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Purpureocillium lilacinum strain AUMC 10620 as a biocontrol agent against the citrus nematode *Tylenchulus semipenetrans* under laboratory and field conditions

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Abstract Three concentrations (1.25, 2.5, and 5×10^7 spores ml^{-1}) (of the biocontrol fungus *Purpureocillium lilacinum* (strain AUMC 10620) were tested on citrus nematode *Tylenchulus semipenetrans* under *in vitro* and field conditions. Larvae and eggs were exposed to the fungal spores *in vitro* for 24, 48, and 72 h, and the findings were recorded at each time point. These results were compared with the application of the nematicide abamectin. Strain AUMC 10620 effectively reduced larval activity and egg hatching of *T. semipenetrans* under laboratory conditions. The highest concentration (5×10^7 spores ml^{-1}) of *P. lilacinum*, resulted in 89.01% immobility in the larvae, compared to abamectin, which resulted in 65.93% immobility after 48 h of exposure. These percentages of immobility were increased after 72 h of exposure (100 and 85.09%) when *P. lilacinum* at a concentration of 5×10^7 spores ml^{-1} and abamectin were used, respectively. On the other hand, the two other *P. lilacinum* concentrations (1.25, and 2.5×10^7 spores ml^{-1}) affected the *T. semipenetrans* larvae to a lesser extent. The highest fungal concentration 5×10^7 spores ml^{-1} inhibited the hatching of *T. semipenetrans* eggs *in vitro* with 71.34, 80, and 86.67% after 24, 48 and 72 h of treatment compared to the abamectin treatment which showed 76.67, 78, and 87% after the abovementioned periods, respectively. In addition, the application of *P. lilacinum* (5×10^7 spores ml^{-1}) or abamectin

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under field conditions significantly ($P<0.05$) reduced the population of the major nematode species (*T. semipenetrans, Tylenchorhynchus* spp., *Helicotylenchus* spp., and *Pratylenchus* spp.) infesting citrus after one, two, and three weeks of treatment compared to the control treatment but with no significant ($P>0.05$) differences between the two treatments. Three weeks after the field application, the percentage of nematode reduction was significantly ($P<0.05$) smaller than the control treatment at concentrations of 5, 2.5, and 1.25×10^7 spores ml^{-1}, respectively, by 78.42, 64.03, and 58.35%. It is evident from these results that the application of *P. lilacinum* strain (AUMC 10620) can be used in integrated pest management programs to control nematodes infesting citrus trees.

Keywords Abamectin · Biological control · Citrus nematodes · *Purpureocillium lilacinum* · *Tylenchulus semipenetrans*

Introduction

Citrus crops are the most exported crops in Egypt, especially oranges and mandarins (Abd-Elgawad et al., 2010). Egypt's harvested area of oranges increased from 93,350 to 127,200 ha from 1999 to 2019. As a result, the production doubled from 1,636,600 tons to 3,197,046 tons from 1999 to 2019 (FAOSTAT, 2019). Egypt was ranked seventh among the top ten citrus crop-producing countries and exported about 1.7 million tons of oranges in 2019, accounting for 38% of the world's exports in 2019 (FAO, 2020).

Many nematode species infect citrus plants both in Egypt and around the world. The most hazardous nematode attacking citrus plants is *Tylenchulus semipenetrans* Cobb, responsible for a disease known as slow decline. The economic yield loss caused by nematode infection was estimated to be 10-30% of total crop losses (Abd-Elgawad et al., 2016; El-Marzoky et al., 2018; Verdejo-Lucas & McKenry, 2004). According to Cohn (1972) and Sasser (1989), the annual economic crop production losses caused by citrus nematode infestation varied between 8.7% and 14%, respectively. On the other hand, the yearly citrus crop output losses in Egypt due to nematodes approached 10%, costing roughly 128.11 million Egyptian pounds annually (Abd-Elgawad, 2014).

Because of their quick action and acceptable results, chemical nematicides are widely used to control plant-parasitic nematodes (PPNs). However, they are costly and pose environmental risks (Tudi et al., 2021). Nematicides have been implicated in numerous reports of groundwater contamination in the interim. This pollution may harm plants directly or indirectly by introducing chemical nematicides into their groundwater (Tudi et al., 2021; Ullah et al., 2020; Zhang et al., 2022).

Regarding global exports, Egyptian citrus fruits face severe competition from other Mediterranean countries (Abd-Elgawad et al., 2010). The main issue is the widespread use of chemical fertilizers and pesticides, which renders these fruits unsuitable for European markets (Bazargan, 2017). Organic farming has grown significantly in recent years and is expected to grow further. As a result, using biocontrol agents to manage nematode infestations in those farms has become an urgent necessity (Abd-Elgawad et al., 2010; European Commission, 2021).

The use of bioproducts, or commercial products having microorganisms as the active component, has proven to be highly effective in the management of nematodes (Radwan et al., 2012). Although numerous bacterial species have the potential to be employed as biological control agents against nematodes, only a small subset of these are incorporated into commercial product formulations (Subedi et al., 2020). *Bacillus firmus*, *Bacillus methylotrophicus, Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus amyloliquefaciens* are some of the *Bacillus* species that are used in commercial products that have already been registered against nematodes. Research on other bacterial genera has increased the number of nematode biological control agents (Subedi et al., 2020).

These bacterial biocontrol agents have direct and indirect effects on nematodes. They can act directly by producing nematicidal compounds such as 3-benzyl-1,4-diaza-2,5-dioxobicyclo[4.3.0]nonane (Yoon et al., 2012), fungichromin (Zeng et al., 2013), and actinomycins (Sharma et al., 2019). They also can colonize plant root tissues (Patel et al., 2018) and parasitize nematode eggs (Jin et al., 2017; Yoon et al., 2012). They can indirectly inhibit nematodes by triggering plant defense mechanisms and increase resistance (Abbasi et al., 2020; Nishad et al., 2020).

Purpureocillium lilacinum (Thom) Samson (formerly *Paecilomyces lilacinus)* is a promising

fungus used widely in controlling nematodes in the soil (Isaac et al., 2021). The fungus belongs to phylum ascomycota, class sordariomycetes, order hypocreales, and family ophiocordycipitaceae (Bennett & Shah, 2022; Girardi et al., 2022; Hibbett et al., 2007; Luangsa-ard et al., 2011). The biological control effect of *P. lilacinum* on PPNs, which has no negative impact on humans, animals, or the environment, sparked interest in developing commercial inputs based on that fungus (Isaac et al., 2021).

The mechanism by which *P. lilacinum* inhibits nematodes has been the subject of extensive research. This fungus kills PPNs through the production of extracellular enzymes (Elsherbiny et al., 2019; Giné & Sorribas, 2017; Kumar & Arthurs, 2021; LaMondia & Timper, 2016; Sharma et al., 2021; Wang et al., 2010; Xu et al., 2021), production of antibiotics (Wang et al., 2016), production of toxic metabolites (LaMondia & Timper, 2016; Park et al., 2004), competition for nutrients and space (Giné & Sorribas, 2017; Khan et al., 2004; Lan et al., 2017), hyphal colonization, mechanical pressure and hyphal penetration (Giné & Sorribas, 2017; LaMondia & Timper, 2016; Zare et al., 2001), parasitism of nematode eggs (Swarnakumari & Kalaiarasan, 2017), prevention of eggs hatching (Khan et al., 2004; Singh et al., 2013), improvement in plant growth parameters (Divya, 2020; Hajji et al., 2017; Silva et al., 2022), and induction of plant defense mechanisms (Elsherbiny et al., 2019; Vega et al., 2008).

The current study was conducted to determine the effectiveness of a strain of *P. lilacinum* (strain AUMC 10620) against the major citrus pest *T. semipenetrans* under *in vitro* and field conditions. This biocontrol agent is also environmentally friendly, safe, and widely accessible. It can be used in integrated pest management programs to reduce the use of chemical pesticides in Egypt and elsewhere.

Materials and methods

Collection of soil and root samples

Ten soil samples and roots were collected from AL-Basha farm, Basatin Barakat, Abu-Hammad district, Sharqia Governorate, Egypt. The location coordinates were 30°27′58.6”N 31°40′04.6″E. The experimental site was about six feddans (one feddan is approximately 4200 square meter) of sandy loam soil, cultivated with 13 years old mandarin trees *Citrus reticulata* grafted on sour orange rootstock *Citrus aurantium* irrigated with the drip irrigation system. Three months before the experiment, no pesticides was applied, and all of the marked trees had been given the recommended horticultural care, including weeding and fertilization (Egyptian Ministry of Agriculture and Land Reclamation, 2022).

Each composite sample consisted of approximately 1 kg of soil, with citrus roots which were collected from randomly selected trees at localized sites. Rainy and hot sunny days were avoided during samples collection. Samples were collected using a hand trowel from a 20-25 cm thick layer under the tree canopy. Collected samples were placed in polyethylene bags and stored in an icebox until sent to the laboratory for nematode extraction (Ravichandra, 2010).

Extraction of citrus nematode juveniles (J2) from soil samples and isolation of eggs from roots for *in vitro* experiments

Active citrus nematode larvae (J2) were extracted as quickly as possible from soil samples. An aliquot sample of 250 g of soil was processed for nematode extraction. Nematodes were extracted using a combination of sieves and the Baermann tray technique (Hooper et al., 2005; El-Marzoky, 2019). A centrifugal flotation process was then used to separate J2 from soil debris. The nematode suspension was placed in a 50 ml tube and centrifuged at 1000 x *g* for 5 min. The supernatant was then removed and the heavy particles in the bottom of the tube were added to a sucrose solution of 50%, and the abovementioned step was repeated. The nematodes were floated in the sucrose solution and were separated from the heavy particles. The supernatant was poured through a 500-mesh sieve, and the contents were washed gently with tap water (Ortiz Paz et al., 2015). Finally, 1 ml of nematode suspension was pipetted into a Hawksley counting slide to identify and count the J2 of the citrus nematode.

The J2 were identified morphologically using and Olympus BH-2 (Olympus Optical Co. Ltd., Tokyo, Japan) light microscope equipped with a digital camera and software (Jenoptik ProgRes Camera, C12plus, Frankfurt, Germany) using 100 X magnification,

according to Siddiqi (1986). One ml of the nematode suspension containing about 500 J2 was added to each 15 cm diameter Petri dish containing water agar (WA) (Lab M Limited, Lancashire, UK) (7.5 g agar in 1 l of distilled water) amended with 5 ml of lactic acid (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) to prevent bacterial growth.

Each treatment was replicated three times to examine the suppressive effect of different concentrations of the biocontrol agent *P. lilacinum* on the number of the citrus nematode *T. semipenetrans*. The treatment used were PC = nematodes + the nematicide abamectin, NP5 = nematodes + *P. lilacinum* (5×10^7 spores ml^{-1}), NP2.5 = nematodes + *P. lilacinum* (2.5×10^7 spores ml^{-1}), and NP1.25 = nematodes + *P. lilacinum* (1.25×10^7 spores ml^{-1}). The negative control treatment consisted of nematodes plus distilled water. In contrast, the positive control treatment consisted of nematodes plus the nematicide abamectin (Tervigo® 2% soluble concentrate) (Syngenta International AG, Basel, Switzerland) as suggested by El-Marzoky et al. (2022) since abamectin is known to be effective against PPNs eggs and larvae. Abamectin was the recommended commercial dose at 1000 ppm (5 ml in 100 ml distilled water). The percentage of immobility (%) was calculated using Schneider-Orelli's formula as recommended by Püntener (1981) (Equations 1 and 2).

$$\text{Percent of Immobility (\%)} = \frac{\text{Treatment J2 immobility (\%)} - \text{Control J2 immobility (\%)}}{100 - \text{immobility incontrol}} \times 100 \quad (1)$$

$$\text{Immobility in control} = \frac{\text{Number of immobile J2}}{\text{Initial population}} \times 100 \quad (2)$$

Data were recorded 24, 48 and 72 h after treatment, and the immobile nematodes were determined as inactive individuals with a straight-like shapes.

According to Van Bezooijen (2006), sodium hypochlorite solution was was used to separate the citrus nematode eggs from the remaining egg masses. This solution was prepared by adding 180 ml of distilled water to 20 ml of commercial Clorox® to achieve 0.5% concentration. The citrus roots were cut into approximately 2 cm each, washed gently with tap water to remove soil debris, and placed in a 500 ml Erlenmeyer flask containing 200 ml of sodium hypochlorite at a concentration of 0.5%. These root segments were gently shaken to separate the eggs for about 3 min. The collected eggs were then transferred to a 100 ml beaker, and the number of eggs was counted in 1 ml of the final suspension. The final suspension was poured through a 200-mesh sieve nestled upon a 500-mesh sieve. The debris above 500-mesh sieves containing the eggs was immediately washed with tap water to release the residual sodium hypochlorite.

One ml of the egg's suspension containing about 500 eggs was added to 15 cm diameter Petri dishes containing WA (Lab M Limited) amended with 5 ml of lactic acid (Sigma-Aldrich) to test the effect of different concentrations of the biocontrol agent *P. lilacinum* on the citrus nematode *T. semipenetrans* egg hatching. Each treatment was replicated three times, and the treatment used were PEC = nematodes eggs + the nematicide abamectin, NEP5 = nematodes eggs + *P. lilacinum* (5×10^7 spores ml^{-1}), NEP2.5 = nematodes eggs + *P. lilacinum* (2.5×10^7 spores ml^{-1}) and NEP1.25 = nematodes eggs + *P. lilacinum* (1.25×10^7 spores ml^{-1}). The negative control treatment consisted of nematodes plus distilled water, while the positive control treatment consisted of nematode plus nematicide abamectin (Tervigo®) (Syngenta). The hatched eggs were recorded 24, 48, and 72 h after application, and the egg hatching rate was calculated using equation (3) according to Sun et al. (2006)

$$\text{The egg hatching rate} = \frac{\text{J2}}{(\text{Eggs} + \text{J2})} \times 100 \quad (3)$$

The reduction in egg hatching was calculated using equation (4) to determine the efficacy of the tested fungal concentrations in reducing egg hatching.

$$\text{The reduction in egg hatching} = \frac{\text{The initial number of the eggs} - \text{Number of hatched eggs}}{\text{The initial number of the eggs}} \times 100 \quad (4)$$

Preparation of different concentrations of the biocontrol agent *P. lilacinum*

The culture of *P. lilacinum* strain (AUMC 10620) was obtained from the Plant Pathology Department, Faculty of Agriculture, Zagazig University, Egypt. Sterilized barley grains were inoculated with spores scrapped from 7 days old growing cultures of *P. lilacinum* grown on potato dextrose agar plates (PDA; Lab M Limited) plates (pH 6.0); supplemented with ampicillin (Sigma-Aldrich). The grains were mixed, and the flasks were incubated for three weeks in the dark at 25 ± 2 °C. Aliquots (500 mg) of *P. lilacinum* strain (AUMC 10620) were mixed in 30 ml of 0.05% sterile potato dextrose broth (Lab M) to obtain a uniform suspension of fungal spores.

The number of spores ml^{-1} was calculated using haemocytometer (Agar Scientific Limited, Essex, UK). The fungus stock solution (NP5 = 5× 10^7 spores ml^{-1}) was prepared, and to achieve the other two different concentrations (NP2.5 = 2.5 × 10^7 spores ml^{-1} and NP1.25 = 1.25 × 10^7 spores ml^{-1}), equation (5) was used (Castaño-Zapata, 1998).

$$\text{Initial concentration of spores ml}^{-1} = \frac{\text{The final volume of the suspension} \times \text{Initial volume of the suspension}}{\text{The final concentration of spores/ml}} \quad (5)$$

One ml of spore suspension of each fungal concentration was added to Erlenmeyer flasks containing WA (Lab M Limited) amended with 5 ml of lactic acid (Sigma-Aldrich). The mixture was homogenized using a sterile glass stirrer. Six ml of the prepared medium was poured into each Petri dish of 15 cm and then allowed to stand for 3 h until the medium became slurry (Ortiz Paz et al., 2015). Finally, the nematode J2 and eggs were added to the slurry for each concentration.

Three replicates were used at each sampling, and the data were recorded after 24, 48, and 72 h of application.

The layout of the field experiment

In the same site as mentioned above, an experiment was carried out to evaluate the effect of different concentrations of the biocontrol agent *P. lilacinum* on PPNs including *T. semipenetrans, Tylenchorhynchus* spp., *Helicotylenchus* spp., and *Pratylenchus* spp. under field conditions. The site was divided into five rows (five treatments); each row consisted of fifteen trees, with untreated rows separated each row (treatment). Inside each marked row, five trees were randomly determined as replicates. The nematicide abamectin was applied as a recommended dose (2 l $feddan^{-1}$), calculated as 13.5 ml $tree^{-1}$. The tested concentrations of the fungal spores were applied as 100 ml of each concentration (1.25, 2.5, and 5 × 10^7 spores ml^{-1}) for each tree. This concentration was applied in a sequence of three different periods. Each application was one day apart from the next in the sequence compared with the control (fungal spores that were autoclaved twice before application). All the tested materials were applied at 50 cm from the tree trunk at a depth of 10 cm in the canopy area. Five replicates were used at each sampling, and the data were recorded after 1, 2, and 3 weeks after treatment.

Soil (500 g) was collected from the marked trees at a depth of 25 cm and transported to the laboratory in polyethylene bags for nematode extraction. The active individuals of the nematode species were extracted from the soil samples by combining sieves and the Baermann trays technique as described above. The nematode species were then identified morphologically according to Mai (1988), and the numbers were counted in the extraction suspension using 250 g of soil.

The percentage of nematode reduction (%) was calculated using equation (6) according to Abbott (1925):

$$\text{Percentage of nematode reduction (\%)} = \frac{\text{Number of nematodes in the control} - \text{Number of nematode in the treatment}}{\text{Number of nematode in the control}} \times 100 \quad (6)$$

Statistical analysis

The field experiment was performed in a completely randomized block design, with five replicats for each treatment. Analysis of variance (ANOVA) and Duncan's multiple range test were used to compare the statistical significance between means at $P \leq 0.05$. For all statistical analyses, SAS Software version 9 (SAS Institute Inc., NC, USA) was used.

Results

The effect of *P. lilacinum* concentration the percentages immobility of *T. semipenetrans* J2 under *in vitro* conditions

The inhibitory effects of different concentrations of the biocontrol agent *P. lilacinum* on the number of the citrus nematode *T. semipenetrans* J2 under *in vitro* conditions are presented in Tables 1 and 2. Results in Table 1 showed that, compared to untreated nematodes and nematodes treated with the nematicide abamectin, the three concentrations of the biocontrol agent had an impact on nematode vitality following three sequential periods of treatment (24, 48, and 72 h).

After 24 h, the nematicide positive control (PC treatment) was effective, and the number of immobile J2 recorded was 300 compared to 238.33, 125.00, and 111.67 in the treatments NP5 (5×10^7 spores ml^{-1}), NP2.5 (2.5×10^7 spores ml^{-1}), and NP1.25 (1.25×10^7 spores ml^{-1}), respectively. After 48 and 72 h, *P. lilacinum* at a concentration of 5×10^7 spores ml^{-1} (NP5) was the most effective treatment. The immobile J2 population reached 450 and 500, respectively. After the same periods, the nematicide positive control treatment (PC) reached 345 and 436.67, respectively. There were no significant ($P > 0.05$) differences only after 24 h of application between NP2.5 (2.5×10^7

Table 1 The effect of different concentrations of the biocontrol agent *Purpureocillium lilacinum* (AUMC 10620) spores and the nematicide abamectin on numbers of immotile *Tylenchulus semipenetrans* J2 after 24, 48, and 72 h of application *in vitro*

Treatments	Number of immotile J2 after 24 h	Number of immotile J2 after 48 h	Number of immotile J2 after 72 h
NC: Negative control (nematodes + distilled water)	30.00 ± 2.88 *d*	45.00 ± 2.88 *e*	75.00 ± 2.88 *e*
PC: Positive control (nematodes + nematicide)	300.00 ± 5.77 *a*	345.00 ± 2.88 *b*	436.67 ± 18.55 *b*
NP5: Nematodes + *P. lilacinum* (5×10^7 spores ml^{-1})	238.33 ± 4.41 *b*	450.00 ± 28.86 *a*	500.00 ± 5.77 *a*
NP2.5: Nematodes + *P. lilacinum* (2.5×10^7 spores ml^{-1})	125.00 ± 10.40 *c*	138.33 ± 4.41 *c*	245.00 ± 7.63 *c*
NP1.25: Nematodes + *P. lilacinum* (1.25×10^7 spores ml^{-1})	111.67 ± 4.41 *c*	128.33 ± 33.70 *d*	158.33 ± 51.98 *d*

Data were from three independent replicates. Values with the same letter within a column are not significantly ($P > 0.05$) different according to Duncan's multiple range test

Table 2 The effect of different concentrations of the biocontrol agent *Purpureocillium lilacinum* (AUMC 10620) spores and the nematicide abamectin on the percent immobility of *Tylenchulus semipenetrans* J2 after 24, 48, and 72 h of application *in vitro*

Treatments	J2 immobility percentages after 24 h (%)	J2 immobility percentages after 48 h (%)	J2 immobility percentages after 72 h (%)
NC: Negative control (nematodes + distilled water)	0.00 *e*	0.00 *d*	0.00 *e*
PC: Positive control (nematodes + nematicide)	57.44 *a*	65.93 *b*	85.09 *b*
NP5: Nematodes + *P. lilacinum* (5×10^7 spores ml^{-1})	44.32 *b*	89.01 *a*	100.00 *a*
NP2.5: Nematodes + *P. lilacinum* (2.5×10^7 spores ml^{-1})	20.21 *c*	20.51 *c*	40.00 *c*
NP1.25: Nematodes + *P. lilacinum* (1.25×10^7 spores ml^{-1})	17.37 *cd*	18.31 *c*	19.60 *d*

Data were from three independent replicates. Values with the same letter within a column are not significantly ($P > 0.05$) different according to Duncan's multiple range test

Table 3 The effect of different concentrations of the biocontrol agent *Purpureocillium lilacinum* (AUMC 10620) spores and the nematicide abamectin on the number of eggs hatching of *Tylenchulus semipenetrans* after 24, 48, and 72 h of application *in vitro*

Treatments	Number of hatched eggs after 24 h	Number of hatched eggs after 48 h	Number of hatched eggs after 72 h
NEC: Negative control (nematodes eggs + distilled water)	201.67 ± 4.41 *a*	205.00 ± 10.40 *a*	240.00 ± 26.45 *a*
PEC: Positive control (nematodes eggs + nematicide)	116.67 ± 8.81 *bc*	110.00 ± 5.77 *bc*	65.00 ± 2.88 *d*
NEP5: Nematodes eggs + *P. lilacinum* (5×10^7 spores ml^{-1})	143.33 ± 4.41 *c*	100.00 ± 20.20 *c*	66.67 ± 4.41 *d*
NEP2.5: Nematodes eggs + *P. lilacinum* (2.5×10^7 spores ml^{-1})	150.00 ± 2.88 *b*	140.00 ± 2.88 *b*	135.00 ± 2.88 *c*
NEP1.25: Nematodes eggs + *P. lilacinum* (1.25×10^7 spores ml^{-1})	205.00 ± 2.88 *a*	195.00 ± 2.88 *a*	185.00 ± 2.88 *b*

Data were from three independent replicates. Values with the same letter within a column are not significantly ($P > 0.05$) different according to Duncan's multiple range test

spores ml^{-1}), and NP1.25 (1.25×10^7 spores ml^{-1}) on the number of immotile J2 (Table 1).

The effects of the different fungal concentrations on J2 mobility are presented in Table 2. Data showed that the percentages immobility increased over time. For example, the *P. lilacinum* concentration (NP5, 5×10^7 spores ml^{-1}) was the most suppressive concentration after 24, 48, and 72 h of treatment compared to NP2.5 (2.5×10^7 spores ml^{-1}) and NP1.25 (1.25×10^7 spores ml^{-1}) (Table 2). This percentage increased to (100%) after 72 h in comparison with the two other fungal concentrations NP2.5 and NP1.25 (40 and 19.6%), respectively, after 72 h (Table 2). On the other hand, under the nematicide treatment (PC), the percentage of J2 that was immobile was found to be 85.09 after 72 h of application.

There were no significant ($P > 0.05$) differences between NP2.5 (2.5×10^7 spores ml^{-1}), and NP1.25 (1.25×10^7 spores ml^{-1}), after 24 and 48 h of application on the percentage immobility of J2 (Table 2).

Inhibitory effect of different concentration of *P. lilacinum* on the percentage egg hatching of *T. semipenetrans* under *in vitro* conditions

The inhibitory effects of different concentrations of the biocontrol agent *P. lilacinum* on the number of eggs hatching and the egg hatching rate of *T. semipenetrans* under *in vitro* conditions are presented in Tables 3 and 4. Results in Table 3 showed that there were no significant ($P > 0.05$) differences between the application of NEP5 (5×10^7 spores ml^{-1}) and the application of the nematicide (PC) after 24, 48, and 72 h on the numbers of hatched eggs (Table 3). The numbers of hatched eggs were reported to be (143.33 and 116.67), (100 and 110), and (66.67 and 65) in (5×10^7 spores ml^{-1}) (NP5) and nematicide treatment (PEC) after 24, 48, and 72, respectively (Table 3). These results were significantly ($P < 0.05$) lower than the negative control (NEC) treatment (Table 3). On the other hand, there were no

Table 4 The effect of different concentrations of the biocontrol agent *Purpureocillium lilacinum* (AUMC 10620) spores and the nematicide abamectin on the eggs hatching rate of *Tylenchulus semipenetrans* after 24, 48, and 72 h of application *in vitro*

Treatments	Eggs hatching rate after 24 h (%)	Eggs hatching rate after 48 h (%)	Eggs hatching rate after 72 h (%)
NEC: Negative control (nematodes eggs + distilled water)	40.33 *a*	41.00 *a*	48.00 *a*
PEC: Positive control (nematodes eggs + nematicide)	23.33 *d*	22.00 *c*	13.00 *d*
NEP5: Nematodes eggs + *P. lilacinum* (5×10^7 spores ml^{-1})	28.66 *bc*	20.00 *c*	13.33 *d*
NEP2.5: Nematodes eggs + *P. lilacinum* (2.5×10^7 spores ml^{-1})	30.00 *b*	28.00 *b*	27.00 *c*
NEP1.25: Nematodes eggs + *P. lilacinum* (1.25×10^7 spores ml^{-1})	41.00 *a*	39.00 *a*	37.00 *b*

Data were from three independent replicates. Values with the same letter within a column are not significantly ($P > 0.05$) different according to Duncan's multiple range test

significant ($P > 0.05$) differences between the application of NEP1.25 (1.25×10^7 spores ml^{-1}) and the control treatment (NEC) after 24 and 48 h, on the numbers of hatched eggs. In comparison, there was a significant ($P < 0.05$) difference between application of NEP1.25 (1.25×10^7 spores ml^{-1}) and the control treatment (NEC) after 72 h (Table 3).

Regarding the inhibitory effects of the biocontrol agent *P. lilacinum* on the eggs hatching rate of *T. semipenetrans* under *in vitro* conditions, only NEP5 (5×10^7 spores ml^{-1}) application followed the same trend in comparison with the application of the nematicide (PEC) (Table 4). Results in Table 4 showed that there were no significant ($P > 0.05$) differences between the application of NEP5 (5×10^7 spores ml^{-1}) and the application of the nematicide (PEC) after 48 and 72 h on the egg hatching rate (Table 4). On the other hand, the application of NEP1.25 (1.25×10^7 spores ml^{-1}) did not show any significant ($P > 0.05$) inhibitory effect on the egg hatching rate of *T. semipenetrans* under *in vitro* conditions after 24, 48, 72 h in comparison to the untreated negative control (NEC) (Table 4).

The inhibitory effects of different concentrations of the biocontrol agent *P. lilacinum* and the nematicide abamectin on the percent reduction in egg hatching of *T. semipenetrans* under *in vitro* conditions are presented in Fig. 1. The tested materials were ranked in descending order by PEC, NEP5, NEP2.5, and NEP1.25 in their effect on reducing egg hatching. After 24 h of exposure, all treatments reduced the nematode egg hatching rate by over 50%, with 76.67, 71.34, 70.00, and 59% for PEC, NEP5, and NEP2.5, and NEP1.25, respectively (Fig. 1). Moreover, these percentages increased after 48 and 72 h from the beginning of the experiment and recorded 87, 86.67, 73, and 63% after 72 h for the abovementioned treatments, respectively (Fig. 1). These data made it abundantly evident that the concentration of *P. lilacinum* at 5×10^7 spores ml^{-1} was the most efficient concentration utilized, as it inhibited the hatching of *T. semipenetrans* eggs in a manner that was comparable to that of the nematicide abamectin (Fig. 1).

The suppressive effect of *P. lilacinum* (5×10^7 spores ml^{-1}) on citrus nematode J2 and eggs was examined using light microscopy after 24, 48, and 72 h (Fig. 2). Abnormal morphological changes were reported in J2 and eggs after exposure to the *P. lilacinum* spores (Fig. 2).

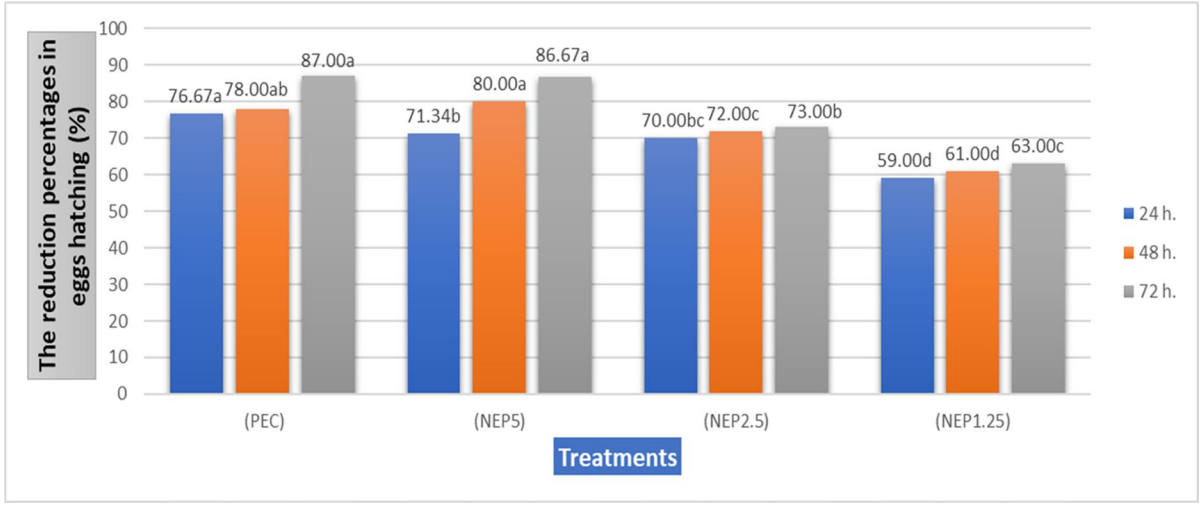

Fig. 1 The effect of different concentrations of the biocontrol agent *Purpureocillium lilacinum* (AUMC 10620) spores and the nematicide abamectin on the reduction percentages in eggs hatching of *Tylenchulus semipenetrans* after 24, 48, and 72 h of application under *in vitro* conditions. PEC = nematodes eggs + the nematicide abamectin, NEP5 = nematodes eggs + *P. lilacinum* (5×10^7 spores ml^{-1}), NEP2.5 = nematodes eggs + *P. lilacinum* (2.5×10^7 spores ml^{-1}) and NEP1.25 = nematodes eggs + *P. lilacinum* (1.25×10^7 spores ml^{-1}). The percentages above the column with the similar color followed by the same letter (s) are not significantly different at ($P \leq 0.05$) according to Duncan's multiple range test. Data were from three independent replicates

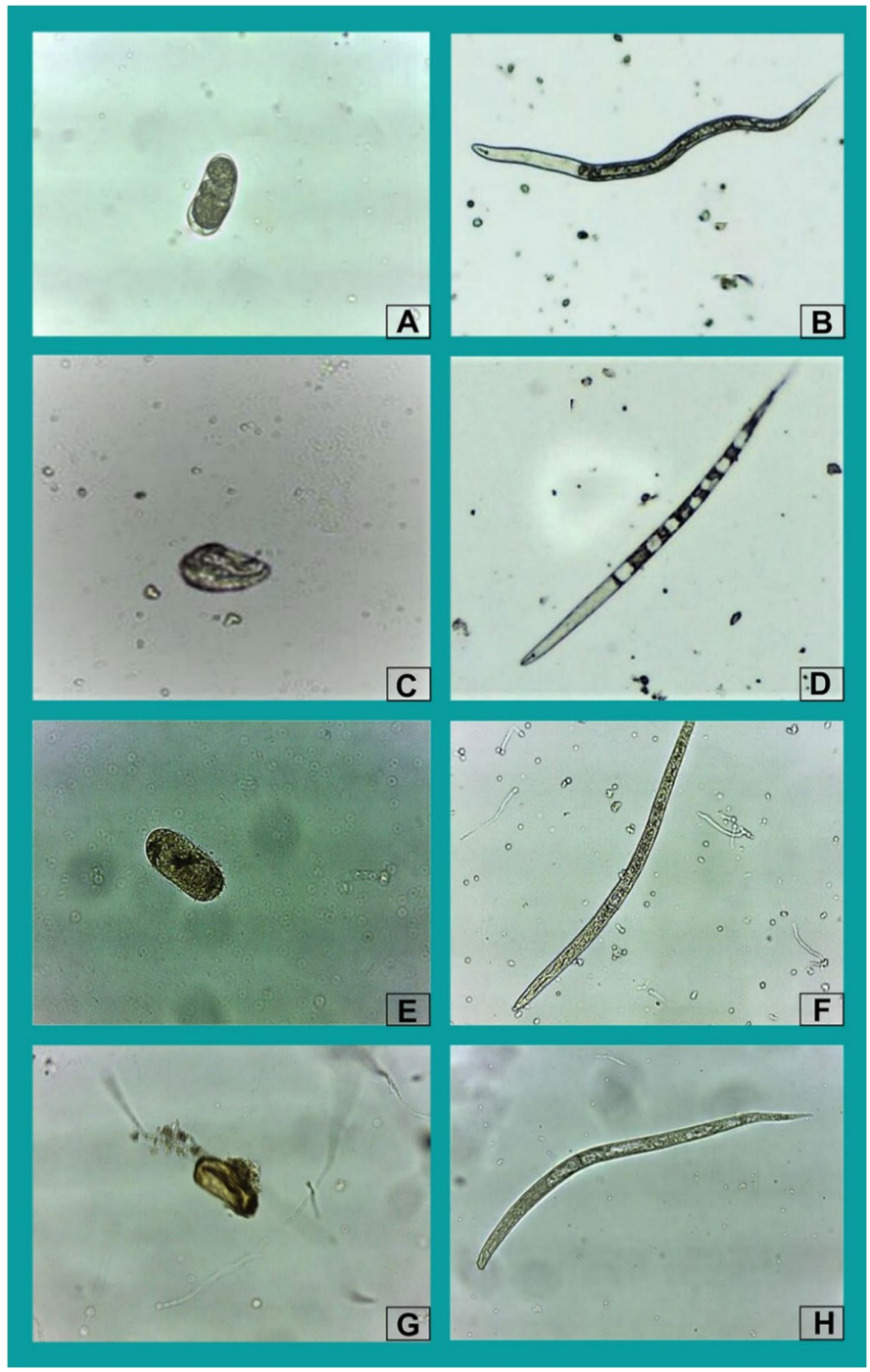

Fig. 2 The effect of *Purpureocillium lilacinum* (AUMC 10620) (5×10^7 spores ml^{-1}), on citrus nematode *Tylenchulus semipenetrans* eggs and J2 after 24, 48, and 72 h of exposure. **A**, egg in the control treatment; **B**, J2 in the control treatment; **C**, egg after 24 h; **D**, J2 after 24 h; **E**, egg after 48 h; **F**, J2 after 48 h; **G**, egg after 72 h; **H**, and J2 after 27 h

The effect of different *P. lilacinum* concentration on PPNs infesting citrus under field conditions

Four species of PPNs associated with citrus trees were surveyed in the experimental area described above. These species were *T. semipenetrans, Tylenchorhynchus* spp., *Helicotylenchus* spp., and *Pratylenchus* spp.

The effect of different concentrations of the biocontrol agent *P. lilacinum* (AUMC 10620) spores and

Table 5 The effect of different concentrations of the biocontrol agent *Purpureocillium lilacinum* (AUMC 10620) spores and the nematicide abamectin on the population of plant parasitic nematodes (PPNs) associated with the citrsus trees after one week of application under field conditions

Treatments	Number of nematodes in 250 g soil^{-1}			
	Tylenchulus semipenetrans	*Tylenchorhynchus* spp.	*Helicotylenchus* spp.	*Pratylenchus* spp.
Control	3719.00 *a* (0.00)	236.00 *a* (0.00)	108.00 *a* (0.00)	54.40 *a* (0.00)
Abamectin	2950.20 *d* (20.67)	165.00 *ed* (30.08)	81.60 *ed* (24.44)	40.00 *e* (26.47)
P. lilacinum (5×10^7 spores ml^{-1})	3010.00 *d* (19.06)	180.00 *d* (23.72)	87.40 *d* (19.07)	44.00 *d* (19.12)
P. lilacinum (2.5×10^7 spores ml^{-1})	3235.00 *c* (13.01)	196.00 *c* (16.95)	92.00 *cb* (14.81)	46.00 *c* (15.44)
P. lilacinum (1.25×10^7 spores ml^{-1})	3390.00 *b* (8.84)	225.00 *b* (4.66)	94. *4 b* (12.59)	49. 6 *b* (8.82)

Data were from five independent replicates.. Values with the same letter within a column are not significantly ($P>0.05$) different according to Duncan's multiple range test. Values in parentheses indicate the reduction percentages (%) according to equation 6

the nematicide abamectin on the population of PPNs associated with the citrus trees after, 1, 2 and 3 weeks of application under field conditions are presented in Tables 5, 6 and 7.

Under field conditions, there was no significant ($P>0.05$) difference between the nematicide abamectin and the biocontrol agent *P. lilacinum* at 5×10^7 spores ml^{-1} in reducing the population of *T. semipenetrans* one week after the application (Table 5). On the other hand, the application of *P. lilacinum* at 5×10^7, 2.5×10^7, and 1.25×10^7 spores ml^{-1} significantly ($P<0.05$) reduced the number of *T. semipenetrans* compared to the control treatment one week after the application (Table 5). The application of *P. lilacinum* at 5×10^7, 2.5×10^7, and 1.25×10^7 spores ml^{-1} significantly ($P<0.05$) reduced the number of *Tylenchorhynchus* spp., *Helicotylenchus* spp., and *Pratylenchus* spp., compared to the control treatment one week after the application (Table 5).

After one week of application, *P. lilacinum* at spore concentrations 1.25×10^7 spores ml^{-1}, 5×10^7 spores ml^{-1} and the nematicide abamectin reduced

Table 6 The effect of different concentrations of the biocontrol agent *Purpureocillium lilacinum* (AUMC 10620) spores and the nematicide abamectin on the population of plant parasitic nematodes (PPNs) associated with the citrus trees after two weeks of application under field conditions

Treatments	Number of nematodes in 250 g soil^{-1}			
	Tylenchulus semipenetrans	*Tylenchorhynchus* spp.	*Helicotylenchus* spp.	*Pratylenchus* spp.
Control	3725.00 *a* (0.00)	245.00 *a* (0.00)	111.00 *a* (0.00)	59.00 *a* (0.00)
Abamectin	2820.00 *ed* (24.29)	116.00 *e* (52.65)	44.00 *d* (60.36)	20.00 *ed* (66.10)
P. lilacinum (5×10^7 spores ml^{-1})	2880.00 *d* (22.68)	131.00 *d* (46.53)	53.00 *cb* (52.25)	23.80 *d* (59.66)
P. lilacinum (2.5×10^7 spores ml^{-1})	2935.00 *cb* (21.20)	147.00 *c* (40.00)	54.4 *c* (50.99)	26.00 *cb* (55.93)
P. lilacinum (1.25×10^7 spores ml^{-1})	2990.00 *b* (19.73)	179.00 *b* (26.93)	61.00 *b* (45.04)	27.60 *b* (53.22)

Data were from five independent replicates.. Values with the same letter within a column are not significantly ($P>0.05$) different according to Duncan's multiple range test. Values in parentheses indicate the reduction percentages (%) according to equation 6

Table 7 The effect of different concentrations of the biocontrol agent *Purpureocillium lilacinum* (AUMC 10620) spores and the nematicide abamectin on the population of plant parasitic nematodes (PPNs) associated with the citrus trees after three weeks of application under field conditions

Treatments	Number of nematodes in 250 g soil^{-1}			
	Tylenchulus semipenetrans	*Tylenchorhynchus* spp.	*Helicotylenchus* spp.	*Pratylenchus* spp.
Control	4733.00 *a* (0.00)	247.00 *a* (0.00)	116.00 *a* (0.00)	64.00 *a* (0.00)
Abamectin	931.00 *ed* (80.32)	56.00 *ed* (77.32)	24.00 *d* (79.31)	0.00 *d* (100.00)
P. lilacinum (5×10^7 spores ml^{-1})	1021.00 *d* (78.42)	68.00 *d* (72.46)	26.00 *d* (77.58)	0.00 *d* (100.00)
P. lilacinum (2.5×10^7 spores ml^{-1})	1702.00 *c* (64.03)	76.00 *c* (69.23)	32.00 *c* (72.41)	5.4 *c* (91.56)
P. lilacinum (1.25×10^7 spores ml^{-1})	1971.2 *b* (58.35)	109.00 *b* (55.87)	34.00 *b* (71.30)	10.00 *b* (84.37)

Data were from five independent replicates. Values with the same letter within a column are not significantly ($P>0.05$) different according to Duncan's multiple range test. Values in parentheses indicate the reduction percentages (%) according to equation 6

the population of *T. semipenetrans, Tylenchorhynchus* spp., *Helicotylenchus* spp., and *Pratylenchus* spp., by (8.84, 4.66, 12.59, 8.82%), (19.06, 23.72,19.07, 19.12%), and (20.67, 30.08, 24.44, and 26.47%), respectively (Table 5).

The suppressive effect of the biocontrol agent *P. lilacinum* increased with time. After two weeks, the number of active *T. semipenetrans* decreased from 3725 in 250 g soil^{-1} in the control treatment to 2820 in the abamectin treatment and 2880 in *P. lilacinum* (5×10^7 spores ml^{-1}) by reduction percentages of 24.09 and 22.68% for the treatments abamectin and *P. lilacinum* (5×10^7 spores ml^{-1}), respectively (Table 6). There were no significant ($P>0.05$) differences between the application of abamectin and *P. lilacinum* (5×10^7 spores ml^{-1}) after 2 weeks on the population of *T. semipenetrans* (Table 6).

The same trend was recorded in the other PPNs, which recorded 116, 44, and 20 in the abamectin and 131, 53, and 23.08 in *P. lilacinum* (5×10^7 spores ml^{-1}) in comparison with 245, 111, and 59 in the control treatment, for species *Tylenchorhynchus* spp., *Helicotylenchus* spp., and *Pratylenchus* spp., respectively (Table 6). After two weeks of application, a noticeable increase in the percentage reduction was recorded when *P. lilacinum* at spore concentrations 1.25×10^7 spores ml^{-1}, 5×10^7 spores ml$^{-1,}$ and the abamectin treatments were applied. These treatments reduced the population of *T. semipenetrans, Tylenchorhynchus* spp., *Helicotylenchus* spp., and *Pratylenchus* spp., by (19.73, 26.93, 45.04, and 53.22%), (22.68, 46.53, 52.25, and 59.66%), and (24.29, 52.65, 60.36, and 66.10%), respectively (Table 6).

The PPNs population experienced the greatest decline following three weeks of treatment, as seen in Table 7. The population of PPNs decreased from 4733, 247, 116, and 64 for *T. semipenetrans, Tylenchorhynchus* spp., *Helicotylenchus* spp., and *Pratylenchus* spp., to become 1021, 68, 26, 0.00, and 931, 56, 24, and 0.00, respectively, when *P. lilacinum* at 5×10^7 spores ml^{-1} and the abamectin treatment were used, respectively after three weeks of application, (Table 7). After three weeks of application, *P. lilacinum* at spore concentrations 1.25×10^7 spores ml^{-1}, 5×10^7 spores ml$^{-1,}$ and the nematicide abamectin reduced the population of *T. semipenetrans, Tylenchorhynchus* spp., *Helicotylenchus* spp., and *Pratylenchus* spp., by (58.35, 55.87, 71.30, 84.37%), (78.42, 72.46, 77.58, 100%), and (80.32, 77.32, 79.31, 100%), respectively (Table 7).

Since there was no significant ($P>0.05$) statistical differences between the highest fungal concentration (5×10^7 spores ml^{-1}) and the nematicide abamectin, these findings support the application of *P. lilacinum* at a concentration of (5×10^7 spores ml^{-1}) either as an individual treatment or possibly the integration of *P. lilacinum* at concentrations (1.25 and 2.5×10^7 spores ml^{-1}) with other biocontrol agents to control *T. semipenetrans* and other PPNs infesting citrus orchards.

Discussion

The biocontrol fungus *P. lilacinum* is a promising bionematicide that is recommended for organic farming to control plant-parasitic nematodes in economically important crops (Das et al., 2023). The current study demonstrated that the *P. lilacinum* strain (AUMC 10620) was highly effective in suppressing *T. semipenetrans* larvae in all three concentrations tested (1.25, 2.5, and 5×10^7 spores ml^{-1}) under *in vitro* and field conditions, with the highest concentration having the most significant impact. The effects were most noticeable in the reduction of egg hatching, which decreased with all three fungal concentrations. Furthermore, these concentrations were effective in reducing the number of major PPNs (*Tylenchorhynchus* spp., *T. semipenetrans, Helicotylenchus* spp., and *Pratylenchus* spp.) that infest citrus trees in the field.

Several studies confirmed our findings that *P. lilacinum* is fatal to adult females, eggs, and J2 citrus nematodes. For instance, when *P. lilacinum* was added to the soil around lemon (*Citrus jambhiri*) trees, J2 levels dropped by 65% and the number of mature females dropped by 76% (Walode et al., 2008). In a similar manner, the number of mature females of *T. semipenetrans* that infects sweet orange was reduced by this biocontrol agent application by 50% (Hanawi, 2016). Furthermore, many authors have reported that *P. lilacinum* is effective against other PPNs. For example, treating soil with *P. lilacinum* at 1×10^6 colony forming units (cfu) g $soil^{-1}$ before and after three days of eggplant transplantation reduced the root gall index by up to 72% and *Megalaima incognita* egg masses by 84% (Sarven et al., 2019). Hajji et al. (2017) also demonstrated that a *P. lilacinum* spore formulation reduced the number of *Globodera pallida* populations in soil by 73% and in the roots of a vulnerable potato variety (Spunta) by 76% compared to an untreated control (Hajji et al., 2017). Ten native *P. lilacinum* strains from Malaysia were tested against a commercial *P. lilacinum* strain for their ability to kill different stages of *M. incognita*. More than 90% of the *M. incognita* nematodes were confirmed to be infected ($P\leq0.01$). The parasitism on the eggs varied from 66 to 78.8% after 7 days of exposure to 10^5 spores ml^{-1}, and the egg-hatching inhibition reached 89% (Leong et al., 2021).

Sharma et al. (2021) discovered that a *P. lilacinum* formulation based on Karanja de-oiled cake and sundried biogas slurry outperformed a wheat-based formulation in controlling *M. incognita*. On the third day, their study revealed an egg mass inhibition of 96.8% and superior colonization ability (100% egg mass colonization). Moreover, *P. lilacinum* was successful in lowering the number of *M. incognita* on tomato (Siddiqui et al., 2000) and black pepper (*Piper nigrum* L.) (Leong et al., 2021). *P. lilacinum* was also highly effective in suppressing the growth of potato cyst-nematode, resulting in a 76% reduction in the roots and a 61% reduction in the soil (Hajji et al., 2017). Furthermore, *P. lilacinum* has been shown to reduce the population of root knots nematodes (*Meloidogyne javanica* and *M. incognita*) (He et al., 2020). All the above-mentioned examples supported the results obtained from the current study that *P. lilacinum* can be used as a bionematicide.

Field trials were carried out in our study to assess the efficacy of *P. lilacinum* against various nematode species. It is noteworthy that the control soil used in the current study was heavily infested with high numbers of different PPNs including *T. semipenetrans*, *Helicotylenchus* and *Pratylenchus*. Our study showed that after one, two, and three weeks of treatment, the application of *P. lilacinum*, particularly at a concentration of 5×10^7 spores ml^{-1} under field conditions, effectively reduced the major nematode species infesting citrus. There were no significant differences ($P\geq0.05$) between the application of *P. lilacinum* and the nematicide abamectin. When compared to the control treatment, the nematode population ($P\leq0.05$) dropped by 78.42, 64.03, and 58.35% after three weeks of the field application at doses of 5, 2.5, and 1.25×10^7 spores ml^{-1}, respectively. Our findings revealed that the *P. lilacinum* strain AUMC 10620 can be successfully exploited as an integral component of IPM techniques to combat nematodes that infest citrus. In addition, other safe and effective biocontrol agents may be also used in citrus orchards as protective treatments against soilborne plant pathogenic fungi and nematodes (Abd-Elgawad et al., 2010).

Similarly, field trials were conducted by Nagachandrabose et al. (2022) to assess the efficacy of a liquid formulation of *P. lilacinum* against the potato cyst nematode *Globodera rostochiensis* and *G. pallida*. The study reported that spraying the soil with 5 l of *P. lilacinum* reduced the reproduction rate, egg density, egg counts of cysts, and root penetration of potato cyst nematodes by 80.7-84.3%, 80.9-85%, 44.3-49.5%, and 62.0-64.4%, respectively (Nagachandrabose et al.,

2022). In our current study, the field trial revealed no significant difference between *P. lilacinum* (5×10^7 spores ml^{-1}) and abamectin nematicide in reducing the primary citrus nematode species at one, two, and three weeks after application under field conditions. However, Isaac et al. (2021) demonstrated the efficacy of *P. lilacinum* strain (AUMC 10149) (10 ml pot^{-1}) at a concentration of 1×10^8 cfu ml^{-1} in reducing the J2 of *M. incognita* on tomato plants by 97.6% and egg hatching by 79.8% after 72 h of exposure.

All the above-mentioned examples carried out under field conditions, supported the results obtained from the current study that *P. lilacinum* can be used as a bionematicide against citrus nematodes. This is because it is less expensive and has similar effectiveness as the nematicide abamectin in preventing PPNs infections in citrus trees. In Egypt, for example, abamectin costs 134$ per feddan to prevent PPN infestations, but Bio-Nematon *P. lilacinum* reduces this cost to 33$ per feddan (Abd-Elgawad, 2020). Moreover it is an environmentally safe product (Davies & Spiegel, 2011; Wilson & Jackson, 2013).

P. lilacinum has been used in conjunction with other biocontrol agents, organic amendment, and chemical control methods to increase its effectiveness against PPNs. For example, Bawa et al. (2020) used bio-formulations of *P. lilacinum* wettable powder (1×10^8 cfu g^{-1}), *P. lilacinum* liquid format (1×10^9 cfu ml^{-1}), *Trichoderma harzianum* wettable powder (2×10^6 cfu g^{-1}), *Trichoderma viride* wettable powder (2×10^6 cfu g^{-1}), combined with chemical control using Furadan 3% G against the eggs and J2 of *M. incognita*. They demonstrated that all formulations inhibited egg hatching of *M. incognita*, with *P. lilacinum* in liquid format achieving the highest egg hatch inhibition of 64%. Furthermore, soil application of *P. lilacinum* (cfu 2×10^6 g^{-1}) combined with organic amendment (neem cake) was found to be equally effective as the fluopyram pesticide in reducing the root-knot nematode population in long pepper (*Piper longum* L.)-cultivated soil (Divya, 2020). Also, when *T. viride*, *Pseudomonas fluorescens*, and *P. lilacinum* were used together, the disease complex caused by *Fusarium oxysporum* f. sp. *conglutinans* and *M. incognita* in cauliflower significantly decreased (Rajinikanth et al., 2013; Sankari Meena et al., 2019). In addition, Dahlin et al. (2019) studied the effectiveness of *P. lilacinum* strain 251 (BioAct WG) and fluopyram nematicides against the root-knot nematode *M. incognita* in tomato plants. Although the nematicide BioAct was able to reduce the nematode population throughout the growing season, the results showed that the nematicide fluopyram was able to reduce *M. incognita* more effectively at planting (Dahlin et al., 2019).

Similarly, Seenivasan et al. (2020) found that using neem seed kernel extract and *P. lilacinum* together through drip irrigation reduced citrus nematode *T. semipenetrans* infestations in acid lime trees much more than using either product alone or carbofuran 3G as a spot treatment. This method also improved root colonization, the chance of egg colonization, fruit yield, and the cost-benefit ratio (Seenivasan et al., 2020). Furthermore, El-Ashry et al. (2021) investigated the control of *M. incognita* on tomato plants using mixtures of *P. lilacinum*, abamectin, rhizobacteria, and botanicals. Using a combination of biocontrol agents and botanicals had a larger effect on *M. incognita* than either treatment alone. The combined approach increased plant growth metrics, decreased galls, and inhibited *M. incognita* reproduction. All of the bioagents and botanicals tested showed nematocidal activity (El-Ashry et al., 2021). Combinations with certain fungal species (*Trichoderma harzianum*, *Verticillium chlamydosporium*, and *P. lilacinum*), the bacterium *Pasteuria penetrans*, some organic amendments (cow manure, compost, and chicken manure), and urea 46% as a nitrogenous fertilizer were successful in lowering nematode levels on guava and fig trees in two field tests in Saudi Arabia (Dawabah et al., 2019).

In general, the use of *P. lilacinum* in conjunction with other biocontrol agents and chemical control approaches has the potential to increase its effectiveness in reducing the number of nematodes and stimulates plant growth. Future research should combine the Egyptian *P. lilacinum* strain (AUMC 10620) used in the present study with other combinations (e.g., biological and/or horticultural) to increase the efficacy of the biocontrol agent, as suggested by Abd-Elgawad et al. (2010).

The mechanism by which *P. lilacinum* inhibits nematodes has been the subject of extensive research. For instance, Khan et al. (2004) reported that the plant's ability to prevent the hatching of eggs of *M. javanica* was due to the secretion of serine proteases that modify the morphological characters of the eggshell. *P. lilacinum*, on the other hand, controls PPNs by colonizing nematode eggshells, the larval cuticle, or through direct hyphal penetration (Giné & Sorribas, 2017). *P. lilacinum* spores, according to Wang

et al. (2010), can penetrate the nematode cuticle by producing hydrolytic enzymes such as proteases and chitinases. *P. lilacinum* has been shown to be effective against *M. incognita* in both *in vitro* and field studies, by parasitizing eggs, preventing egg hatching, and increasing juvenile mortality (Singh et al., 2013).

Swarnakumari and Kalaiarasan (2017) described the *Meloidogyne* spp. egg infection mechanism by *P. lilacinum*. On the first day after inoculation, they noticed fungal hyphae attached to the egg surface. Appressorium formation initiated mycelial penetration on eggshells on the second day, and eggs were completely colonized by the fourth day. The egg contents was compressed, and development came to a halt at the gastrula stage (Swarnakumari & Kalaiarasan, 2017). In a similar study, Kumar and Arthurs (2021) investigated the effects of eight biocontrol agents, including *P. lilacinum*, and found that this fungus successfully inhibited nematodes related to orange plantations. The fungus secretes extracellular enzymes, specifically chitinases, collagenases, and serine proteases, that facilitate cuticle/eggshell penetration and host cell breakdown. *Meloidogyne* spp. egg surfaces were colonized within 24 h, with penetration via appressorium and mycelial colonization of egg contents occurring within four days (Kumar & Arthurs, 2021). Despite its ability to penetrate the cuticle, *P. lilacinum* can infect all sedentary stages of the nematode, though appressoria were only seen developing on eggs. The fungus is thought to use both mechanical pressure and enzymes to break through the nematode cuticle and eggshell (LaMondia & Timper, 2016). The production of proteases and chitinases by the fungus was linked to the infectious process as suggested by Xu et al. (2021).

Moreover, Sharma et al. (2021) demonstrated that the protease enzyme was a key pathogenic factor that contributes to the parasitic activity of *P. lilacinum* against nematode eggs. Other research has demonstrated that *P. lilacinum* exhibits phytotoxic properties through the production of the antibiotic leucinostatin (Wang et al., 2016). Furthermore, *P. lilacinum* produces extracellular enzymes such leucine arylamidase, esterase, acid phosphatase, and esterase-lipase (Elsherbiny et al., 2019; Giné & Sorribas, 2017). Additionally, *P. lilacinum* competes with nematodes for nutrients and space, limiting their population growth (Giné & Sorribas, 2017; Khan et al., 2004; Lan et al., 2017). In many occasions, *P. lilacinum* kills nematodes with toxins before infecting them. Acetic acid and leucinostatins have been identified as the principal toxic metabolites in *P. lilacinum* culture filtrates (LaMondia & Timper, 2016; Park et al., 2004). Another mechanism is the fungus's ability to induce plant defense mechanisms against nematodes. *P. lilacinum* releases elicitors that activate the systemic acquired resistance (SAR) pathway in plants, resulting in the production of PR proteins (Elsherbiny et al., 2019; Vega et al., 2008). The mechanism of action of our Egyptian *P. lilacinum* strain (AUMC 10620) used in this study can be attributed to any of the above-mentioned mechanisms. Future research into the mode of action of *P. lilacinum* (AUMC 10620) is required.

Based on the results of the present study, integrated pest management programs should include the use of *P. lilacinum* strain (AUMC 10620) to manage citrus-nematode interactions. It can diminish nematode infection *in vitro* and *in vivo*, making it a promising practical bioagent for controlling Egypt's citrus nematode *T. semipenetrans*. Our findings could be applied to sustainable agriculture and the environmentally friendly management of citrus nematodes.

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Author contribution A. El-M, A. E, K El-T, M. A. conceived and designed the research and supervised the study, A. El-M, A. E, K El-T, M. A., M. J., M. A. assisted with data evaluation, A. El-M, A. E, M.A. performed field experiments, A. El-M, A. E, M. A., M. J., M. A. analyzed the data, A. El-M, A. E, K El-T, wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Data Availability The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

Declarations

Conflict of interest The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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