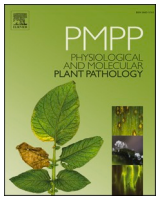
Physiological and Molecular Plant Pathology 139 (2025) 102791

Contents lists available at ScienceDirect

Physiological and Molecular Plant Pathology

journal homepage: www.elsevier.com/locate/pmpp



Enhancing tomato immunity against root-knot nematodes using PGPF and Melithorin® through biochemical and molecular approaches

Mohamed S. Attia [a,*], Amer M. Abdelaziz [a], Mostafa I. Abdelglil [b], Eslam K. Kandil [a], Muge Ergun [c], Salah M. Elsayed [d], Maryam M. Elsayed [e], Noha M. Ashry [f], Mohamed M. Nofel [a]

[a] Botany and Microbiology Department, Faculty of Science, Al-Azhar University, 11884, Nasr City, Cairo, Egypt
[b] Al-Farahidi University- Baghdad, Iraq
[c] International Industrial Property Rights Regulation Unit Administrator.AGROBEST Group, Turkey
[d] Horticulture Research Institute, Agricultural Research Center, Giza, Egypt
[e] Research and Development Department, ALSALAM International for Development & Agricultural Investment, Egypt
[f] Agriculture Microbiology Department, Faculty of Agriculture, Benha University, Qalubia, 13736, Egypt

ARTICLE INFO

Keywords:
GC-MS analysis
Meloidogyne incognita
PPO isoforms
Secondary metabolites

ABSTRACT

This article focused on assessing the biocontrol effectiveness of four plant growth-promoting fungal (PGPF) species—*Verticillium lecanii*, *Penicillium buchwaldii*, *Alternaria photistica*, and *Aspergillus niger*—alongside the commercial formulation Melithorin®(fosthiazate 90 %), against the root-knot nematode *Meloidogyne incognita* in tomato plants. Laboratory bioassays demonstrated that Melithorin® had the most potent nematocidal effect, causing 94 % juvenile mortality after 96 h. Among the fungal isolates, *A. niger* showed the highest activity (92.6 %). Under greenhouse conditions, Melithorin® significantly reduced root gall formation and juvenile nematode populations by 96.6 % and 84.9 %, respectively. The fungal treatments also exhibited suppressive effects, with *V. lecanii* and *A. niger* performing better than *P. buchwaldii* and *A. photistica*. Chemical analysis using gaschromatography–mass spectrometry (GC-MS) revealed the presence of several bioactive metabolites in ethyl acetate extracts of the fungal isolates. Noteworthy compounds included 1H-benzotriazole, 5-nitro in *V. lecanii*; desulphosinigrin in *P. buchwaldii*; and a shared phenolic compound—2,2′-methylenebis[6-(1,1-dimethylethyl)-4-methylphenol]—in both *A. photistica* and *A. niger*. These metabolites are likely contributors to the observed nematocidal activity. Biochemical assessments of the treated tomato plants indicated that nematode infestation triggered oxidative stress, as reflected by elevated malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) levels. Applications of PGPF and Melithorin® alleviated this stress by significantly lowering MDA and H_2O_2 content, while enhancing total phenolics and the activities of peroxidase (POD) and polyphenol oxidase (PPO) enzymes.

Furthermore, isozyme profiling revealed increased expression of PPO isoforms, particularly PPO2, PPO3, and PPO4, with the highest intensities observed in plants treated with Melithorin®.In summary, Melithorin® proved to be the most effective agent in reducing nematode damage and activating plant defense responses. PGPF showed promising potential, both in suppressing nematodes and enhancing the plant's biochemical resistance mechanisms.

1. Introduction

Tomato production faces significant challenges, with root-knot nematodes (*Meloidogyne* spp.) being one of the most challengingpests [1].Root-knot nematodes (RKNs) infest plant roots, causing the formation of root galls, which disrupt nutrients and water uptake. Yield losses from root-knot nematode infestations can range from 30 % to 80 %, depending on the severity of the infestation. [2]. Research indicates that severely infested plants may suffer a 20–40 % decrease in fruit size, resulting in significant economic losses. [3]. Traditional methods of controlling RKN rely on chemical nematicides, which have a number of drawbacks, such as beneficial insects and wildlife. Organophosphate nematicides like Melithorin® (fosthiazate 90 %) are another option for agricultural pest control of plant-parasitic nematodes. [4]. A variety of nematode species, particularly root-knot nematodes, can be effectively controlled with fosthiazate. [5].Furthermore, the rising resistance of

* Corresponding author.
E-mail address: drmohamedsalah92@azhar.edu.eg (M.S. Attia).

https://doi.org/10.1016/j.pmpp.2025.102791
Received 5 May 2025; Received in revised form 9 June 2025; Accepted 12 June 2025
Available online 14 June 2025

nematodes to standard nematicides has rendered traditional management measures less reliable [6]. Consequently, there has been a growing interest in alternative strategies that minimize dependence on chemical inputs while promoting long-term sustainability. Among these, biological control methods have attracted considerable attention for their environmental safety, specificity in targeting pests, durable effectiveness, and minimal risk to human health. [7,8].As a result, biological control strategies have become increasingly favored for their ecological advantages, precision in pest management, sustained efficacy, and reduced health hazards compared to conventional chemical pesticides. [9]. One promising group of biological agents is plant growth-promoting fungi (PGPF [10].These beneficial soil fungi colonize the rhizosphere and establish symbiotic relationships with plant roots. Fungican promote plant growth, activate resistance of host plants, enhancing their defense mechanisms against a range of foliar and root pathogens [11]. Some fungi, including species of *Aspergillus*, have been found to play an important role in controlling nematode in agricultural environments. This is largely attributed to their ability to produce secondary metabolites that are lethal to pathogenic nematodes. Likewise, *Verticillium lecanii*, Zare& Gams, 2001(Sordariomycetes:Hypocreales:Cordycipitaceae), although primarily known as an entomopathogenic fungus, has demonstrated potential as a biological control agent against nematodes through parasitism [12]. Both *Aspergillus* and *Verticillium* species exhibit multiple mechanisms of nematode suppression, including direct parasitism, competition, and the production of toxic compounds. *Aspergillus* is capable of secreting enzymes that break down the nematode cuticle [13,14]. The innovation in our research lies in the application of PGPF specifically for the management of root-knot nematodes (RKNs). While PGPF have been studied extensively for their role in controlling plant diseases, their potential in targeting RKNs has not been widely explored. This study aims to enhance sustainable agricultural productivity by (PGPF) to boost plant development and reduce the harmful effects of root-knot nematodes (RKNs. Furthermore, the study investigates the additional benefits of fosthiazate in enhancing plant performance and providing further protection against nematode-induced stress.

2. Materials and methods

The present study was conducted in 2024 at the Plant Pathology Research Lab and Botanical Garden, Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt. Three-weeks-old tomato seedlings (*Solanum lycopersicum* L. var. 023) were planted as model plant.

The root-knot nematode inoculum, *Meloidogyne incognita,* Kofoid& White, 1919(Secernentea:Tylenchida:Heteroderidae), was obtained from the Plant Pathology Institute at the ARC. Infected tomato plant roots were used to isolate the nematode eggs, which were then treated with 0.5 % NaOCl following the method of Natio et al., [15].

2.1. Sources of treatments

The nematicide Melithorin® (containing 90 % fosthiazate), produced by AGROBEST Grup-Turkey, The fungal isolates were isolated and identified through both morphological and molecular characterization in the our previous study Kandil et al., [16].

2.2. In vitro study

To determine the impact on the mortality of nematode juveniles, 1 mL the freshly hatched juvenile suspension (50 juveniles/mL) was conducted to 1 mL of each PGPF culture filtrate in sterile Petri dishes, then incubated at four times (24, 48, 72, and 96 h). Every treatment was replicated three times. The non-moving juveniles were observed under a low-power stereomicroscope, and the mortality rate was subsequently determined.

2.3. In vivo study of RNKs

Three-weeks-old *Solanum lycopersicum* L. var. 023 were transplanted into plastic pots (40 × 40 cm) containing 6 kg of a sterilized sand-clay mixture in a 1:3 (w/w) ratio. The pots were placed inside a plastic greenhouse, where day and night temperatures were maintained at 22 °C and 18 °C, respectively. The pot experiment was designed to evaluate the effectiveness of four PGPFin addition to the nematicide Melithorin®, in managing *Meloidogyne incognita* infections in tomato plants. A total of 111 tomato seedlings were transplanted, one/pot. The pots were divided into 11 treatment groups, each consisting of 10 seedlings. Treatments were applied seven days after transplanting. *Meloidogyneincognita*second-stage juveniles (J2) were inoculated by injecting 50 mL of a suspension containing 40 J2/mL (equivalent to 2000 J2 per plant) into the soil through three incisions around each plant's root zone using a pipette. The greenhouse temperature was maintained at 25 ± 2 °C. After 60 days, plants were carefully uprooted and cleaned to remove soil residues. Nematode infestation was assessed by measuring the number of galls per gram of root and the number of J2 per 250 g of soil. The second-stage juveniles were isolated using the sieving method, as outlined by Hopper et al. [17].

2.4. Bioactive compounds extraction

PGPF isolates were grown in Potato Dextrose Broth (PDB) (Sigma-Aldrich, Germany) at 27 ± 2 °C for 21 days. Following incubation, the fungal cultures were filtered, and the resulting filtrates were extracted with ethyl acetate (EtOAc) at a 1:1 ratio. The organic (EtOAc) layer was separated from the aqueous phase using a separating funnel and subsequently evaporated at 40 °C using a rotary evaporator (Heidolph VV2001, Germany), as described by Abdelaziz et., [18].

2.5. (GC–MS) investigation

The metabolites present in the fungal extracts were analyzed using gas GC–MS, following the protocol of Shehabeldine et al., [19].The chemical constituents were identified by comparing the obtained spectra with those in the WILEY 09 (Wiley, New York, NY, USA) and NIST 11 mass spectral libraries. Identified compounds were reported along with their chemical names, molecular formulas, and molecular weights.

2.6. Physiological and molecular resistance indicators

Phenolic compounds were extracted following Folin-Denis method [20] by soaking 1 g of dried, defatted leaves in 5–10 ml of 80 % ethanol for 24 h at 0 °C, followed by three re-extractions, with the final extract adjusted to 50 ml. Then 0.5 ml of the extract was combined with 0.5 ml of Folin-Denis reagent and agitated for 3 min. Subsequently, 1 ml of saturated sodium carbonate solution and 3 ml of distilled water were added to the mixture. Following an incubation period of 1 h, the absorbance was recorded at a wavelength of 725 nm. The method used by Hu, Richter [21] was applied to determine the amount of MDA in fresh tomato leaves. Fresh tomato leaves were tested for hydrogen peroxide H_2O_2 content [22].

2.7. Assay of antioxidant enzyme activity

For the assay of antioxidant enzymes activity, 2 g of tomato tissue was homogenized in 10 mL of phosphate buffer (pH 6.8, 0.1 M). The mixture was then centrifuged at 2 °C for 20 min at 20,000 rpm. To measure peroxidase activity (POD), 0.2 mL of the enzyme extract was mixed with 5.8 mL of phosphate buffer (pH 7), 2 mL of 20 mM pyrogallol, and 2 mL of 20 mM hydrogen peroxide (H2O2). The rate of increase in absorbance as pyrogallol was measured spectrophotometrically by UV spectrophotometer (Jenway) within 60 s

at 470 nm and 25 °C [23]. The method employed by Matta and Dimond [24] was utilized to calculate the activity of PPO.

2.8. Electrophoresis isozymes

Native polyacrylamide gel electrophoresis (Native-PAGE) was conducted to detect differences in PPO isozymes between control and treated samples. The analysis was performed using leaf tissue (100 mg fresh weight), following the methodology described by Barcelo et al. [25].

2.9. Statistical analysis

The experimental data were analyzed using one-way ANOVA to assess significant differences between treatment means. Allexperimentswereconducted using three replicates for each treatment. When significant differences were observed, mean comparisons were conducted using the LSD test at a 5 % significance.

3. Results

3.1. Evaluation of nematode juvenile mortality

The data illustrated in Fig. 1 indicate that all tested elicitors contributed to increased juvenile nematode mortality, particularly with extended incubation periods. Among the treatments, Melithorin® demonstrated the most potent nematicidal effect, achieving a 94 % mortality rate after 96 h. This was closely followed by *Aspergillus niger,* van Tieghem 1867(Eurotiomycetes: Eurotiales: Aspergillaceae), which caused 92.6 % mortality. *Penicillium buchwaldii*, Frisvad& Samson, 2013 (Eurotiomycetes: Eurotiales: Aspergillaceae) and *V lecanii* also showed significant nematode inhibition, with mortality rates of 87.7 % and 73.3 %, respectively. The least effective among the tested agents was *Alternariaphotistica*,E.G. Simmons, 1986 (Dothideomycetes: Pleosporales: Pleosporaceae), which still produced a notable 47.4 % mortality rate after the same exposure period, highlighting varying levels of efficacy.

3.2. In vivo nematode parameters

Represented data in Table (1) demonstrated that all tested elicitors significantly reduced numbers of infected tomato root galls as well asnumbers of second stage juveniles in soil. Melithorin® showed the most effective control against root-knot nematodes, achieving a 96.6 % reduction in gall formation and an 84.9 % reduction in second-stage juveniles. It was followed in effectiveness by *Verticillium lecanii* (83.8 % and 70.6 %), *Aspergillus niger* (75.1 % and 61.1 %), *Penicillium buchwaldii* (69.9 % and 55.1 %), and *Alternariaphotistica* (40.2 % and 36.7 %) for gall and juvenile reduction, respectively.

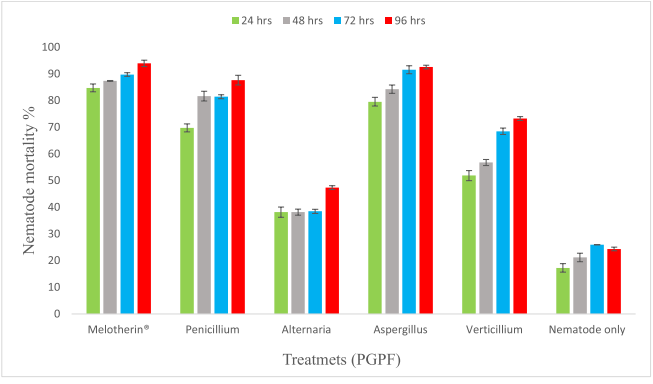


Fig. 1. Impact of plant growth promoting fungi (PGPF) on nematode mortality in vitro.

Table (1)
Impact of tested treatments on galls and second stage juveniles of *Meloidogyne incognita.*

Treatment	Factors				
	Galls/1g of root			Second stage juveniles/ 250 g soil	
	Average No.	Reduction %	RGI	Average No.	Reduction %
Melithorin®	4.1 ± 0.5 e	96.6	2	30.6 ± 1.2 e	84.9
P. buchwaldii	36.6 ± 1.6 c	69.9	4	91.3 ± 2.4 c	55.1
A. photistica	72.6 ± 1.8 b	40.2	4	128.6 ± 4.1 b	36.7
A. niger	30.3 ± 1.4 c	75.1	3	79.3 ± 2.3 c	61.1
V. lecanii	19.6 ± 0.8 d	83.8	3	59.6 ± 1.4 d	70.6
Nematode only	121.6 ± 4.4a	–	5	203.4 ± 7.7 a	–
LSD (5 %)	6.7	–	–	12.1	–

*The Root Gall Index (RGI) was assessed based on the scale; where: 0 = no galls, 1 = 1–2 galls, 2 = 3–10 galls, 3 = 11–30 galls, 4 = 31–100 galls, and 5 = more than 100 galls.

3.3. Gas Chromatography-Mass Spectrometry (GC-MS)analysis

3.3.1. GC-MS analysis of Aspirgilliusniger

Table 2 and Fig. 2 present the results of a GC-MS analysis of *Aspirgilliusniger*, listing 19 compounds. The fungal metabolite contains a diverse mixture of compounds including alcohols, esters, fatty acids, hydrocarbons. The majour components were Docosenamide (13.31), Hexadecanoic acid, 2,3-dihydroxypropyl ester (5.39 %), Octadecanoic acid, 2,3-dihydroxypropyl ester (4.41 %), Ethanol, 2-(2-butoxyethoxy) (4.20 %), and Phenol, 2,2′-methylenebis [6-(1,1-dimeth ylethyl)-4-methyl (2.38 %).

3.3.2. GC-MS analysis of Verticillium lecnaii

Table 3 and Fig. 2 summarize the results of a GC-MS analysis of *Verticillium lecnaii* indicating the presence of 15 compounds belonging to carbohydrate metabolism products. A series of fatty acids, including oleic, eicosenoic, and hexadecanoic acids, appear between 40 and 60 min, reflecting lipid-related components likely produced or accumulated by the fungus. The most prominent compounds based on peak area

Table (2)
Gas Chromatography-Mass Spectrometry (GC-MS) analysis of *Aspirgilliusniger.*

No	Compound name	RT (Min)	peak area %
1	2,4-Pentanediol	12.08	1.01
2	Butanoyl chloride	12.14	0.29
3	Methyl 5,7-hexadecadiynoate	18.07	1.39
4	Ethanol, 2-(2-butoxyethoxy)	18.80	4.20
5	Fluoroacetic acid, dodecyl ester	19.82	0.11
6	Nonadecane	24.02	0.25
7	1-Deoxy-d-mannitol	23.63	0.18
8	4-Octadecenal	28.50	0.18
9	Heptadecane	33.02	0.52
10	Hexadecanoic acid	48.77	1.23
11	Oleic Acid	50.15	0.21
12	Octadecanoic acid, 2,3-dihydroxypropyl ester	55.05	4.41
13	Phenol, 2,2′-methylenebis [6-(1,1-dimeth ylethyl)-4-methyl	62.28	2.38
14	Adrenosterone	62.67	1.07
15	Hexadecanoic acid, 2,3-dihydroxypropyl ester	65.52	5.39
16	Glycerol 1-palmitate	65.60	1.41
17	Thiocarbamic acid, N, N-dimethyl, S-1,3-diphenyl-2-butenyl ester	65.87	1.24
18	Prostaglandin A1-biotin	68.18	1.46
19	13-Docosenamide, (Z)	72.47	13.31

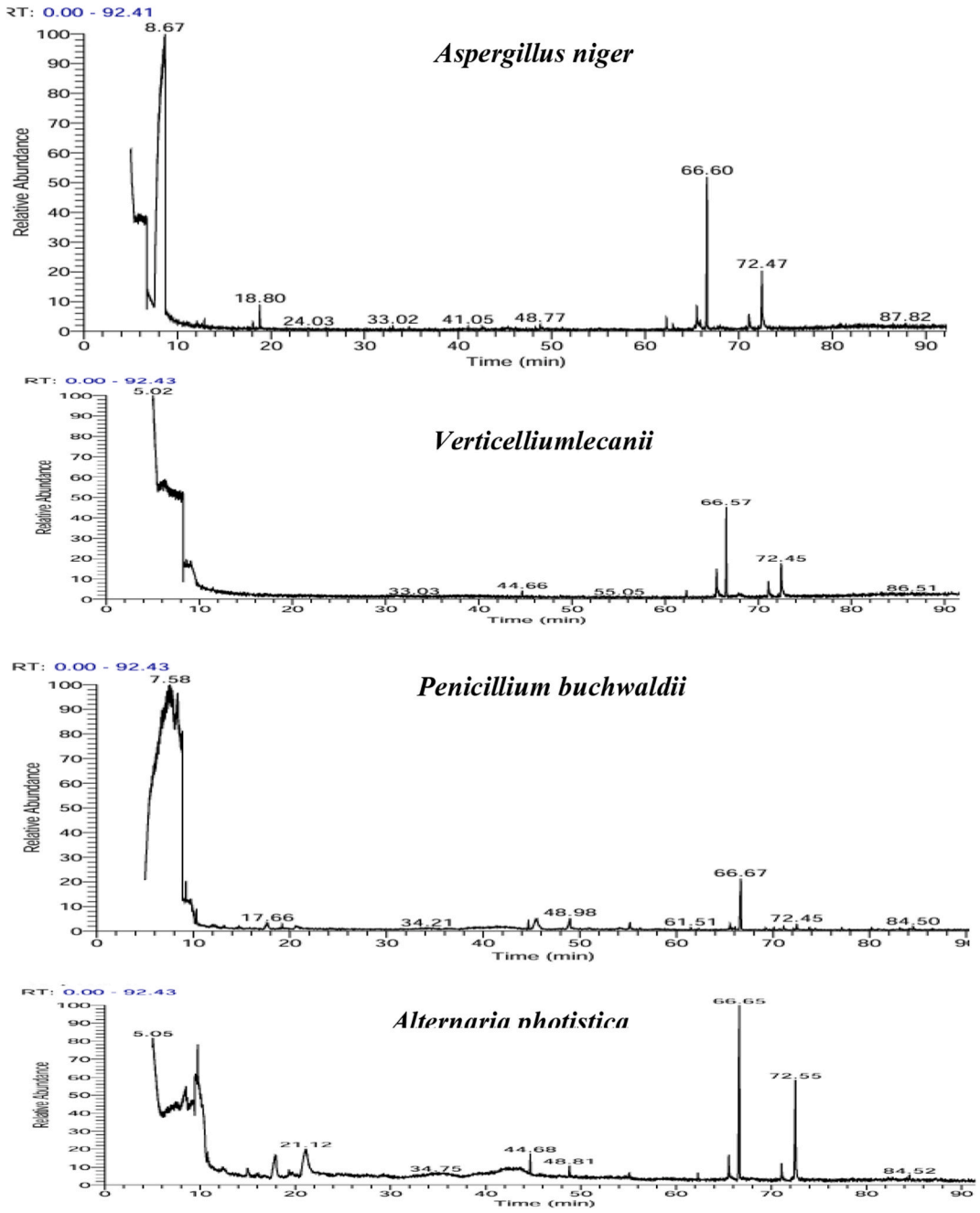


Fig. 2. Gas Chromatography-mass spectrometry (GC-MS)analysis of PGPF.

percentage include Docosenamide (12.79 %), and Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (11.40 %). These two make up over half of the total detected components, suggesting they are either major metabolic products of *V. lecnaii*. Prostaglandin A1-biotin (4.34 %) and Octadecanoic acid, 2,3-dihydroxypropyl ester (6.81 %) also contribute significantly, both being complex lipids or derivatives.

3.3.3. GC-MS analysis of Penicillium buchwaldii

Table 4 and Fig. 2 present the GC-MS analysis results for *Penicillium buchwaldii*, highlighting 17 distinct chemical compounds. The data suggests a diverse chemical profile consisting of phenols, organic acids, sugars, esters, and fatty acid derivatives. The majour components were Citronellyl oleate (8.88 %), Hexadecanoic acid (5.31 %), Kojic Acid (4.97 %), Octadecanoic acid (3.45 %), 5-Hydroxymethylfurfural (3.09 %), 1,2-Benzenedicarboxylic acid (2.63 %), and Docosenamide(2.01 %).

3.3.4. GC-MS analysis of Alternaria photistica

The GC-MS analysis of *Alternaria photistica* reveals a diverse mixture of primary and secondary metabolites, including alcohols, sugars, acids, esters, and fatty acid derivatives (Table 5 and Fig. 2). The most abundant compound in the entire fungus metabolites is 13-Docosenamide (Z), present at 14.95 %. Additionally, esterified fatty acids such as Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (3.08 %) and Octadecanoic acid, 2,3-dihydroxypropyl ester (2.26 %) highlight the fungus biosynthetic capacity for complex lipid derivative.

3.4. Total phenolic compounds

Fig. 3 demonstrates that nematode infection led to a significant increase of 76 % in total phenol levels in tomato plants. In healthy tomato plants, the highest increase was observed with *A. niger* (162.1 %)

Table (3)
GC-MS analysis of *Verticilliumlecnaii*

No	Compound name	RT (Min)	peak area %
1	Melezitose	27.90	0.15
2	9-Octadecenoic acid (Z)	40.10	1.02
3	cis-11-Eicosenoic acid	43.61	0.11
4	Phthalic acid, 5-methylhex-2-yl isobutyl ester	44.65	2.04
5	Hexadecanoic acid	48.76	0.70
6	Heptadecanoic acid, 9-methyl-, methyl ester	53.82	0.17
7	L-Ascorbic acid, 6-octadecanoate	55.05	0.42
8	Oleic Acid	56.80	0.15
9	Phenol, 2,2′-methylenebis[6-(1,1-dimeth ylethyl)-4-methyl	62.28	2.59
10	Hexadecanoic acid, 3[(trimethylsilyl)oxy] propyl ester	65.25	0.95
11	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) eth yl ester	65.51	11.40
12	Prostaglandin A1-biotin	67.85	4.34
13	Oleic acid, eicosyl ester	70.56	0.43
14	Octadecanoic acid, 2,3-dihydroxypropyl ester	71.08	6.81
15	13-Docosenamide, (Z)	72.45	12.79

Table (4)
GC-MS analysis of *Penicillium buchwaldii*.

No	Compound name	RT (Min)	peak area %
1	Phenol, 3-fluoro	11.96	1.13
2	Pentanoyl chloride	13.23	1.22
3	Melezitose	13.53	0.14
4	cis-2-Ethyl-2-hexen-1-ol	14.71	1.09
5	Kojic Acid	17.68	4.97
6	Ethanol, 2-(2-butoxyethoxy)	19.20	1.28
7	5-Hydroxymethylfurfural	20.67	3.09
8	Bromotetradecanoic acid	36.41	0.30
9	Oleic Acid	38.45	0.22
10	Octaethylene glycol monododecyl ether	40.56	0.42
11	1,2-Benzenedicarboxylic acid	44.68	2.63
12	Citronellyl oleate	45.50	8.88
13	Hexadecanoic acid	48.98	5.31
14	Octadecanoic acid	55.21	3.45
15	Glycerol 1-palmitate	65.55	1.88
16	Octadecanoic acid, 2,3-dihydroxypropyl ester	71.10	1.26
17	13-Docosenamide, (Z)	72.46	2.01

Table (5)
GC-MS analysis of *Alternaria photistica*.

No	Compound name	RT (Min)	peak area %
1	Cis-2-Ethyl-2-hexen-1-ol	14.98	1.51
2	Melezitose	16.05	0.58
3	Kojic Acid	17.93	8.64
4	Hexofuranoside	20.25	0.26
5	5-Hydroxymethylfurfural	21.07	12.04
6	Octadecenoic acid (z)	41.68	0.11
7	Oleic Acid	42.13	0.25
8	Phthalic acid, butyl tetradecyl ester	44.68	2.16
9	Hexadecanoic acid	48.80	1.45
10	Stearic acid	55.06	0.74
11	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) eth yl ester	65.54	3.08
12	Prostaglandin A1-biotin	67.85	1.53
13	Octadecanoic acid, 2,3-dihydroxypropyl ester	71.09	2.26
14	13-Docosenamide, (Z)	72.54	14.95

followed by*A. photistica* (76.5 %), *V. lecanii* (68.1.6 %),and *P. buchwaldii* (59.5 %) respectively compared to the healthy control. Similarly, in nematode-infected plants,*P. buchwaldii* induced the greatest rise in phenol content by (44.6 %)followed by*A.niger* (19.8 %), Melithorin® (14.4 %),and *V. lecanii* (3.8 %)respectively. These findings indicate that *A. niger*was the most effective treatment in healthy plants, while

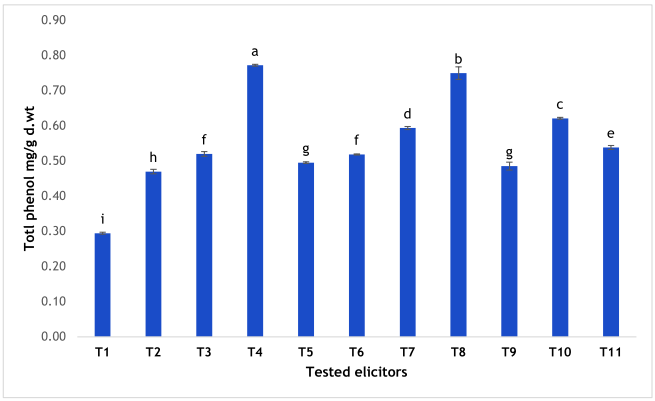


Fig. 3. Effect of tested elicitors on phenol contents in tomato plants infected with *Meloidogyne incognita.* T1:Healthy control., T2: Healthy + *P. buchwaldii,* T3: Healthy + *A. photistica*, T4: Healthy + *A. niger,* T5: Healthy + *V. lecanii,* T6: Infected control, T7: Infected + Melithorin®, T8: Infected + *P. buchwaldii,* T9: Infected + *A. photistica,* T10: Infected + *A. niger,* and T11: Infected + *V. lecanii.*

*P. buchwaldii*showed the strongest effect in enhancing phenol levels in infected plants.

3.5. MDA and H_2O_2contents

Data in Fig. 4showed that root-knot nematode infection caused a significant increase in malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) levels in both shoots and roots of tomato plants, indicating elevated oxidative stress. Specifically, MDA levels increased by 46 % in infected plants compared to the health control. The most notable reduction in MDA levels was observed with*A. photistica* (25 %),*V.lecanii* (22.9 %), followed by*P. buchwaldii* (7.9 %) and *A. niger* (7.5 %), compared to the healthy control. In nematode-infected plants,

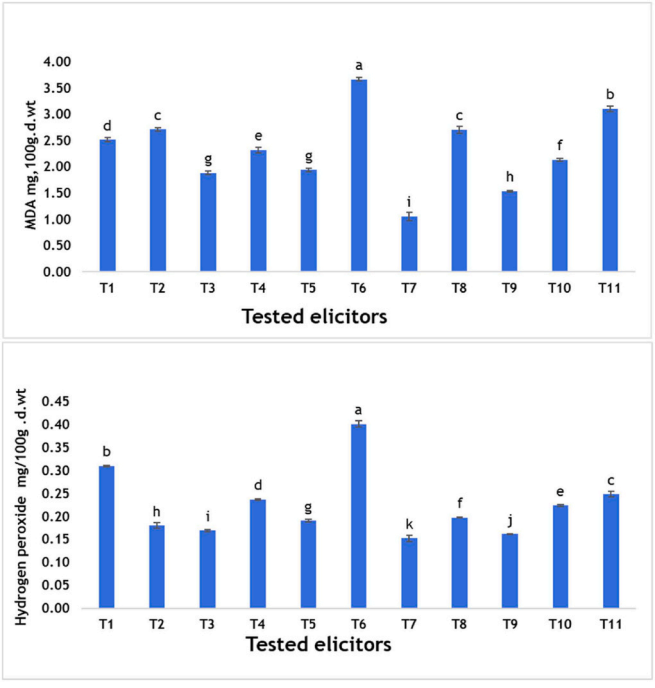


Fig. 4. Effect of PGPF and Melithorin® on malonaldehyde and hydrogen peroxide (H_2O_2) contents in shoots and roots of tomato plants in response to nematode infection.T1: Healthy control., T2: Healthy + *P. buchwaldii,* T3: Healthy + *A. photistica*, T4: Healthy + *A. niger,* T5: Healthy + *V. lecanii,* T6: Infected control, T7: Infected + Melithorin®, T8: Infected + *P. buchwaldii,* T9: Infected + *A. photistica,* T10: Infected + *A. niger,* and T11: Infected + *V. lecanii.*

Melithorin® showed the highest reduction in MDA (71 %),*A.photistica* (58.2 %),*A.niger* (41 %)followed by *P. buchwaldii* (26.2 %) and *V. lecanii* (15.2 %). These results suggest that *P. buchwaldii* was the most effective treatment in minimizing oxidative damage in healthy plants, while Melithorin® and *A. photistica* were most effective in mitigating stress in infected plants.Also, Fig. 4 shows that root-knot nematode infection caused a significant increase of 29 % in hydrogen peroxide (H_2O_2) levels in tomato plants compared to the healthy control, reflecting enhanced oxidative stress. The greatest reduction in H_2O_2 was observed with *A. photistica* (45 %), compared to the healthy control. In nematode-infected plants, Melithorin® led to the highest reduction in H_2O_2 levels (62 %), followed by *A. photistica* (59.7 %),*P. buchwaldii* (51 %), *A. niger* (44 %), and *V. lecanii*(38 %). These results highlight *A. photistica* as the most effective treatment in reducing oxidative stress in healthy plants, while Melithorin® and *A. photistica* showed the strongest effects in infected plants.

3.6. Oxidative enzyme activities

The results in Fig. 5 show that root-knot nematode infection triggered a significant increase in the activity of oxidative stress-related enzymes, including peroxidase (POD) and polyphenol oxidase (PPO), reflecting the plant's response to infection. Among infected treatments, Melithorin® recorded the highest activities of both enzymes, with POD reaching 1.284 units/g f.wt and PPO at 1.10 units/g f.wt. The highest peroxidase activity was observed with *V. lecanii* (1.247 units/g f.wt), followed by *A. photistica* (1.105), *A. niger* (0.536), and *P. buchwaldii* (0.5915). For PPO activity, *A. niger* led to the highest increase (0.75), followed closely by *V. lecanii* (0.74), *A. photistica* (0.61), and *P. buchwaldii* (0.38).In infected plants, Melithorin® again showed the strongest induction of antioxidant enzymes, followed by *A. niger* (POD: 1.194, PPO: 1.04), *V. lecanii* (POD: 0.78, PPO: 1.03), and *A. photistica* (POD: 0.817, PPO: 0.85). These findings suggest that *V. lecanii* and *A. niger* were the most effective in boosting antioxidant activities in healthy plants, while Melithorin® and *A. niger* provided the strongest defense in infected plants.

3.7. Polyphenol oxidase (PPO)isozymes

The isozyme analysis further reinforces the biochemical findings, highlighting the differential expression of PPO isozymes (PPO1 to PPO6) across all treatments (Fig. 6). Isozymes PPO2, PPO3, and PPO4 exhibited strong and consistent expression ("++" band intensity) in nearly all treatments, with the most pronounced bands observed in infected plants treated with Melithorin®, *A. photistica, A. niger*, and *V. lecanii*. Meanwhile, PPO1 and PPO5 showed moderate expression levels but were notably more intense in infected plants treated with PGPF and Melithorin®, compared to the untreated controls. Interestingly, PPO6 displayed a unique pattern of reduced intensity ("–") in the healthy control and in infected plants treated with *A. photistica* and *A. niger*, suggesting possible selective enzyme suppression or post-translational regulation. These isozyme expression patterns confirm that Melithorin®, *A. niger,* and *A. photistica* stimulate a diverse and robust polyphenol oxidase response, likely contributing to enhanced resistance against nematode-induced oxidative stress. Notably, Melithorin® stood out with the most intensified expression of key PPO isozymes (PPO2, PPO3, PPO4), indicating a heightened enzymatic defense response. Compared to the infected control, Melithorin® clearly emerges as the most effective treatment, surpassing both untreated and biologically treated plants in its ability to activate the antioxidant defense system.

4. Discussion

Scientists have been exploring the use of plant growth-promoting microorganisms as bio-nematicides [26].This study highlights the promising role of PGPF as an effective biological agents in enhancing agricultural productivity and strengthening the plant's natural defense mechanisms against pathogenic nematode attacks. PGPF can mediate plant growth by different direct and indirect mechanisms [27,28].The present study's GC-MS analysis revealed various bioactive compounds in

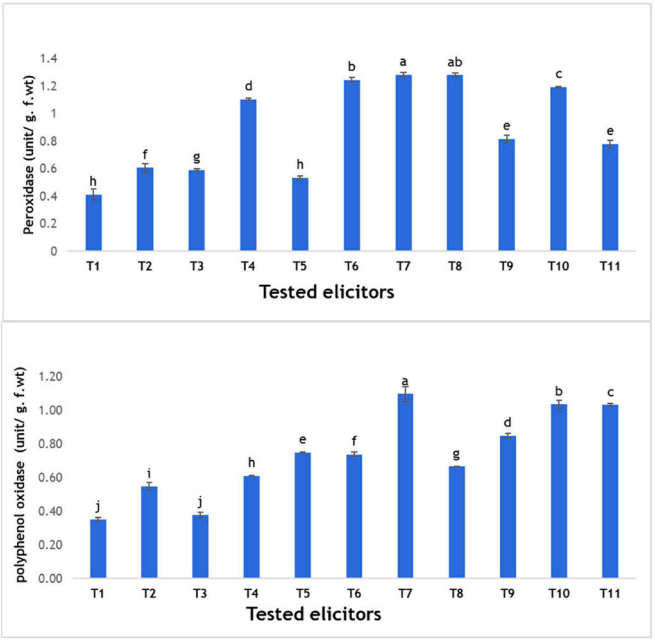


Fig. 5. Effect of tested PGPF and melithorin® on peroxidase (POD) and polyphenol oxidase (PPO) activities.T1: Healthy control., T2: Healthy + *P. buchwaldii,* T3: Healthy + *A. photistica*, T4: Healthy + *A. niger,* T5: Healthy + *V. lecanii,* T6: Infected control, T7: Infected + Melithorin®, T8: Infected + *P. buchwaldii,* T9: Infected + *A. photistica,* T10: Infected + *A. niger,* and T11: Infected + *V. lecanii.*

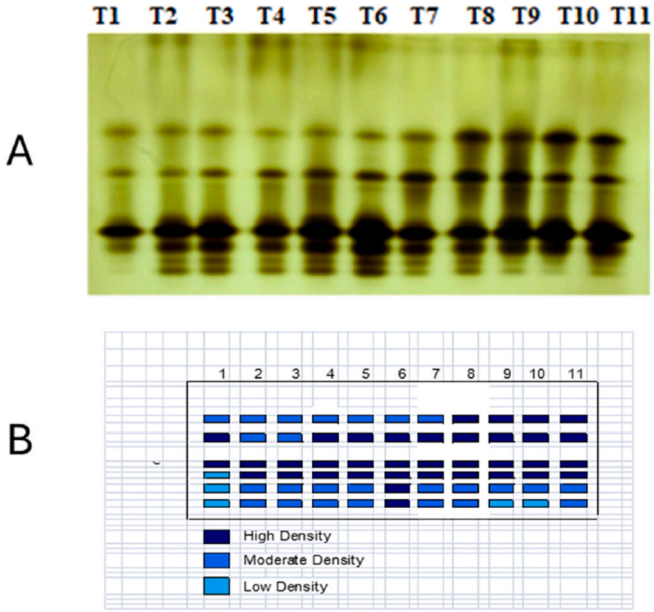


Fig. 6. Effect of tested PGPF and melithorin® on (A) polyphenol oxidase isozyme and (B) Isogram analysis of polyphenol oxidase isozyme of tomato plants.TT1: Healthy control., T2: Healthy + *P. buchwaldii,* T3: Healthy + *A. photistica*, T4: Healthy + *A. niger,* T5: Healthy + *V. lecanii,* T6:Infected control, T7: Infected + Melithorin®, T8: Infected + *P. buchwaldii,* T9: Infected + *A. photistica,* T10: Infected + *A. niger,* and T11: Infected + *V. lecanii.*

ethyl acetate extracts from PGPF *Verticillium lecanii*, *Penicillium buchwaldii*, *Alternaria photistica*, and *Aspergillus niger*. The GC-MS analysis of ethyl acetate extract from *Aspergillus niger* revealed a diverse profile of bioactive compounds, several of which show potential antinematode relevance through their pesticidal and insecticidal properties. Notably, docosenamide (13.31 %) demonstrated strong insecticidal activity [29,30]. Other compounds like ethanol, 2-(2-butoxyethoxy) (4.20 %), octadecanoic acid, 2,3-dihydroxypropyl ester (4.41 %), and adrenosterone (1.07 %) also exhibit pesticidal properties [31,32], which further supports the nematicidal potential of the extract. The presence of antimicrobial agents such as hexadecanoic acid (1.23 %) and glycerol 1-palmitate (1.41 %) [33,34] adds value by possibly contributing to gut microbiome disruption in nematodes. The GC-MS analysis of *Verticillium lechnii*reveals a metabolite profile rich in compounds with documented anti-insect activity, supporting its potential as a biocontrol agent. Notably, Docosenamide (12.79 %) and Octadecanoic acid, 2,3-dihydroxypropyl ester (6.81 %) have demonstrated significant insecticidal effects [35,36]. Additionally, compounds such as Melezitose (0.15 %) and L-Ascorbic acid, 6-octadecanoate (0.42 %) have been associated with anti-insect properties [37,38], potentially contributing to defense mechanisms or deterring herbivory. Compounds with broader bioactivities such as 9-Octadecenoic acid (Z), Hexadecanoic acid, and Oleic acid, while known for antimicrobial and antioxidant functions, may also indirectly affect insect survival by disrupting microbial symbionts or weakening immune defenses [39]. The GC-MS analysis of *Penicillium buchwaldii* (Table 4, Fig. 2) indicates a chemically diverse profile, with several compounds possessing notable biological activities relevant to insect control. Among the major components, Citronellyl oleate (8.88 %) is recognized for its insect-repellent properties due to its citronellol backbone, commonly found in botanical insecticides. Hexadecanoic acid (5.31 %) and Octadecanoic acid (3.45 %) have both been associated with insecticidal and antimicrobial properties, potentially acting through membrane disruption or enzyme inhibition [33]. Kojic Acid (4.97 %), primarily known for its antioxidant and antimicrobial functions, may indirectly affect insect survival by targeting microbial symbionts [40]. The presence of 5-Hydroxymethylfurfural (3.09 %) a compound toxic to various insect species further supports the potential insecticidal activity [41]. Notably, Docosenamide (2.01 %), also found in *V. lechnii,* is a known insecticidal agent that may impair nervous system function [35]. The detection of 1,2-Benzenedicarboxylic acid (2.63 %) adds to the extract's antimicrobial and possibly insect-deterrent capabilities [42]. The GC-MS profile of *Alternaria photistica* demonstrates a strong potential for anti-insect activity, driven by a rich diversity of bioactive compounds. The most prominent metabolite, 13-Docosenamide (Z) (14.95 %), is a well-documented insecticidal agent known to impair insect neural and developmental functions [43]. Complementing this are several other potent insecticidal constituents, including 5-Hydroxymethylfurfural (12.04 %), both of which have been linked to larvicidal and pesticidal actions [30,44]. Notably, Kojic Acid (8.64 %) and Melezitose (0.58 %) contribute to insect growth inhibition and deterrence [45]. Additionally, esterified fatty acids like Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (3.08 %) and Octadecanoic acid, 2,3-dihydroxypropyl ester (2.26 %) are known to disrupt insect cellular membranes [36].

Docosenamide is the majour component in the four PGPF isolates have several mechanisms as the following; It may interfere with neuropeptide signaling pathways or disrupt ion channel function in nematodes, leading to paralysis or impaired locomotion [43]. It my inhibit acetylcholinesterase, which plays a crucial role in nematode neurotransmission [46].It may be compromise the integrity of the cuticle or intestinal epithelium, leading to desiccation or internal leakage [47]. The results of the current study indicate a significant increase in the total phenolic content in nematode-infected plants. This increase is due to the fact that tomato plants increase their phenolic production as a defense strategy to prevent the spread of damage caused by root-knot nematodes [48,49].This increase in phenolic compounds likely helps in strengthening cell walls and may contribute to the direct toxicity against nematodes, as phenolics can be involved in producing toxic intermediates during plant-pathogen interactions [50].Interestingly, in infected plants)PGPF and Melithorin®) treatments lead to a notable increase in phenolic content, much higher than in untreated infected plants. This implies that these fungi, often known for their plant growth-promoting properties, might amplify the plant's natural defense response when under nematode attack. This effect could be mediated by the production of signaling molecules or enzymes that trigger a heightened defensive response in the presence of nematodes [51].The present study showed that in nematode-infected plants,*P. buchwaldii* induced the greatest rise in phenol content by (44.6 %) followed by*A. niger* (19.8 %), Melithorin® (14.4 %), and *V. lecanii* (3.8 %) respectively. These findings indicate that *A. niger*was the most effective treatment in healthy plants, while *P. buchwaldii*showed the strongest effect in enhancing phenol levels in infected plants. These responses still indicate that these fungi likely aid the plant in mounting a defense against nematodes but may operate through different or less intense pathways. Melithorin® (fosthiazate 90 %), known for its nematocidal properties, also resulted in a high increase in phenolics in infected plants. Fosthiazate could be inducing the plant's defense mechanisms more directly, leading to a heightened accumulation of defense-related compounds [52]. The pronounced increase in phenolic content in infected plants treated with these fungi suggests potential synergistic effects in which biocontrol fungi prime or improve plant defenses against nematodes. The analysis of Malondialdehyde (MDA) content in both shoots and roots of tomato plants provides insight into the oxidative stress responses caused by root-knot nematode infection and the role of biocontrol treatments in modulating this stress. The present study indicated that, in nematode-infected plants, Melithorin® showed the highest reduction in MD, followed by PGPF. These results suggest that *P. buchwaldii* was the most effective treatment in minimizing oxidative damage in healthy plants, while Melithorin® and *A. photistica* were most effective in mitigating stress in infected plants.MDA is a commonly used biomarker for lipid peroxidation and general oxidative stress, with higher levels indicating cell membrane damage and an intensified stress response [53,54]. Infection with root-knot nematodes significantly increased MDA levels in both shoots and roots of infected plants, reflecting heightened oxidative stress. This increase in MDA indicates that the nematode infection damages cellular membranes through lipid peroxidation, which is a typical response to biotic stress as the plant attempts to defend itself [55]. Reductions of MDA ranged from 6 % to 50 %, depending on the specific treatment and plant part. This decrease in MDA levels in healthy plants treated with biocontrol agents suggests that these treatments could have a protective effect, possibly by enhancing antioxidant activities or other mechanisms that reduce ROS accumulation [56,57]. The current results show that root-knot nematode infection triggered a significant increase in the activity of oxidative stress-related enzymes, including peroxidase (POD) and polyphenol oxidase (PPO), reflecting the plant's response to infection. Among infected treatments, Melithorin® recorded the highest activities of both enzymes. The present study provides compelling evidence that both Melithorin® and the plant growth-promoting fungi (PGPF), particularly *A. photistica* and *A. niger*, play a crucial role in mitigating oxidative stress and enhancing the defense mechanisms of tomato plants infected with root-knot nematodes. The observed reduction in oxidative stress biomarkersMDA and H_2O_2accompanied by an increase in total phenols and the activities of peroxidase (POD) and polyphenol oxidase (PPO), suggests that these treatments stimulate the plant's internal antioxidant defense system. The enhanced phenolic accumulation in treated plants can be attributed to the activation of the phenylpropanoid pathway, which is well-known for its role in plant defense against pathogens [58]. Phenolic compounds act as both antimicrobial agents and free radical scavengers, thereby limiting cellular damage under stress conditions [59]. This aligns with the findings of [60], who reported increased phenolic content and enzyme activities in plants treated with resistance

inducers under pathogen pressure. The isozyme analysis further reinforces these biochemical results. The strong expression of PPO2, PPO3, and PPO4 in infected plants treated with Melithorin®, *A. photistica*, and *A. niger* points to the activation of specific PPO isoforms involved in the oxidative polymerization of phenolics into lignin and other defense-related compounds. This lignification process strengthens cell walls and creates a physical barrier against nematode invasion—a mechanism well documented in studies such as [61,62]. Among the tested treatments, Melithorin® demonstrated the most consistent and significant effects, not only in reducing stress markers but also in upregulating enzyme activity and isozyme expression. This may be due to its chemical composition, which possibly acts as a systemic acquired resistance (SAR) inducer, triggering broad-spectrum defense responses [63]. These results are in agreement with [64], who highlighted the efficacy of Melithorin® in boosting plant immunity. In summary, the results suggest that Melithorin®, alongside *A. photistica* and *A. niger*, can effectively enhance tomato plant resistance to nematode infection by modulating oxidative stress responses and activating phenolic-based defense pathways. These findings highlight the potential of integrating chemical inducers and PGPF as sustainable and eco-friendly strategies in integrated pest management (IPM) programs. In our study, the anti-nematode activity of the tested elicitors appears to operate through two main mechanisms. The first is a direct effect on root-knot nematodes, likely due to the presence of bioactive compounds in the extracts such as docosenamide, ethanol, 2-(2-butoxyethoxy)-, octadecanoic acid, 2, 3-dihydroxypropyl ester, and adrenosterone. The second is an indirect effect, mediated by the elicitors' ability to enhance the plant's antioxidant defense system, thereby mitigating the damage caused by root-knot nematode infection. This defense activation was evident in the increased activity of antioxidant enzymes and levels of stress-related markers such as MDA, H_2O_2, and phenolics, which was confirmed by antioxidant izozyme determination.

5. Conclusion

This study demonstrates that Melithorin® and the tested plant growth-promoting fungi (PGPF) possess significant nematicidal potential against *Meloidogyne incognita*, both in vitro and under greenhouse conditions. Among the biocontrol agents, *Verticillium lecanii* and *Aspergillus niger*showed notable effectiveness in reducing nematode populations and associated root galling. GC-MS analysis of fungal metabolites revealed a range of bioactive compounds likely contributing to their antinematode activity. Furthermore, the treatments modulated host defense responses, including enhanced phenolic content, proline levels, and antioxidant enzyme activities.

CRediT authorship contribution statement

Mohamed S. Attia: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Amer M. Abdelaziz:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mostafa I. Abdelglil:** Writing – review & editing, Writing – original draft. **Eslam K. Kandil:** Writing – original draft, Visualization, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Muge Ergun:** Writing – original draft, Visualization, Data curation, Conceptualization. **Salah M. Elsayed:** Funding acquisition, Formal analysis, Conceptualization. **Maryam M. Elsayed:** Validation, Formal analysis, Data curation, Conceptualization. **Noha M. Ashry:** Writing – review & editing, Writing – original draft, Resources, Formal analysis, Conceptualization. **Mohamed M. Nofel:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Plant collection

The plant collection and use were by all the relevant guidelines.

Ethics approval and consent to participate

All authors approved.

Consent for publication

All authors agree for publication.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank the Faculty of Science, Al-Azhar University for promoting this research. The authors are grateful to Eng. Mahmud Elsayed, for his help during the study.

Data availability

Data will be made available on request.

References

[1] A.M. Abdelaziz, et al., Biostimulation of tomato growth and biocontrol of fusarium wilt disease using certain endophytic fungi, Bot. Stud. 63 (1) (2022) 34.
[2] R. Hussey, K. Barker, A comparison of methods of collecting inocula of Meloidogyne spp. Including a New Technique, 1973.
[3] D.-D. Niu, et al., Application of PSX biocontrol preparation confers root-knot nematode management and increased fruit quality in tomato under field conditions, Biocontrol Sci. Technol. 26 (2) (2016) 174–180.
[4] Y. Liu, et al., Organophosphorus nematicide potentiated nematicidal effect by changing rhizosphere bacterial and fungal communities, Rhizosphere (2024) 100936.
[5] H.Y. Wu, et al., Nematicidal activity of fosthiazate against soybean cyst nematode, J. Nematol. 51 (1) (2019) 1–9.
[6] V. Phani, M.R. Khan, T.K. Dutta, Plant-parasitic nematodes as a potential threat to protected agriculture: current status and management options, Crop Prot. 144 (2021) 105573.
[7] A.M. Abdelaziz, et al., Potential of biosynthesized zinc oxide nanoparticles to control Fusarium wilt disease in eggplant (Solanum melongena) and promote plant growth, Biometals 35 (3) (2022) 601–616.
[8] A.H. Hashem, et al., Bacillus megaterium-mediated synthesis of selenium nanoparticles and their antifungal activity against Rhizoctonia solani in faba bean plants, J. Fungi 7 (3) (2021) 195.
[9] M.S. Attia, et al., Eco-friendly preparation of thyme essential oil nano emulsion: characterization, antifungal activity and resistance of Fusarium wilt disease of Foeniculum vulgare, Not. Bot. Horti Agrobot. Cluj-Napoca 51 (3) (2023), 13312-13312.
[10] M.T. El-Saadony, et al., Plant growth-promoting microorganisms as biocontrol agents of plant diseases: mechanisms, challenges and future perspectives, Front. Plant Sci. 13 (2022) 923880.
[11] R. Hamrouni, et al., Current status and future trends of microbial and nematode-based biopesticides for biocontrol of crop pathogens, Crit. Rev. Biotechnol. (2024) 1–20.
[12] A. Khalil, et al., Fungal endophytes from leaves of Avicennia marina growing in semi-arid environment as a promising source for bioactive compounds, Lett. Appl. Microbiol. 72 (3) (2021) 263–274.

[13] M.S. Attia, M.S. Salem, A.M. Abdelaziz, Endophytic fungi Aspergillus spp. reduce fusarial wilt disease severity, enhance growth, metabolism and stimulate the plant defense system in pepper plants, Biomass Convers. Biorefinery 14 (14) (2024) 16603–16613.
[14] R. Devi, et al., Beneficial fungal communities from different habitats and their roles in plant growth promotion and soil health, Microb. Biosystems 5 (1) (2020) 21–47.
[15] J.K. Nitao, S.L. Meyer, D.J. Chitwood, In-vitro assays of Meloidogyne incognita and Heterodera glycines for detection of nematode-antagonistic fungal compounds, J. Nematol. 31 (2) (1999) 172.
[16] E.K. Kandil, et al., Effectual role of plant growth-promoting fungi and fosthiazate in controlling tomato root-knot nematode infection: in vivo and in vitro studies, Physiol. Mol. Plant Pathol. 134 (2024) 102463.
[17] B. Hopper, J. Fell, R. Cefalu, Effect of temperature on life cycles of nematodes associated with the mangrove (Rhizophora mangle) detrital system, Mar. Biol. 23 (4) (1973) 293–296.
[18] A.M. Abdelaziz, A.S. Mohamed, M.S. Attia, Protective role of Claroideoglomus etunicatum and Trichoderma harzianum to improve growth and physiological immune responses of Olea europaea tolerance against Fusarium solani, Physiol. Mol. Plant Pathol. (2025) 102593.
[19] A.M. Shehabeldine, et al., Antimicrobial characteristics of endophytic Aspergillus terreus and acute oral toxicity analysis, Electron. J. Biotechnol. 72 (2024) 1–11.
[20] G. Dai, et al., Polyphenols and resistance of grapevines to downy mildew, in: International Symposium on Natural Phenols in Plant Resistance, 381, 1993.
[21] Z. Hu, et al., Physiological and biochemical effects of rare Earth elements on plants and their agricultural significance: a review, J. Plant Nutr. 27 (1) (2004) 183–220.
[22] S. Mukherjee, M. Choudhuri, Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings, Physiol. Plantarum 58 (2) (1983) 166–170.
[23] H.-U. Bergmeyer, Determination with glucose oxidase and peroxidase, Methods Enzym. 2 (1965) 1205–1215.
[24] A. Matta, A. Dimond, Symptoms of Fusarium wilt in relation to quantity of fungus and enzyme activity in tomato stems, Phytopathology 53 (5) (1963), 574-&.
[25] A.R. Barceló, R. Muñoz, F. Sabater, Lupin peroxidases. I. Isolation and characterization of cell wall-bound isoperoxidase activity, Physiol. Plantarum 71 (4) (1987) 448–454.
[26] M.M. Abd-Elgawad, T.H. Askary, Fungal and bacterial nematicides in integrated nematode management strategies, Egypt. J. Biological Pest Control 28 (1) (2018) 1–24.
[27] A.M. Abdelaziz, et al., Protective role of endophytic fungi and salicylic acid as therapeutic nutrients to improve immune responses of tomato plants against fusarial wilt disease, Not. Bot. Horti Agrobot. Cluj-Napoca 52 (1) (2024), 13497-13497.
[28] M.S. Attia, et al., Application of Rhizopus microsporus and Aspergillus oryzae to enhance the defense capacity of eggplant seedlings against Meloidogyne incognita, Not. Bot. Horti Agrobot. Cluj-Napoca 51 (3) (2023) 1–23.
[29] E. Odeyemi, et al., Chemical composition and insecticidal activity of volatile oils ofAllium cepa bulb and Carica papaya leaf against Balanogastris kolae infestation on stored Kolanuts, IFE J. Sci. 26 (2) (2024) 397–407.
[30] K.C. Shilaluke, A.N. Moteetee, Insecticidal activities and GC-MS analysis of the selected family members of Meliaceae used traditionally as insecticides, Plants 11 (22) (2022) 3046.
[31] J. Adesina, Y. Rajashaker, T.I. Ofuya, Potentiality and chemical composition of Bridelia micrantha (Berth) extracts and its fractions as biofumigant against economically important stored grain insect pests, J. Horticulture Postharvest Res. 3 (1) (2020) 61–72.
[32] A. Litwin, M. Nowak, S. Różalska, Entomopathogenic fungi: unconventional applications, Rev. Environ. Sci. Biotechnol. 19 (1) (2020) 23–42.
[33] A.H. Hashem, et al., Bacterial endophytes from Moringa oleifera leaves as a promising source for bioactive compounds, Separations 10 (7) (2023) 395.
[34] M.S. Ali, et al., Phytol and glycerol derivatives from the marine green alga Codium iyengarii of the Karachi Coast (Arabian Sea), Z. Naturforsch. B Chem. Sci. 56 (8) (2001) 837–841.
[35] S.S. Dey, et al., Comparative analysis of four different solvent extracts of Annona muricata bark based on bioactive compounds and antibacterial effectiveness, Heliyon 11 (4) (2025) e42930.
[36] C. Okonkwo, S. Moses, Insecticidal potentials and chemical composition of ethanol extracts from the leaves of Acanthus montanus on selected insect pests, J. Chem. Pharmaceut. Res. 9 (10) (2017) 111–116.
[37] M.K. Ya'kobovitz, T.D. Butters, E. Cohen, Inhibition of α-glucosidase activity by N-deoxynojirimycin analogs in several insect phloem sap feeders, Insect Sci. 23 (1) (2016) 59–67.
[38] S. Farooq, et al., Isolation and characterization of Aspergillus oryzae from Withania coagulans rhizosphere: metabolite profiling and bioactivity assessment, J. Plant Biochem. Biotechnol. (2024) 1–17.
[39] F.M. Elkady, et al., Unveiling the Launaea nudicaulis (L.) hook medicinal bioactivities: phytochemical analysis, antibacterial, antibiofilm, and anticancer activities, Front. Microbiol. 15 (2024) 1454623.
[40] M. Kannan, I. Opatovsky, A review on nutritional and non-nutritional interactions of symbiotic and associated fungi with insect, Symbiosis 91 (1) (2023) 139–149.
[41] X. Chen, et al., 5-Hydroxymethylfurfural mediated developmental toxicity in Drosophila melanogaster, Food Chem. Toxicol. 189 (2024) 114738.
[42] O. Osuntokun, G. Cristina, Bio-guided isolation, chemical purification, identification, antimicrobial and synergistic efficacy of extracted essential oils from stem bark extract of Spondias mombin (Linn), Int J Mol Biol Open Access 4 (4) (2019) 135–143.
[43] R. Saranya, M.S. Ali, V. Anuradha, Phytochemical, fluorescence screening and GC-MS analysis of various crude extracts of Anastatica hierochuntica, J. Pharm. Chem. Biol. Sci 7 (2018) 44–56.
[44] S.-A. Choi, et al., Acaricidal properties of 5-methylfurfural identified from Valeriana fauriei and its structural analogues against synanthropic mites and Asian longhorned tick with color alterations, Applied Biological Chemistry 64 (2021) 1–13.
[45] A. Berestetskiy, Q. Hu, The chemical ecology approach to reveal fungal metabolites for arthropod Pest management, Microorganisms 9 (7) (2021) 1379.
[46] S. Mitra, et al., Pesticides in the environment: degradation routes, pesticide transformation products and ecotoxicological considerations, Sci. Total Environ. (2024) 173026.
[47] A. Habteweld, et al., Understanding the dynamic interactions of root-knot nematodes and their host: role of plant growth promoting bacteria and abiotic factors, Front. Plant Sci. 15 (2024) 1377453.
[48] H.K. Kirwa, Behavioral response of root-knot nematode, Meloidogyne incognita, to tomato. Solanum Lycopersicon, Root Exudates, 2020.
[49] D. Pitabas, Molecular Dissection of Rice-Meloidogyne Graminicola Interactions: Effectors and Their Targets, Division of Nematology Icar-Indian Agricultural Research Institute, 2022. NEW DELHI.
[50] S. Kaur, et al., How do plants defend themselves against pathogens-biochemical mechanisms and genetic interventions, Physiol. Mol. Biol. Plants 28 (2) (2022) 485–504.
[51] J. Poveda, P. Abril-Urias, C. Escobar, Biological control of plant-parasitic nematodes by filamentous fungi inducers of resistance: Trichoderma, mycorrhizal and endophytic fungi, Front. Microbiol. 11 (2020) 992.
[52] J. Yeon, et al., Control of a Tomato Plant root-knot Nematode by Induced Resistance of Oxalic Acid Derived from Aspergillus Niger, 2021.
[53] K. Dhama, et al., Biomarkers in stress related diseases/disorders: diagnostic, prognostic, and therapeutic values, Front. Mol. Biosci. 6 (2019) 91.
[54] E.A. de Almeida, et al., Oxidative stress in perna perna and other bivalves as indicators of environmental stress in the Brazilian marine environment: antioxidants, lipid peroxidation and DNA damage, Comp. Biochem. Physiol. Mol. Integr. Physiol. 146 (4) (2007) 588–600.
[55] K. Khanna, et al., Impact of plant growth promoting rhizobacteria in the orchestration of Lycopersicon esculentum mill. resistance to plant parasitic nematodes: a metabolomic approach to evaluate defense responses under field conditions, Biomolecules 9 (11) (2019) 676.
[56] N. Sharma, et al., Insights into the role of Streptomyces hydrogenans as the plant growth promoter, photosynthetic pigment enhancer and biocontrol agent against Meloidogyne incognita in Solanum lycopersicum seedlings 9 (9) (2020) 1109.
[57] Y. Yassin, et al., Foliar application of Rhizobium leguminosarum bv. Viciae strain 33504-Borg201 promotes faba bean growth and enhances systemic resistance against bean yellow mosaic virus infection 81 (8) (2024) 220.
[58] N. Saini, et al., Exploring Phenolic Compounds as Natural Stress Alleviators in plants-a Comprehensive Review, 2024 102383.
[59] K.J.B. Kulbat, F. Science, The role of phenolic compounds in plant resistance 80 (2) (2016) 97–108.
[60] E. Mahmoud, S.Y. Shokri, Z.N. Hussin, Induction of resistance in peanut plants against root rot diseases under greenhouse conditions by some chemical inducers 31 (6) (2006) 3511–3524.
[61] J. Yu, et al., Silicon mediated plant immunity against nematodes: summarizing the underline defence mechanisms in plant nematodes interaction 23 (22) (2022) 14026.
[62] R. Saikia, et al., Biochemical changes in host plant invaded by opportunistic fungi and nematodes, in: Opportunistic Fungi, Nematode and Plant Interactions: Interplay and Mechanisms, Springer, 2024, pp. 53–68.
[63] M. Kamle, et al., Systemic Acquired Resistance (SAR) and Induced Systemic Resistance (ISR): Role and Mechanism of Action Against Phytopathogens, 2020, pp. 457–470.
[64] E. Kandil, et al., Promising Nematocidal Efficacy of Verticillium lecanii, Hydrogen Peroxide, and Melithorin® against Root-Knot Nematode, Meloidogyne incognita in the Tomato Plant, Egyptian J. Botany 65 (1) (2025) 266–275.