin decline area (Fig.1). Three fungal isolates displayed the highest chitinase activity in the current study, while four isolates displayed negative activity, two isolates recorded low activity, and two isolates displayed moderate activity (Table 1). Five fungal isolates, designated DAMF1, DAMF2, DAMF3, DAMF4, and DAMF5, were chosen for additional research after exhibiting the most noticeable chitinase activity (width of colored zone ≥50 mm), and were purified on PDA medium (Fig 2).

Table 1: Chitinolytic enzyme activity of fungal isolates as determined by qualitative test.

Fungal isolate No.	Diameter of colored zone mm	Activity Ratio
1	27.30^{f}	L
2	0.00^{g}	N
3	0.00^{g}	N
4	79.66^{ab}	H
5	67.30^{c}	M
6	0.00^{g}	N
7	30.30^{e}	L
8	82.00^{a}	H
9	58.00^{d}	M
10	0.00^{g}	N
11	79.33^{b}	H
L.S.D	1.22	-

High activity is denoted by H, moderate activity by M, low activity by L, weak activity by W, and no activity by N.

Duncan's multiple range test revealed that the means in each column that had the same letter or letters did not differ at $P \leq 0.05$.

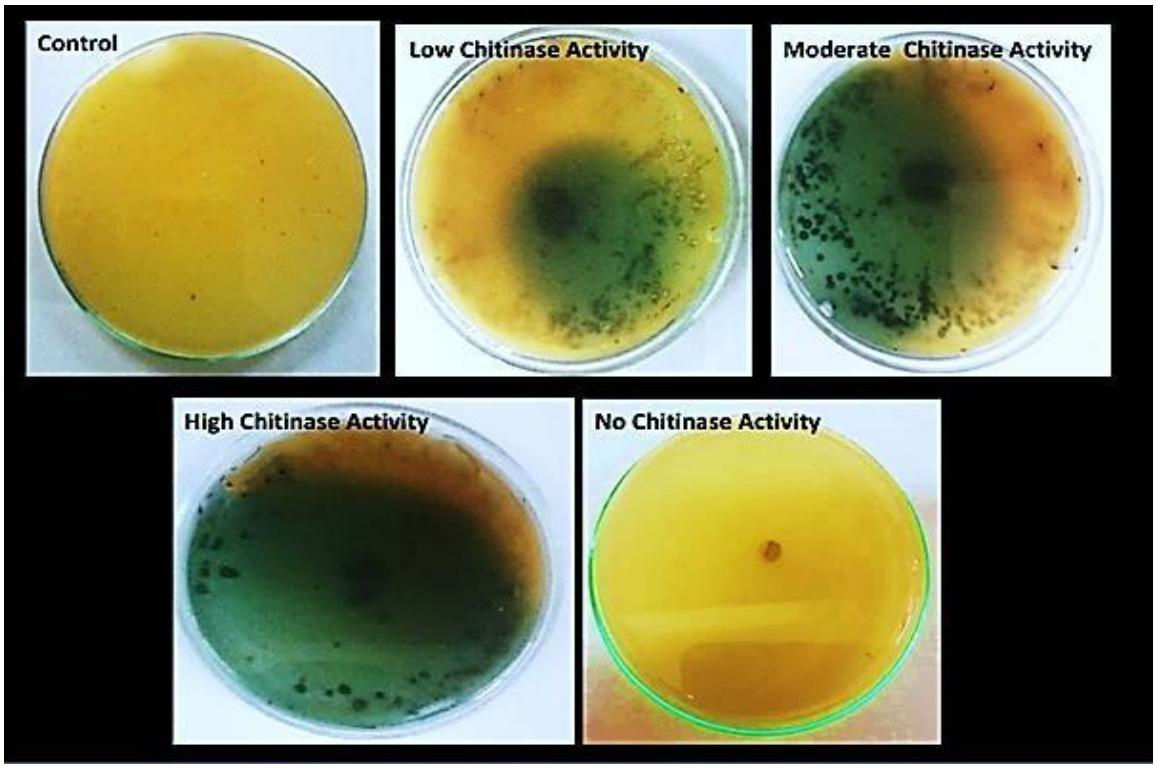


Fig.1. Primary Screening of fungal isolates for chitinase activity

Fig.2: The selected chitinolytic fungal isolates on PDA medium

2.2. Molecular identification of the selected fungal isolates:

Fig (3) demonstrates Phylogenetic Analysis of isolates that illustrates the relationships inferred from ITS gene sequences of present isolates (*Fusarium incarnatum* strain DAMF1 (OM877315), *Trichoderma yunnanense* strain DAMF2 (OM874629), *Aspergillus niveus* strain

DAMF3 (OM965355), *Aspergillus oryzae* DAMF4 (OM876875), *Purpureocillium lilacinum* DAMF5 (OM876240), as well as other related species with GenBank accession numbers shown in parenthesis alongside the strain names. Branch-level bootstrap values based on 1000 replicates are displayed above the branches. The number of nucleotide changes per site is indicated by the bar scale. MEGA11 software was used to calculate the evolutionary distances using the Tamura-Nei model.

BLASTn search revealed 99% nucleotide identity with *F. incarnatum* isolates. Close relationships were observed with isolates from Nigeria (MN882829), China (KX184815), China (MW659187) and India (KY776645) and on grape fruit from China (MT565585). Since our isolate (accession number OM877315) formed well-supported clade (99 - 100%) with other *F. incarnatum* sequences, consistent with the results of the BLASTn results. Also our *Trichoderma yunnanense* strain DAMF2 (OM874629) formed a well-supported clade (97%) with other *T. yunnanense* sequences. Close relationship were observed with *T. yunnanense* isolates from India (LC573454), Malaysia (OK584476 and MW543027). Phylogenetic analysis results were consistent with BLASTn results.

While our *Aspergillus niveus* strain DAMF3 (OM965355) belonged in a well-supported clade (100%) with other *A. niveus* sequences. Phylogenetic analysis results were consistent with BLASTn data (96.83%). Close relationship were observed with *A. niveus* isolates from Egypt (MT319815), USA (MH865243), Tanzania (KM979503), Italy (MK053576), and Algeria (MH109544).And our *Aspergillus oryzae* strainDAMF4 (OM876875) Formed a well-supported clade (100%) with other *A. oryzae* sequences. The phylogenetic analysis results were consistent with the results of the BLASTn search (98.68%). Close relationships were observed with *A. niveus* isolates reported from China.

Finally, Our *Purpureocillium lilacinum* strain DAMF5 (OM876240) belonged in a well-supported clade (100%) with other *P. lilacinum* sequences. The phylogenetic analysis results were identical with the results of the BLASTn search (100%). Close relationships were observed with *P. lilacinum* isolates from Taiwan (MK724000, MK723999), China (MH483683), India (KJ862077) and Malaysia (KM246754).

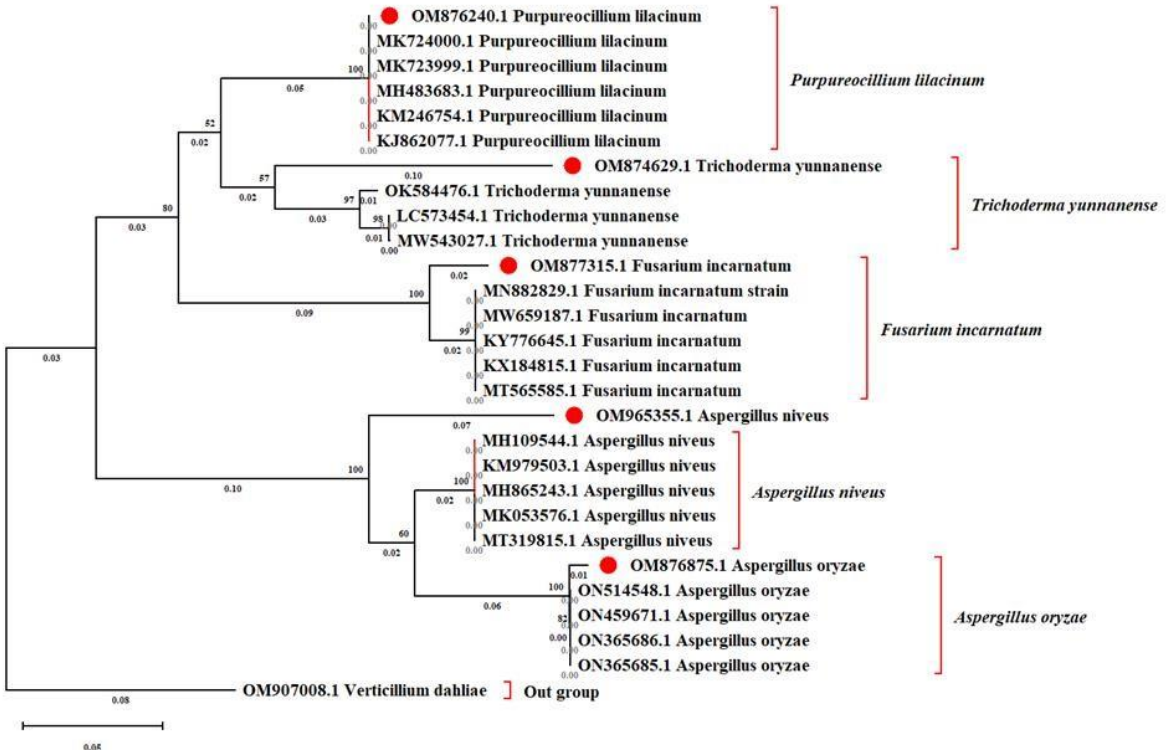


Fig. 3: Phylogenetic trees related to the tested fungal strains A) *Purpureocillium lilacinum*, B) *Trichoderma yunnanense*, C) *Fusarium incarnatum*, D) *Aspergillus oryzae*, and E) *Aspergillus niveus.*

2.3. Chitinase activity induction and measurement:

Using chitinase inducers and accepted techniques for chitinase testing, fungal chitinolytic activity was induced and quantitatively assessed. The highest quantitative chitinolytic activity fungus was found to be *A. oryzae* (0.8427), which outperformed other examined isolates (Table 2). *P. lilacinum*, on the other hand, had the lowest quantitative fungal chitinolytic activity.

Table 2: Induction and assaying of chitinase activity for isolated chitinolytic fungi

	Chitinase				
	Treatment	N	Subset		
			1	2	3
Tukey HSD[a,b]	*Purpureocillium lilacinum*	3	0.1980		
	Trichoderma yunnanense	3		0.3710	
	Aspergillus niveus	3		0.3740	
	Fusarium incarnatum	3		0.4243	
	Aspergillus oryzae	3			0.8427
	Sig.		1.000	0.207	1.000

Means for groups in homogeneous subsets are displayed. The error term is Mean Square (Error) = .001. Alpha = .05.

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

Table (3) provides the necessary information, including the chemical formula, area sum (%), retention time (RT), and compound name for each fungal isolate. GC-MS analysis identified the presence of important chemical components based on the National Institute of Standards and Technology's (NIST) database. Major chemicals for *F. incarnatum* are identified as L-(-)-Sorbose, pentakis (trimethylsilyl) ether, methyloxime (anti) and D-(-)-Fructose, pentakis (trimethylsilyl) ether, methyloxime (syn), with corresponding area sum of 32.93 and 34.65%. However, *T. yunnanense* has two main compounds with area sum of 27.66 and 33.43%, respectively: sucrose, octakis (trimethylsilyl) ether, and D-(+)-turnose, octakis (trimethylsilyl) ether. D-(-)-Tagatose, pentakis (trimethylsilyl) ether, and methyloxime (syn) were the main compounds with an area sum of 51.62% in the chitinolytic isolates of *A. niveus*. The primary chemicals for *A. oryzae* were identified as D-Psicose, pentakis (trimethylsilyl) ether, methyloxime (anti) and D-(-)-Tagatose, pentakis (trimethylsilyl) ether, methyloxime (syn), with respective area sum of 29.91 and 31.93%. With an area sum of 90.44%, silanol and trimethylphosphate (3:1) were identified as the main components for the fungal isolate *P. lilacinum*.

Table 3: Volatile Organic Compounds (VOCs) of the selected fungal isolates

Sample	Secondary metabolites or Volatile Organic Compounds (VOCs)	Retention Time(RT)	Area sum %	Chemical Formula
Fusarium incarnatum	2,3-Butanediol, 2TMS derivative	6.983	0.39	$C_{10}H_{26}O_2Si_2$
	(2S,3R)-3-[(4E,7E)-Nona-4,7-dienoyl]-N,N-bis(trimethylsilyl)oxirane-2-carboxamide	9.882	0.2	$C_{18}H_{33}NO_3Si_2$
	Silanol, trimethyl-, phosphate (3:1)	14.67	12.5	$C_9H_{27}O_4PSi_3$
	Butanedioic acid, bis(trimethylsilyl) ester	15.459	0.39	$C_{10}H_{22}O_4Si_2$
	D-Glucopyranoside, methyl 2,3,4,6-tetrakis-O-(trimethylsilyl)-	17.432	0.69	$C_{19}H_{46}O_6Si_4$
	Malic acid, 3TMS derivative	18.76	0.27	$C_{13}H_30O_5Si_3$
	Erythritol, 4TMS derivative	19.155	0.33	$C_{16}H_{42}O_4Si_4$
	Tartaric acid, 4TMS derivative	20.825	4.1	$C_{16}H_{38}O_6Si_4$
	Xylitol, 5TMS	21.773	2.81	$C_{20}H_{52}O_5Si_5$
	D-(-)-Erythrose, tris(trimethylsilyl) ether, ethyloxime (isomer 1)	23.132	0.85	$C_{15}H_{37}NO_4Si_3$

	3-Deoxyhexitol, 5TMS derivative	23.321	1.1	$C_{21}H_{54}O_5Si_5$
	L-(-)-Sorbose, pentakis(trimethylsilyl) ether, methyloxime (anti)	23.437	32.93	$C_{22}H_{55}NO_6Si_5$
	D-(-)-Fructose, pentakis(trimethylsilyl) ether, methyloxime (syn)	23.541	34.65	$C_{22}H_{55}NO_6Si_5$
	D-(-)-Tagatose, pentakis(trimethylsilyl) ether, methyloxime (anti)	23.724	1.82	$C_{22}H_{55}NO_6Si_5$
	(2R,3R,4R,5S)-Hexane-1,2,3,4,5,6-hexaol, 4TMS	23.936	6.94	$C_{18}H_{46}O_6Si_4$
Trichoderma yunnanense	(2S,3R)-3-[(4E,7E)-Nona-4,7-dienoyl]-N,N-bis(trimethylsilyl)oxirane-2-carboxamide	9.866	1.32	$C_{18}H_{33}NO_3Si_2$
	Silanol, trimethyl-, phosphate (3:1)	14.64	7.42	$C_9H_{27}O_4PSi_3$
	Tartaric acid, 4TMS derivative	20.832	6.23	$C_{16}H_{38}O_6Si_4$
	.beta.-D-Fructofuranose, 1,2,3,4,6-pentakis-O-(trimethylsilyl)-	21.895	0.25	$C_{21}H_{52}O_6Si_5$
	3-Deoxyhexitol, 5TMS derivative	23.283	0.18	$C_{21}H_{54}O_5Si_5$
	D-(+)-Xylose, tetrakis(trimethylsilyl) ether, methyloxime (anti)	23.412	0.26	$C_{18}H_{45}NO_5Si_4$
	D-Psicose, pentakis(trimethylsilyl) ether, methyloxime (syn)	23.488	0.28	$C_{22}H_{55}NO_6Si_5$
	d-Galactose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, o-methyloxyme, (1E)-	23.625	0.18	$C_{22}H_{55}NO_6Si_5$
	(2R,3R,4R,5S)-Hexane-1,2,3,4,5,6-hexaol, 4TMS	23.913	0.36	$C_{18}H_{46}O_6Si_4$
	Sucrose, octakis(trimethylsilyl) ether	29.222	27.66	$C_{36}H_{86}O_{11}Si_8$
	Rosiridin, 5TMS derivative	29.385	1.93	$C_{31}H_{68}O_7Si_5$
	D-(+)-Turanose, octakis(trimethylsilyl) ether	29.688	33.43	$C_{36}H_{86}O_{11}Si_8$
	.alpha.-D-Glucopyranoside, 1,3,4,6-tetrakis-O-(trimethylsilyl)-.beta.-D-fructofu	30.052	17.3	$C_{36}H_{86}O_{11}Si_8$
	D-Trehalose, 7TMS derivative	30.121	3.19	$C_{33}H_{78}O_{11}Si_7$
Aspergillus niveus	(2S,3R)-3-[(4E,7E)-Nona-4,7-dienoyl]-N,N-bis(trimethylsilyl)oxirane-2-	9.699	0.82	$C_{18}H_{33}NO_3Si_2$

	carboxamide			
	Silanol, trimethyl-, phosphate (3:1)	14.549	3.16	$C_9H_{27}O_4PSi_3$
	Erythritol, 4TMS derivative	19.147	0.71	$C_{16}H_{42}O_4Si_4$
	Tartaric acid, 4TMS derivative	20.794	2.44	$C_{16}H_{38}O_6Si_4$
	Adonitol, 5TMS	21.758	0.63	$C_{20}H_{52}O_5Si_5$
	D-Fructose, 1,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime	23.412	28.26	$C_{22}H_{55}NO_6Si_5$
	D-(-)-Tagatose, pentakis(trimethylsilyl) ether, methyloxime (syn)	23.503	51.62	$C_{22}H_{55}NO_6Si_5$
	d-Galactose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, o-methyloxyme, (1E)-	23.625	3.24	$C_{22}H_{55}NO_6Si_5$
	L-(-)-Sorbose, pentakis(trimethylsilyl) ether, methyloxime (anti)	23.693	5.74	$C_{22}H_{55}NO_6Si_5$
	d-Mannose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, o-methyloxyme, (1Z)-	23.776	0.64	$C_{22}H_{55}NO_6Si_5$
	(2R,3R,4R,5S)-Hexane-1,2,3,4,5,6-hexaol, 4TMS	23.906	2.74	$C_{18}H_{46}O_6Si_4$
	(2S,3R)-3-[(4E,7E)-Nona-4,7-dienoyl]-N,N-bis(trimethylsilyl)oxirane-2-carboxamide	9.707	2.88	$C_{18}H_{33}NO_3Si_2$
	Silanol, trimethyl-, phosphate (3:1)	14.549	8.89	$C_9H_{27}O_4PSi_3$
	Hexadecane-1,2-diol, 2TMS derivative	14.662	2.49	$C_{22}H_{50}O_2Si_2$
	Malic acid, 3TMS derivative	18.76	3.09	$C_{13}H_{30}O_5Si_3$
	Erythritol, 4TMS derivative	19.147	1.04	$C_{16}H_{42}O_4Si_4$
	Tartaric acid, 4TMS derivative	20.794	5.68	$C_{16}H_{38}O_6Si_4$
	Adonitol, 5TMS	21.758	0.75	$C_{20}H_{52}O_5Si_5$
	D-Psicose, pentakis(trimethylsilyl) ether, methyloxime (anti)	23.382	29.91	$C_{22}H_{55}NO_6Si_5$
Aspergillus oryzae	D-(-)-Tagatose, pentakis(trimethylsilyl) ether, methyloxime (syn)	23.488	31.93	$C_{22}H_{55}NO_6Si_5$
	Fructose benzoyl oxime, pentakis(trimethylsilyl)-	23.685	1.07	$C_{28}H_{59}NO_6Si_5$
	(2R,3R,4R,5S)-Hexane-1,2,3,4,5,6-hexaol, 4TMS	23.905	5.08	$C_{18}H_{46}O_6Si_4$
	Juniperoside III, 4TMS derivative	30.007	0.79	$C_{27}H_{52}O_7Si_4$
	D-Psicofuranose,	30.432	1.12	$C_{21}H_{52}O_6Si_5$

	pentakis(trimethylsilyl) ether (isomer 1)			
	D-(+)-Turanose, octakis(trimethylsilyl) ether, methyloxime (isomer 1)	30.53	3.08	$C_{37}H_{89}NO_{11}Si_8$
	Umbroside, 5TMS	35.061	1.02	$C_{34}H_{68}O_{12}Si_5$
Purpureocilliumlilacinum	2,3-Butanediol, 2TMS derivative	6.937	0.63	$C_{10}H_{26}O_2Si_2$
	3,6-Dioxa-2,7-disilaoctane, 2,2,4,5,7,7-hexamethyl-, (R*,S*)-	7.218	0.74	$C_{10}H_{26}O_2Si_2$
	(2S,3R)-3-[(4E,7E)-Nona-4,7-dienoyl]-N,N-bis(trimethylsilyl)oxirane-2-carboxamide	9.859	2.77	$C_{18}H_{33}NO_3Si_2$
	1-(Trimethylsilyl)-3-[(trimethylsilyl)oxy]urea	10.474	0.48	$C_7H_{20}N_2O_2Si_2$
	Silanol, trimethyl-, phosphate (3:1)	14.693	90.44	$C_9H_{27}O_4PSi_3$
	Tartaric acid, 4TMS derivative	20.809	4.96	$C_{16}H_{38}O_6Si_4$

2.5. Toxicology studies for five prominent chitinase fungal isolates:

On five prominent fungal isolates, acute and subacute oral toxicity tests were conducted. Results showed that administering doses equivalent to 1 ml/rat as a single oral dose, caused no signs of toxicity or mortality in the tested fungal isolates (Fig 4).

Fig 4: Toxicity of five fungal isolates in male rats under laboratory conditions, light/dark cycle (12 h/12 h), temperature (22 ± 3 °C), and 42 % humidity.

2.6. Evaluation of fungal isolates against *M. javanica in vitro*:

2.6.1. Egg hatching inhibition:

Data in figure (5A&5B) represent the influence of five fungal culture (FC) and fungal culture filtrate (FCF) of chitinolytic isolates on egg hatching inhibition of *M. javanica* under laboratory conditions. It was noticed that all fungal isolates had positive effect on hatching inhibition but the most effective treatments were *A. niveus* and *A. oryzae* respectively whether fungal culture or fungal culture filtrate.

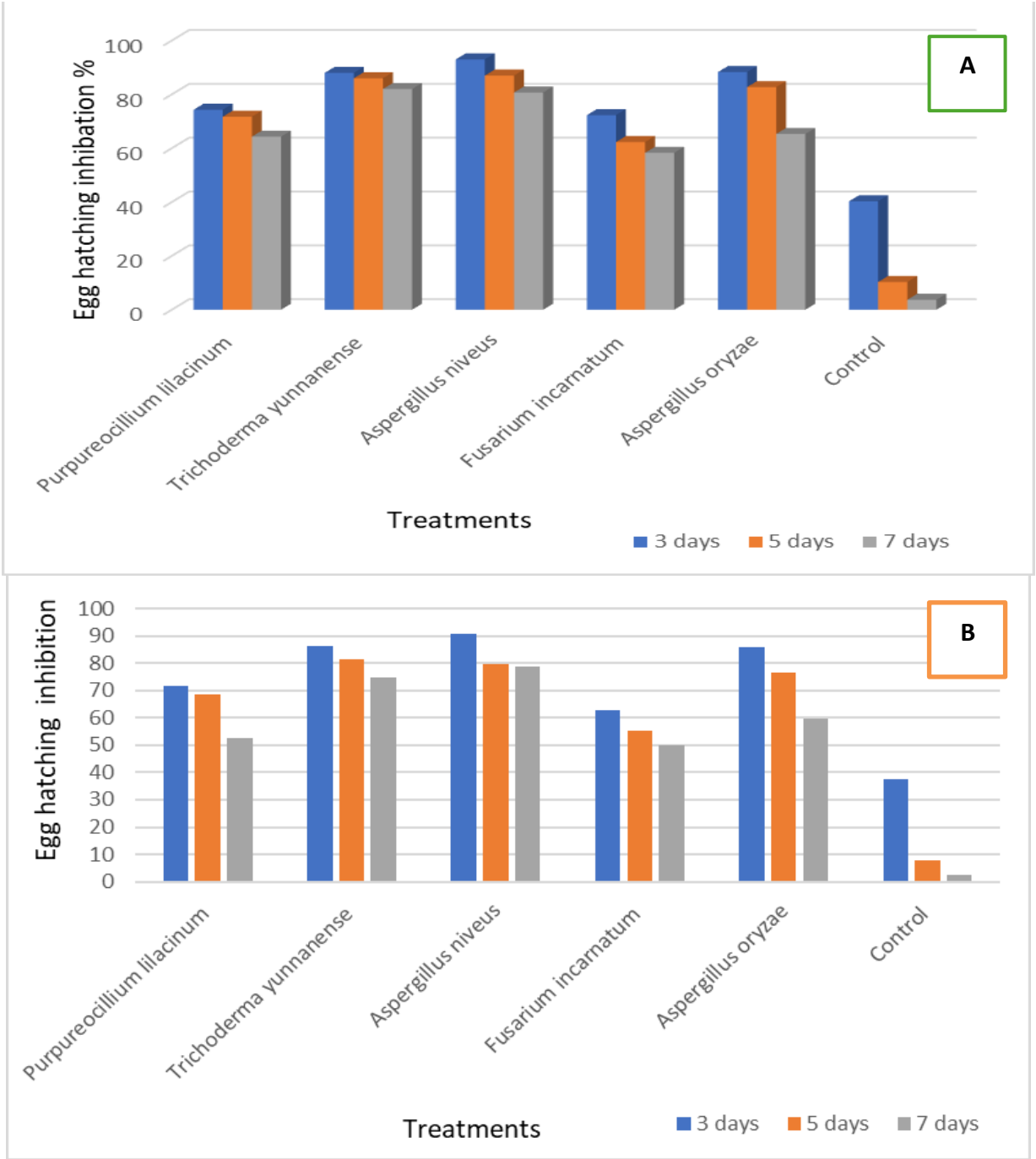


Figure (5): Egg hatching inhibition percentages of *Meloidogyne javanica* by fungal cultures (FCs,5A) and fungal culture filtrate (FCF,5B) of five chitinolytic isolates after 3, 5 and 7 days of exposure *in vitro*.

2.6.2. Juveniles (J_2s) mortality:

The identified fungal isolates were examined for their nematicidal activities via the effect of lytic enzymes on juvenile's mortality *in vitro*. Obtained results, after 72 h of exposure, revealed that all fungal culture (FC) and fungal culture filtrate (FCF) caused mortality in *M. javanica* J2s as compared to control. *T. yunnanense* and *A. niveus* exhibited the highest mortality percentage (Fig 6).

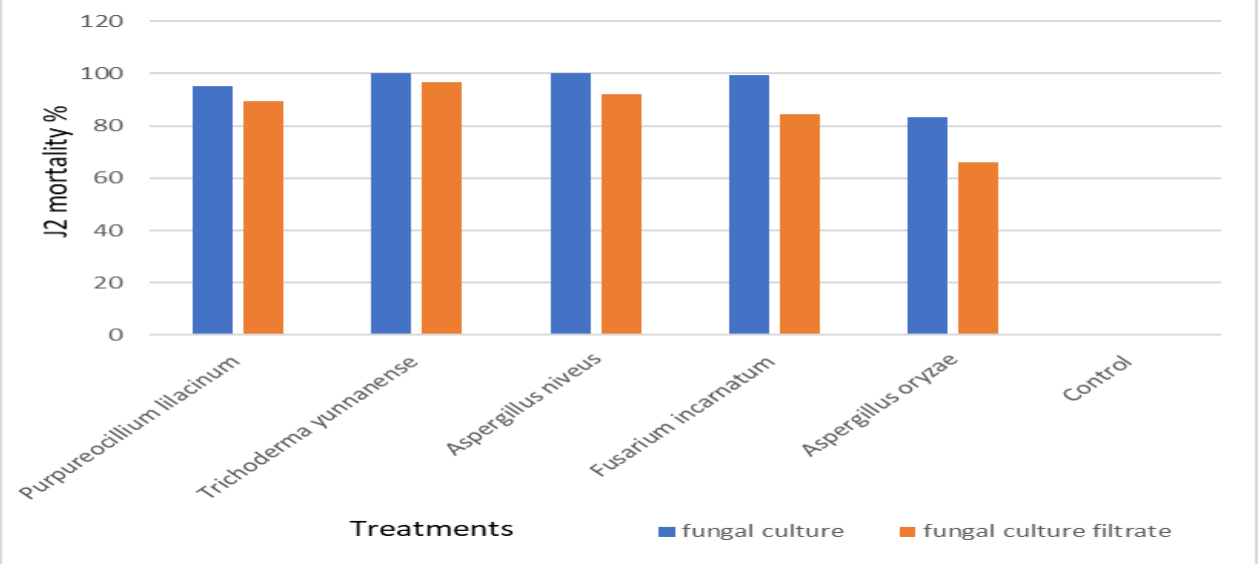


Figure (6): Mortality percentages of *Meloidogyne javanica* by cell fungal culture (FC) and fungal culture filtrate (FCF) of five chitinolytic fungal isolates after72 hours of exposure *in vitro*.

2.7. Evaluation of fungal isolates against *M. javanica in vivo*:

2.7.1. Greenhouse test:

Table (4) shows the effects of five chitinolytic fungal isolates on *M. javanica* infected strawberries grown in a greenhouse. The results showed that all tested treatments significantly decreased the overall nematode population, with decline percentages ranging from 45.5% to 92.3%. Among the studied treatments, *A. niveus* demonstrated the best performance, reducing the overall population of root-knot nematode by 70.0%. *T. yunnanense* came in second rank with a reduction percentage of 65.5%. The amount of egg masses and root galling were both dramatically reduced by all treatments. The most notable reduction in root galling was seen in *A. niveus* (28.5 galls/root). Nevertheless, the nematode population as a whole, the number of galls, and the egg masses were all reduced the least by the fungal isolate *P. lilacinum*.

Table 4: Five chitinolytic fungal isolates' effects on the prevalence and multiplication of *Meloidogyne javanica* infections in strawberries grown in greenhouse

Treatments	Nematode population in			Red. %	No. galls	RGI*		EI*
	Soil	Root						
		Developmental stages	Females				No. egg masses	
***Fusarium incarnatum* DAMF1**	2367.5^{c}	11.0^{d}	61.0^{c}	49.4	68.3^{c}	4	41.5^{c}	4
***Trichoderma yunnanense* DAMF2**	1622.5^{d}	7.0^{de}	36.0^{d}	65.5	38.8^{d}	4	25.0^{d}	3
***Aspergillus niveus* DAMF3**	1410.0^{e}	10.8^{d}	25.0^{e}	70.0	28.5^{e}	3	20.0^{d}	3
***Aspergillus oryzae* DAMF4**	1670.0^{d}	18.0^{c}	59.0^{c}	63.8	60.0^{c}	4	41.3^{c}	4
***Purpureocillium lilacinum* DAMF5**	2515^{b}	30.0^{b}	83.3^{b}	45.5	92.3^{b}	4	52.2^{b}	4
Fenamiphos	355.0^{f}	3.3^{e}	14.3^{f}	92.3	15.0^{f}	3	4.5^{e}	2
Nematode alone	4630.0^{a}	53.5^{a}	140.5^{a}	--	160.8^{a}	5	111.0^{a}	5
LSD	58.9	4.06	4.09		8.5	--	7.8	

Each value presented the mean of four replicates

The Duncan's multiple range test revealed no statistically significant differences at $P \leq 0.05$ between the means in any column that had the same letter (s).

* The root gall index (RGI) or egg masses index (EI) was rated on a scale of 0 to 5, where 0 =no galls or egg masses, 1 = 1-2, 2= 3-10, 3=11-30, 4=31-100, and 5 = more than 100 galls or egg masses/root system.

3.7.2. Field experiment:

The findings in Tables (5 and 6) show how five chitinolytic fungal isolates affect root-knot reproduction and, as a result, the development of strawberry plants when used in the field. It was discovered that all treatments significantly improved plant growth parameters, though to varying degrees. Of the five isolates, *A. oryzae* and *P. lilacinum* showed the greatest improvements in shoot and root weight, fruit number, and fruit weight per plant.

Table (5): **Impact of five chitinolytic fungal isolates on growth parameters of strawberry infected with *Meloidogyne* spp. under field conditions**

Treatments	Shoot weight (g)	Root weight (g)	No. fruit / plant	Fruit weight / plant (g)
***Fusarium incarnatum* DAMF1**	41.63^{bc}	5.24^{b}	16.67^{c}	187.3^{d}
***Trichoderma yunnanense* DAMF2**	41.61^{bc}	4.48^{bc}	27.67^{b}	291.7^{c}
***Aspergillus niveus* DAMF3**	49.23^{b}	5.11^{b}	30.33^{b}	298.3^{c}
***Aspergillus oryzae* DAMF4**	51.40^{b}	5.28^{b}	42.66^{a}	519.0^{a}
***Purpureocillium lilacinum* DAMF5**	71.47^{a}	8.15^{a}	40.76^{a}	413.3^{b}
Fenamiphos	49.11^{b}	5.59^{b}	33.33^{b}	340^{c}
Nematode alone	34.93^{c}	3.63^{c}	18.31^{c}	128.3^{e}
LSD	10.67	1.17	6.26	54.34

Each value presented the mean of four replicates

The Duncan's multiple range tests revealed no statistically significant differences at $P \leq 0.05$ between the means in any column that had the same letter (s).

According to Table (6), the application of chitinolytic fungal isolates lowered root-knot nematode characteristics such as the number of galls, egg masses/root system of strawberry, and nematode population in soil and root. Fenamiphos reduced reproduction by 71.0%, demonstrating a stronger suppressive effect. When compared to nematode alone, all treatments significantly decreased nematode final populations; the average decrease values ranged from 25.3% for *P. lilacinum* treatment to 63.1% for *A. oryzae* treatment. The *A. oryzae* treatment yielded the fewest galls (32.3 galls/3g root), followed by the *A. niveus* treatment (54.0 galls/3g root). Egg masses/root decreased with treatments of fenamiphos, *A. oryzae*, and *A. niveus*, which had reduction percentages of 91.2, 78.0, and 67.7%, respectively.

Table (6): **Effect of five chitinolytic fungal isolates on population density of *Meloidogyne* spp. infected strawberry under field conditions:**

Treatments	Final population	Red. %	Galls	Red. %	Egg masses	Red.%
***Fusarium incarnatum* DAMF1**	1564.0^{b}	35.2	98.4^{b}	39.6	73.7^{bc}	41.0
***Trichoderma yunnanense* DAMF2**	1270.0^{c}	47.4	80.5^{bc}	50.6	64.3^{c}	48.6
***Aspergillus niveus* DAMF3**	1133.3^{cd}	53.0	54.0^{cd}	66.9	40.4^{d}	67.7
***Aspergillus oryzae* DAMF4**	890.0^{de}	63.1	32.3^{d}	80.2	27.5^{de}	78.0
***Purpureocillium lilacinum* DAMF5**	1803.3^{b}	25.3	108.0^{b}	33.7	91.3^{b}	27.0
Fenamiphos	700^{e}	71.0	33.3^{d}	79.6	11.0^{e}	91.2
Nematode alone	2413.3^{a}	--	163.0^{a}	--	125.0^{a}	--
LSD	273.6		28.53		23.35	

Each value presented the mean of four replicates

The means in each column that had the same letter (s) did not differ statistically significantly at P ≤0.05, according to the Duncan's multiple range tests.

The data in Table (7) compare strawberry plants that had not been treated to those treated with the aforementioned chemicals in order to determine the fruit quality feature. *A. niveus*, *T.*

yunnanense, and *A. oryzae* had the best results and increased the level of total sugar. Similarly, all chitinolytic fungal isolates had an impact on increasing total soluble solids (TSS), in strawberry fruits. It was discovered that *A. oryzae*, followed by *T. yunnanense*, were the most successful in increasing fruit firmness. The treatment plants had much higher vitamin C content than the untreated plants. *T. yunnanense*, followed by *A. oryzae*, produced the greatest rise in vitamin C levels.

Table (7): Influence of five chitinolytic fungal isolates on fruit quality characteristic in strawberry plants as affected by root -knot nematode under field conditions

Treatments	Total sugar (mg/g f wt)	TSS (Brix)	Fruit firmness (g/cm^2)	Vitamin C (mg/100g fruit)
***Fusarium incarnatum* DAMF1**	3.43^{b}	8.323^{bc}	2.730^{c}	43.5^{cd}
***Trichoderma yunnanense* DAMF2**	4.95^{a}	8.566^{abc}	3.516^{ab}	51.6^{a}
***Aspergillus niveus* DAMF3**	4.763^{a}	8.533^{abc}	3.066^{bc}	40.3^{d}
***Aspergillus oryzae* DAMF4**	4.963^{a}	8.863^{a}	3.843^{a}	48.4^{ab}
***Purpureocillium lilacinum* DAMF5**	3.950^{b}	8.660^{ab}	2.853^{bc}	45.6^{bc}
Fenamiphos	3.606^{b}	8.180^{c}	3.033^{bc}	41.1^{d}
Nematode alone	3.306^{b}	8.146^{c}	2.363^{c}	36.8^{e}
LSD	0.901	0.457	0.746	3.2

Each value presented the mean of four replicates

The Duncan's multiple range tests revealed no statistically significant differences at P 0.05 between the means in any column that had the same letter (s).

The results in Figures 7 and 8 demonstrate that the activity of peroxidase (PO) and polyphenol oxidase (PPO) in strawberry leaves are indications of the severity of the infection, compared to the activity of PO and PPO in untreated plants. At the same time, treatment with *A. oryzae* led to an increase in PPO and PO activity.

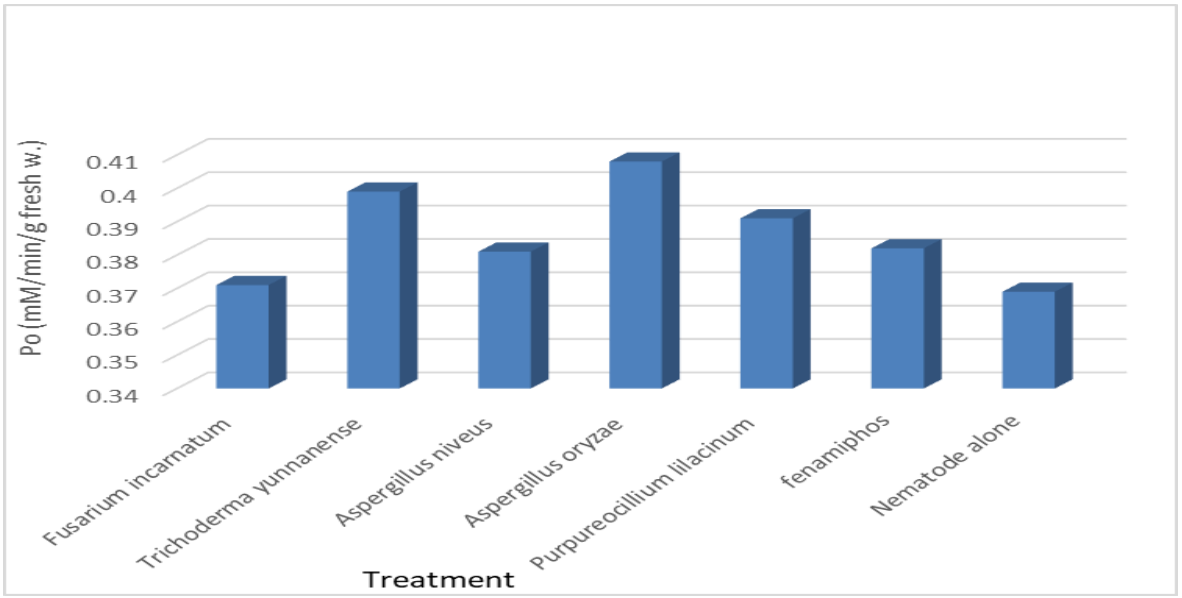


Figure (7): Peroxidase (PO) activity in strawberry leaves as influenced by the administration of five fungal chitinolytic isolates with the root- knot nematode, *Meloidogyne* spp.

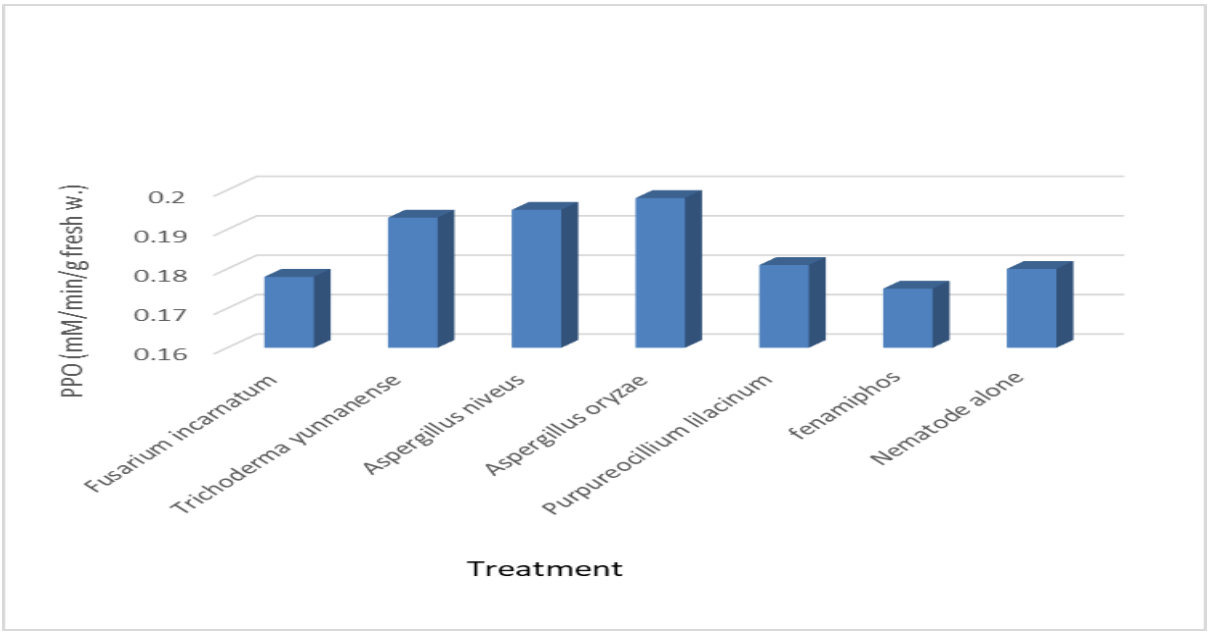
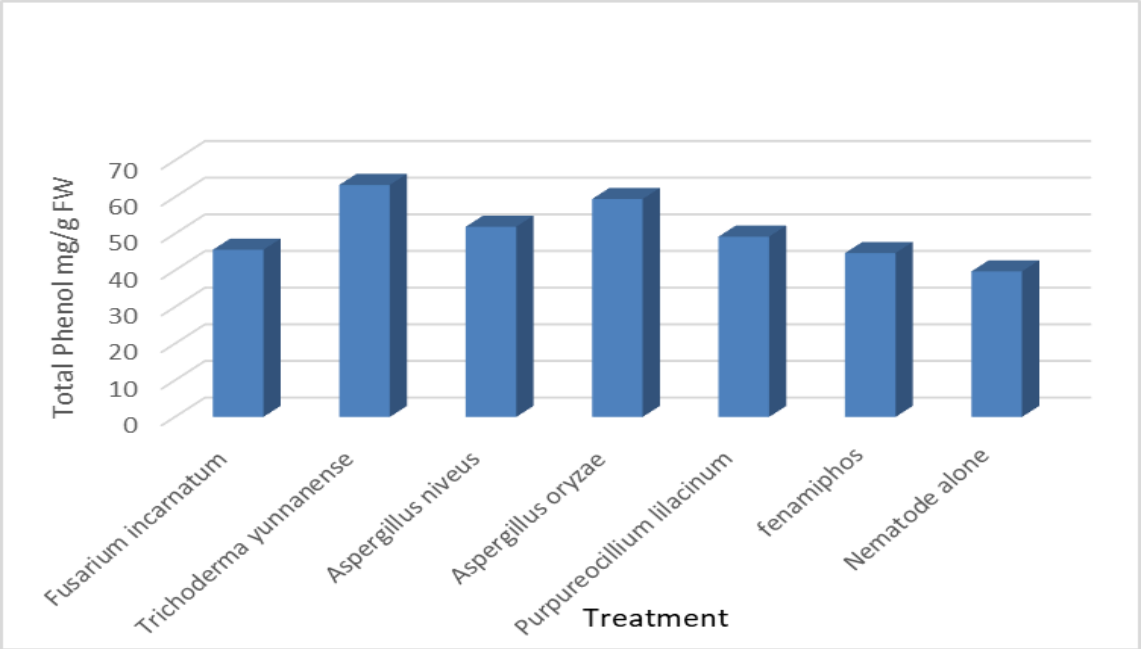


Figure (8): Polyphenol oxidase (PPO) activity in strawberry leaves as influenced by the administration of five fungal chitinolytic isolates with the root- knot nematode, *Meloidogyne* spp.

Figure (9) demonstrated the strawberry's total phenol content. Comparing treated plants to untreated plants, all examined treatments showed an increase in phenolic components. The maximum amount of phenolic component was produced by *T. yunnanense* treatment, which was



followed by treatments with *A. oryzae* and *A. niveus*.

Figure (9): Influence of five chitinolytic fungal isolates on total phenols in strawberry as affected by root- knot nematode under field conditions.

It was discovered that strawberry plants that received no treatments had higher proline contents than those received chitinolytic fungal isolates. Moreover, treatment with *P. lilacinum* and *A. niveus* demonstrated a notable increase in proline content among all treatments (Fig.10).

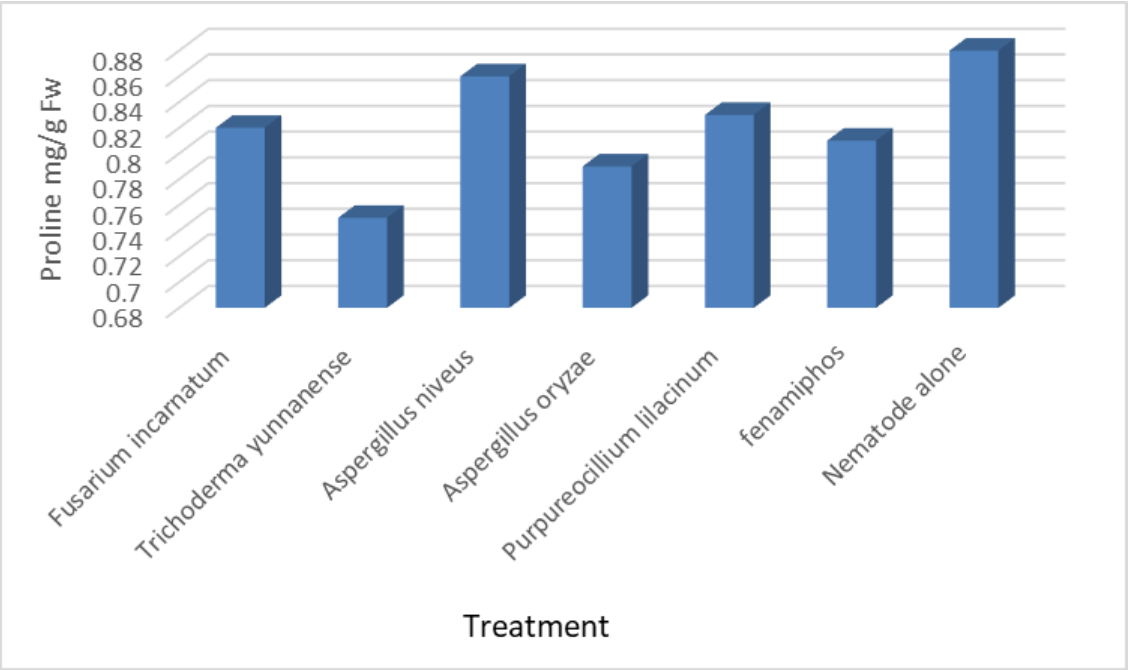


Figure (10): Effect of five chitinolytic fungal isolates on proline content in strawberry as affected by root- knot nematode under field conditions.

3. Discussion

Numerous researchers have conducted extensive research on the function of chitinase and other lytic enzymes in nematode biocontrol. All of the fungi isolated in the current investigation had chitinase activity, efficiently prevented egg hatching, and killed *M. javanica* juveniles. In order to use chitin as a source of carbon and nitrogen for their metabolic processes, fungi and bacteria primarily degrade it **[55, 56]**. The results of the GC-MS analysis demonstrated the collected metabolites belonged to several chemical functional groups. In general, volatile organic molecules with long chains (C16-C18) are thought to be more biologically important than shorter hydrocarbon side chains Dodecanoic acid (C12), according to **[57]**. Conferring to **[58]**, toxicity increases with the number of carbon atoms in the chain; for example, C17 homologues are 20 times more active than C14 forms in *T. yunnanense* and *A. oryzae*, respectively.

Numerous biotechnological uses, such as plant-associated metabolites and their equivalents, can be produced by chitinolytic fungi **[59, 60, 61, 62, 63]**. Additionally, they can create a wide range of volatile organic compound (VOC) combinations that are utilized as insecticides directly **[64].** Many studies demonstrated the significance of fungi's secondary metabolism. According to **[65]** and **[66]**, the second juvenile stage root-knot nematode, *M. incognita*, is killed by the VOCs of *F. oxysporum*. This fatality ranges from 88 to 96%. Also,

findings by **[67]**, reported that eggs from *M. incognita* that were exposed to VOCs of *F. oxysporum* for 72 hours show a 43% decrease in the proportion of eggs that hatched .

Numerous scientists came to the conclusion that some species of *Fusarium* have nematicidal activity after observing that *F. culmorum* culture filtrate has greater mortality rates on *Pratylenchus neglectus, P. penetrans,* and *P. thornei* **[68]**. **[69]**, documented that of the 294 screened isolates, galls produced by *M. incognita* in a greenhouse test were considerably decreased by treatments with *Fusarium*, *Acremonium*, *Trichoderma*, *Paecilomyces*, *Chaetomium*, and *Phyllosticta*.

Trichoderma is a genus of fungi that also creates PPN-inhibiting metabolites. According to **[70]**, *Trichoderma* sp.'s organic molecule, 6-pentyl-2H-pyran-2-1, is nematicidal and kills over 85% of *Panagrellus redivivus*, *Bursaphelenchus xylophilus*, and *Caenorhabditis elegans* **[71]**.

In order to establish a complex molecular dialogue with the plant-host, *Trichoderma* can colonise the roots (but only the outermost layers; it is unable to enter the vascular bundle). In addition to their easy adaptation to various climatic and edaphic conditions and their quick development rate, this relationship gives this genus an edge over many other fungi, making them excellent candidates for biological control agents **[72]**. It is now understood that *Trichoderma* functions as a biological control agent through a variety of mechanisms, such as competition with the pathogen, mycoparasitism, antibiosis, promotion of plant growth, enhanced plant resistance to abiotic stressors, and activation of the plant's defences against infections **[73, 74]**.

Our research revealed that *A. niveus* and *A. oryzae* considerably decreased the nematode population. This result is in line with the findings of **[75]**, who found that *A. niger* improves the biocontrol ability of the bacterial inoculants in tomatoes and amplifies the effect of *Pseudomonas fluorescens in vitro*. On the other hand, *P. lilacinum* improves the development parameters of strawberry plants. This result aligns with the findings of *P. lilacinum*, which enhanced tomato plant length, fresh and dry weight, and reduced the number of nematodes on the roots and in the soil **[76, 77, 78]**.

4. Conclusion

Biocontrol techniques are an efficient alternative to toxic chemical nematicides for plant-parasitic nematodes. As a result, numerous effective and varied techniques based on the use of chitinolytic fungi as biological control agents are offered. These fungi work through two distinct classes of action mechanisms, specifically those involving lytic enzymes and secondary metabolite formation (antibiosis). By parasitizing the nematodes, paralyzing the juveniles, creating lytic enzymes, engaging in antibiosis, and competing for space with the worms,

chitinolytic fungi reduce the attack of plant-parasitic nematodes. Additionally, GC-MS analysis was performed to ascertain whether specific bioactive compounds were present in various isolates of chitinolytic fungi. Fungal extracts are a safe and efficient green pest management substitute for synthetic nematodes, according to a toxicological study. The study's conclusions indicate that the bioactive compounds present in fungi may protect plants from disease-causing infections.

5. The conflict of interest

All authors declare that there is no conflict of interest in submitting this manuscript.

6. Acknowledgement

"Financial support has been provided to **SIRAM by PRIMA, a program supported by H2020**, the European Programme for Research and Innovation and funded by **The Academy of Scientific Research & Technology (ASRT)**".

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