Isolating a group of fungi from soil with the ability to control root-knot nematodes (*Meloidogyne* spp.) damage in vegetables

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ABSTRACT

Vegetable cultivation is essential to Vietnam's agricultural development strategy. *Meloidogyne* spp. has caused a root-knot disease which is dangerous due to decreased plant vitality, yield, and quality. This research aimed to select fungal strains controlling *Meloidogynes* spp., which causes root knots in vegetable plants. As a result, strains of the fungi *Paecilomyces sp.*, *Mariannaea* sp., and *Penicillium* sp. effectively inactivated second-stage juveniles (J2) after 72 h of inoculum. Specifically, *Paecilomyces sp.* was immobilized in 64.5% of the nematodes, *Mariannaea* sp. was 72%, and *Penicillium* sp. was 70%, compared to only 13.5% in the control group without fungal inoculation. In a net house experiment investigating the influence of fungal strains on J2 in Malabar spinach (*Basella alba* L.), the *Mariannae* sp. strain inoculated on plants at a density of 10^7 cfu/mL produced the best results in terms of root length (25.3 cm) and root weight (0.4 g) compared to the control uninoculation (18.5 cm; 0.3 g) and the control with only the nematode (11.2 cm; 0.2 g). Additionally, the *Mariannaea* sp. strain significantly reduced the number of juvenile nematodes per 50 g of soil (15.7 J2) compared to the control (160 J2) in Malabar spinach (*Basella alba* L.) in a net house.

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1. Introduction

Meloidogyne spp. is a root-knot nematode that causes serious damage to many crops in the tropical and subtropical; they have attached roots and reduced vegetable yields (Luc et al., 2005). Due to root-knot nematodes wide host range, controlling these nematodes can be challenging. Pesticides have been used to control nematodes; however, they are toxic to the environment and banned from use (Hutchinson et al., 1999; Nico et al., 2004). Many mechanisms use plant parasitic fungi to control root-knot nematodes. Sharon et al. (2007) reported reduced tomato root damage caused by *Meloidogyne javanica* if tomato pre-planting soil was treated with *Trichoderma harzianum*. Meyer (1999) studied the ability to exhaust egg hatching of *M. incognita* on watermelon of *Verticillium lecanii*. Kerry and Hidalgo-Diaz (2004) developed a management system using *Pochonia chlamydosporia* (*Verticillium chlamydosporium*) to control the root-knot nematode in organic vegetable production. Maehara & Futai (2000) studied the change in the nematode population of the pinewood when applying nine species of fungi isolated from pine trees, in which *Mariannaea elegans* strain was used, however, there was no significant difference in nematode population compared to the control treatment. In Korea, *Mariannaea elegans* was published in 2004 as the anamorph stage of *Cordyceps pruinosa*, an insect pathogenic fungus (Shin *et al.,* 2004). The *Paecilomyces lilacinus* was extensively tested for the biological control of plant parasitic nematodes (Khan et al., 2005). Martez et al. (1996) reported that *P. lilacinus* significantly reduced *M. incognita* in soil and root tomatoes, thereby increasing tomato yield. Sikandar et al. (2020) reported that second-stage juveniles of *M. incognita* increased with increasing fungal concentration and exposure time. The highest mortality of juveniles was recorded at 97.8% in 72 h, respectively, and the highest ovicidal activity was at 100% concentration with 5.2% egg hatching. The study also concluded that *P. chrysogenum* Snef 1216 can be a biocontrol agent against *M. incognita*.

Swe et al. (2011) grouped nematophagous fungi (NF) into group nematode-trapping fungi (NTF), which include: (1) using adhesive or mechanical traps fungi; (2) endoparasitic fungi; (3) egg-parasitic fungi; and (4) toxin-producing fungi (Liu et al., 2009). Nordbring-Hertz et al. (2000) grouped NF into three categories, including nematode-trapping fungi (NTF), endoparasitic, and toxic-compound-producing (NPF). Nematophagous fungi have over 200 species, which are fungi strains that can capture, kill, or parasitize nematodes and use nematodes as an alternative or supplementary source of nutrition. They were classified according to the method of attacking nematodes: nematode-trapping fungi using glue or mechanical traps; endoparasitic fungi using spores; and fungi that parasitize eggs or females with the tips of hyphae and produce toxins and fungal factors that immobilize nematodes before the invasion (Nordbring-Hertz el al., 2006; Hsueha et al., 2013). The egg-parasitic fungi are also one of the potential directions for effective nematode control. The researched strains, including *Paecilomyces* sp., *Pochonia chlamydosporia*, and *Trichoderma viride,* were particularly effective against the root-knot nematodes *Meloidogyne* sp. and *Heterodera* spp. (Moreno-Gavíra et al., 2020; Poveda et al., 2020). Cobb (1917) suggested that NF could be a biological control agent in plant-parasitic nematodes. Linford et al. (1937) used NF for the biological control of nematodes. Currently, both chemical and biological products

are used to control crop-damaging nematodes but are not effective in killing nematodes and cause long-term toxicity to the environment. Meanwhile, biological products used alone, such as Abamectin, Tervigo 20SC, *Bacillus thuringiensis,* and *Trichoderma* supplements, are not as effective in killing nematodes as expected. Therefore, the research aimed to select native nematode-trapping fungi strains that can control root-knot nematodes on vegetable crops.

2. Materials and Methods

2.1. Materials

A total of 35 soil and root of vegetable samples (500 g/sample) were collected in the presence of root-knot nematodes at a depth of about 10 - 20 cm around the root zone of vegetable crops such as carrots, amaranth, spinach, tomatoes, and peppers in Lam Dong province (Da Lat), Ho Chi Minh City (Cu Chi), and Dong Nai (Cam My) for isolating fungi.

Meloidogyne spp. was collected in soil and root Malabar spinach, 10 - 25 cm deep, in Cam My district, Dong Nai province, and identified by morphology according to VS (2020) and Perry et al. (2009). The PDA medium (Himedia) (Potato 200 g, Dextrose 20 g, Agar 20 g), additional 0.1 g/L streptomycin sulfate, and 0.1 g/L penicillin were isolated nematode-trapping fungi.

2.2. Method isolation nematode-trapping fungi

Isolation of the nematode-trapping fungi was performed according to the modified method of Berhanu et al. (2022). Nematodes were collected from soil and roots using a Baermann funnel (Giuma & Cooke, 1971). Weigh 100 g of soil, then put it on a Baermann funnel (mesh diameter of 2 mm) lined with two layers of filter paper. Add sterile water until the water level touches the soil sample. After 24 to 48 h, the nematode suspension was centrifuged at 1.000 rpm for 3 min. The supernatant was then removed, and the pellet was resuspended in 3 mL of distilled water and incubated at room temperature (27°C). After four days, the nematodes were transferred to a 10 mL falcon tube containing 5 mL of sterile distilled water and concentrated (do this step twice). The remaining nematode residue was then transferred to PDA plates (Himedia) supplemented with 0.05 g/L of streptomycin sulfate and 0.05 g/L of penicillin to inhibit bacteria. After 2 - 7 days, the mycelia were checked for fungus growth and purified on PDA. The isolated sample was stored at 4°C in a PDA with glycerin at 15% in the test tube medium.

2.3. Preparation of nematodes and egg material

Nematodes are filtered using the improved Bearman static filtration method. Weigh 100 g of soil and place it on a Baermann sieve (a mesh diameter of 2 mm) lined with two layers of filter paper. Add sterile water until the water level touches the soil sample. The nematodes will move through the filter paper and fall into the tray. Filter for 24 - 48 hours at 28 - 30°C, then examine nematodes under a 10X - 40X stereo microscope.

Nematodes were cultured in an agar medium (1%) after 7 - 10 days, observed under a stereo microscope at 10X magnification, and localized on the agar block with eggs. The agar block containing nematode eggs was cut into a new agar plate (remove the agar block and keep nematode eggs). Besides, root samples with galls suspected to have gall nematodes inside the roots were separated directly on an Olympus stereo microscope to collect adult females and eggs, then cultured on an agar medium and stored in the pots of grown Malabar spinach (Southey, 1986).

2.4. Affected by nematode-trapping fungi to parasitize eggs with *Meloidogyne* spp.

Aspirate 500 µL of fungal spore solution (2 x 10^7 cfu/mL) was cultured in PD (Potato 200 g, dextrose 20 g, water 1000 mL) medium for 5 - 10 days, and then added 1 mL gently spread on a petri dish with 30 nematode eggs. The eggs were placed on 1% water agar plates containing 1% ampicillin (Liang et al., 2020). The experiment was repeated three times for one type of fungus, corresponding to three petri dishes. The control was inoculated with sterile distilled water at 28°C. After 1 - 7 days, the petries were observed under a microscope, stained with methylene blue, and recorded as the percentage of infected eggs until the eggs hatched into juveniles.

2.5. The efficacy of nematode-trapping fungi on second stage junvenlies (J2) (*Meloidogyne* spp.)

The test was in sterile Petri dishes containing 20 µL each of fungal spore suspension at a concentration of 2 x 10^7 spores/mL and 500 µL of nematode suspension containing 50 J2 *Meloidogyne* spp. (Singh & Mathur, 2010). Each treatment was replicated 4 times. The control was sterile distilled water. The effect of fungal spores on nematode activity was recorded after 24 and 72 h. Immobile J2 were transferred to sterile distilled water to check their potential for revival by blue LED light (450 - 490 nm). After 120 sec, nematodes that stopped moving when exposed to blue LED light were considered dead (Rajasekharan et al., 2018). Besides, distinguishing between live and dead J2 was the method by Xiang & Lawrence (2016); nematodes with immobile movement within 2 min exposed to NaOH 1N were considered dead.

2.6. Application of nematode-trapping fungi to *Meloidogyne* spp. on Malabar spinach in a net house

This experiment was performed according to the modified method of Messa et al. (2020), and Naz et al. (2020). Malabar spinach plants were grown in pots measuring 15 - 20 cm, placed 60 cm above the ground, watered daily, and fertilized with NPK (20 - 20 - 15) two days before inoculating nematodes *Meloidogyne* spp. At that time, it had two leaves and was three days older. The inoculum density was adjusted to 1000 eggs and juveniles per 100 cm^3 of soil and incorporated into the soil. The experiment was designed as a completely randomized single factor in a net house. Fungi were cultured in Potato Dextrose Broth medium on a shaker for 5 - 7 days, adjusted spore density to 2 x 10^7 cfu/mL, with the first fungal inoculation occurring ten days after nematode inoculation. Each treatment was replicated 10 times, corresponding to 10 Malarba spinach plants. The treatments were as follows: T0: uninoculated; T1: *Meloidogyne* spp.; T2: *Paecilomyces*. sp.; T3: *Mariannaea* sp.; T4: *Penicillium* sp.; T5: *Meloidogyne* spp. + *Paecilomyces* sp. T6: *Meloidogyne* spp. + *Mariannaea* sp.; T7: *Meloidogyne* spp. + *Penicillium* sp. Measure root length and mass, count the number of galls/root, J2/5g of roots, egg masses/root, and J2/50 g of soil after 40 days of nematode inoculation.

2.7. Data analysis

Data were subjected to analysis of variance (ANOVA) using the SPSS v.22 software and Ducan's test.

3. Results and Discussion

3.1. Isolation nematode-trapping fungi

A total of 12 fungal samples with varying morphology and color colonies were isolated from 35 soil samples collected in vegetable-growing areas in Da Lat (Lam Dong), Cam My (Dong Nai), and Cu Chi (Ho Chi Minh City) and divided into four groups based on morphological characteristics. Group 1 was identified by green fungal colonies, oval spores, and septated spore branches, which occurred in 25% of the samples. Group 2 was determined by purple colonies with elliptical spores growing in chains, flask-shaped, and swollen at the bottom; they grow singly or in clusters of 2 - 4, accounting for 33.3%. Group 3 consisted of purple-red colonies on PDA medium with elliptical to diamond-shaped spores, which grew in phialides chains that were similar vase-shaped and in clusters of 3 - 6 (16.7%). Group 4 were gray or brown colonies on PDA medium, spherical-shaped spores growing in chains, flask-shaped, bulging at the bottom, and gradually getting smaller towards the top (25%).

On PDA, colonies grew slowly, 55 - 60 mm in diameter, after 28 days at 25°C. The colony was round with serrated edges; the mycelium raised above the agar surface, and many dark brown drops appeared. The front of the petri colonies was light orange to brown, and the back was light yellow to orange-brown (Figure 1). The mycelium grows straight, transparent, and without septa. The flasks grow in clusters of 3 - 5 on hyphae and are transparent. The vessel was vase-shaped, bulging at the bottom and gradually getting smaller towards the top, forming a neck shape measuring 4.3 - 15.0 x 2.3 - 2.6 µM. Spores were spherical or subspherical, transparent, and 2.3 - 3.5 x 2.0 - 3.0 µM in size. The morphological characteristics were consistent with those of *Penicillium* sp. (Visagie et al., 2014).

Colonies of fungi in group 2 grew quickly, reaching 75 - 80 mm after 21 days at 25°C. The colony was round with many concentric rings; the color of the colonies changed from white to purple-pink to purple when producing spores, and the mycelium in the center and edges was white. The font of the colony was smooth and covered with a layer of powder. The back was light gray with concentric rings (Figure 2). The mycelium was straight, branched, transparent, and had many septa. The flask grows in clusters of 2 - 4 along the mycelium. The vessels are vase-shaped, short, bulging in the middle, gradually getting smaller towards the tip, measuring 4.1 - 6.7 x 1.6 - 2.2 µM. Spores are spherical, sometimes slightly pointed at both ends and grow in long chains. The chains are often linked to form large, measuring 2.3 - 3.2 x 1.7 - 2.7 µM. The morphological characteristics are consistent with the morphological characteristics of *Paecilomyces lilacinus* (Luangsa-Ard et al., 2011).

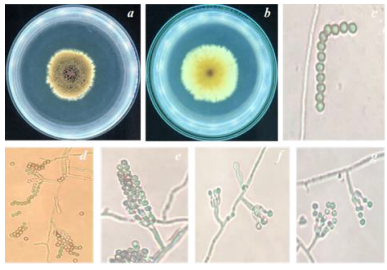

Figure 1. Morphology of *Penicillium* sp. (Group 1). a: front colony on potato dextrose agar (PDA) medium; b: reverse colony on PDA medium; **c**: conidia; d - g: conidia and phialides. Scale bar = 10 µM.

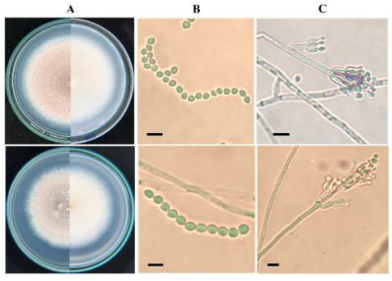

Figure 2. Morphology of *Paecilomyces* sp. (Group 2). A: Colony on potato dextrose agar (PDA) medium, B: Conidia, C: Polyphialides in the aerial mycelium. Scale bar = 10 µM.

The colonies of fungi in group 3 grew quite quickly and reached a diameter of 75 - 80 mm after 14 days at 25°C. The colony was round, divided into many patches with concentric rings; the front of the colony was white; the back surface was dark purple, fading towards the edge; and the mycelium grew close to the agar surface (Figure 3). The hyphae were irregular, complex, and branched, ranging in width from 3 to 7 µM. The flask-shaped body grows from fungal hyphae in clusters of 3 - 6, with dimensions of 9.1 - 25.0 x 1.9 - 3.2 µM. Spores were transparent, elliptical to oblong, swollen in the middle, growing in chains, measuring 4.1 - 8.9 x 2.2 - 3.5 µM. The morphological characteristics were consistent with those of *Mariannaea* sp. (Samson, 1974).

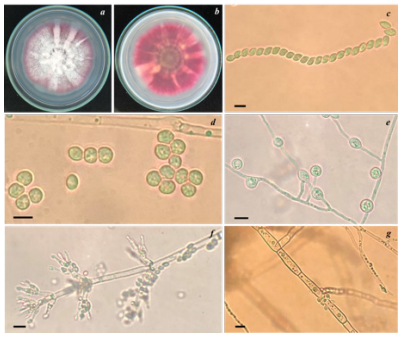

Figure 3. Morphology of *Mariannaea* sp. (group 3). a: Front colony on potato dextrose agar (PDA) medium; b: Reverse colony on PDA medium, c: Conidia, d - e: chlamydospore; f: Polyphialides in the aerial mycelium; g: Mycelium. Scale bar = 10 µM.

In group 4, the colony was round with serrated edges; the upper surface colony was pink with a white line; and the lower surface was yellow-brown. It grew at an average daily rate of 2.19 ± 0.81 mm and reached a diameter of 39 mm after 14 days on PDA (Figure 4). The mycelium was transparent and had no septa. The vessels were 5.32 - 7.64 x 2.03 - 2.72 µM and grew at the top of the mycelium with a dense fan-shaped cluster. Spores were 2.60 - 3.01 x 2.49 - 2.86 µM in size (average 2.80 ± 0.30 x 2.68 ± 0.28 µM, n = 30), spherical. The morphological characteristics were consistent with those of *Aspergillus* sp. (Raper & Fennell, 1965).

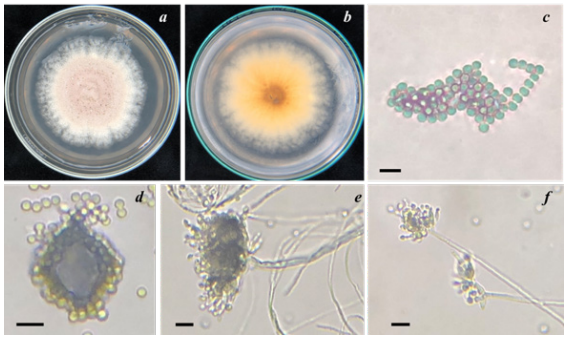

Figure 4. Morphology of *Aspergillus* sp. (group 4); Front colony on potato dextrose agar (PDA) medium; b: Reverse colony on PDA medium, c-d: conidia; e - f: Phialides. Scale bar = 10 µM.

3.2. Parasitism of *Meloidogyne* spp. eggs by nematode-trapping fungi

Table 1. Effect of fungi isolaties on *Meloidogyne* spp. eggs

Treatment	Fugal group	Nematode eggs not hatched after fungal inoculation (%)			
		1 day	3 days	5 days	7 days
LD-CR-7.01	*Mariannaea* sp.	3.00^{ef}	4.33^{cd}	5.67^{f}	9.67^{f}
DN-MT-35.01	*Penicillium* sp.	1.67^{f}	2.67^{d}	3.33^{fg}	5.00^{g}
LD-CC-15.01	*Paecilomyces* sp.	39.33^{a}	53.67^{a}	73.33^{a}	90.67^{a}
LD-OT-11.01	*Mariannaea* sp.	4.33^{e}	7.00^{c}	16.67^{e}	18.33^{e}
CC-CC-8.02	*Paecilomyces* sp.	25.33^{d}	36.67^{b}	49.00^{d}	80.33^{b}
CC-OT-20.01	*Paecilomyces* sp.	29.00^{c}	4.67^{cd}	53.00^{c}	68.33^{d}
LD-RD-6.01	*Penicillium* sp.	0.00^{g}	0.00^{e}	0.00^{g}	0.00^{h}
DN-DL-3.01	*Aspergillus* sp.	0.00^{g}	0.00^{e}	0.00^{g}	0.00^{h}
DN-CC-3.02	*Aspergillus* sp.	0.00^{g}	0.00^{e}	0.00^{g}	0.00^{h}
DN-RC-30.01	*Paecilomyces* sp.	34.33^{b}	50.67^{ab}	68.33^{b}	75.00^{c}
LD-CR-1.01	*Aspergillus* sp.	0.00^{g}	0.00^{e}	0.00^{g}	0.00^{h}
CC-OT-6.03	*Penicillium* sp.	0.00^{g}	0.00^{e}	0.00^{g}	0.00^{h}
Control		0.00^{g}	0.00^{e}	0.00^{g}	0.00^{h}

$^{a-h}$Different letters indicate significant differences within a column at $P < 0.05$.

Four strains of *Paecilomyces* sp. controlled the hatching of nematode eggs, with the percentage of unhatched eggs ranging from 68.33 to 90.67%, of which sample LD-CC-15.01 showed the percentage of eggs not hatching (Table 1), with the highest rate of eggs not hatching being 90.67%. *Mariannaea* sp. (LD-CR-7.01) could control egg hatching, but the rate was low compared to *P. lilacinus*. *Aspergillus* sp. was not able to control nematode egg hatching. According to Hanawi (2016), species in the genus *Paecilomyces*, especially the *P. lilacinus,* were to be able to parasitize *Tylenchulus semipenetrans* eggs. Moreno-Gavíra et al. (2020) and Poveda et al. (2020) recorded that *Paecilomyces* could penetrate eggshells and structural components of juveniles and adult stages in different nematode species through the germination of spores, the branching of mycelium, and the formation of attachments, based on the ability to secrete extracellular enzymes due to destroy the eggshell structure, thereby decreasing the rate of eggs hatching into juveniles. The mycelium was contacted by the host; it penetrated the host through appressorium or secreted enzymes such as cellulase, glucanase, laccase, leucinoxin, lipase, pectinase, protease, chitinase, or xylanase to dissolve the protective layer and protect the outside of the host during infection. Besides, *Paecilomyces* spp. had an active mechanism that controlled the nervous system of nematodes, especially *Meloidogyne* spp., and several other nematode genera such as *Globodera, Rotylenchulus, Heterodera, Xiphinema*, and *Pratylenchus* (Favre-Bonvin et al., 1991). *Paecilomyces lilacinus* decreased *M. incognita* populations in soil and roots and increased tomato yield (Lara et al., 1996). *Paecilomyces lilacinus* has also been reported to infect female nematodes (*Meloidogyne* spp.) and cysts of *Heterodera* spp., and *Globodera* spp. (Jatala, 1986).

3.3. Effect of nematode-trapping fungi on the mobility of J2 *Meloidogyne* spp.

Table 2. Effect of spore suspensions of fungal strains on the mobility of J2 *Meloidogyne* spp.

Treatment	Fugal group	Nematodes (J2) were immobilized at different times (individual)		Percentage of nematodes (J2) immobilized after 72 h (%)
		24 h	72 h	
LD-CR-7.01	*Mariannaea* sp.	$22.3^{bc} \pm 2.2$	$36.0^{ab} \pm 2.9$	72.0
DN-MT-35.01	*Penicillium* sp.	$31.8^{a} \pm 1.3$	$35.0^{ab} \pm 0.82$	70.0
LD-CC-15.01	*Paecilomyces* sp.	$18.3^{cdef} \pm 2.9$	$32.3^{abc} \pm 3.6$	64.6
LD-OT-11.01	*Mariannaea* sp.	$16.0^{fg} \pm 1.15$	$27.3^{cdefg} \pm 1.3$	54.6
CC-CC-8.02	*Paecilomyces* sp.	$20.8^{cd} \pm 1.3$	$28.5^{cdef} \pm 2.1$	57.0
CC-OT-20.01	*Paecilomyces* sp.	$12.0^{hijkl} \pm 1.4$	$30.0^{bcd} \pm 2.8$	60.0
LD-RD-6.01	*Penicillium* sp.	$16.5^{defghi} \pm 2.4$	$26.5^{cdefgh} \pm 3.7$	53.0
DN-DL-3.01	*Aspergillus* sp.	$13.3^{ghijk} \pm 1.5$	$18.0^{jklmn} \pm 1.4$	36.0
DN-CC-3.02	*Aspergillus* sp.	$9.8^{klmn} \pm 2.2$	$18.5^{ijklmn} \pm 2.4$	37.0
DN-RC-30.01	*Paecilomyces* sp.	$18.3^{cdef} \pm 0.5$	$24.3^{defghi} \pm 1.0$	48.6
LD-CR-1.01	*Aspergillus* sp.	$7.0^{mno} \pm 1.6$	$15.3^{lmn} \pm 1.5$	30.6
CC-OT-6.03	*Penicillium* sp.	$4.8^{o} \pm 1.0$	$13.3^{no} \pm 2.8$	26.6
Control		$3.8^{o} \pm 0.5$	$6.8^{p} \pm 1.3$	13.6

$^{a-o}$Different letters indicate significant differences within a column at $P < 0.05$.

The effects of fungal strains on immobile J2 were significantly different ($P < 0.05$) compared to the control treatment after 72 h (Table 2). The results showed that three fungal strains caused the highest inactivation of J2 *Meloidogyne* spp. after 72 h, including *Mariannaea* sp. (LD-CR-7.01) 72%, *Penicillium* sp. (DN-MT-35.01) 70%, and *Paecilomyces* sp. (LD-CC-15.01) 64.6% compared to the control (13.6%). Zarrin et al. (2015) noted that among 13 tested fungi, both *Penicillium* sp. and *Paecilomyces* sp. strains significantly reduced the live larvae of *Trichostrongylidae* after seven days of incubation ($P < 0.01$). The number of larvae (%) was inoculated with the *Penicillium* sp. reduced by 95% and inoculated with the *Paecilomyces* sp. reduced by 91.5% compared to the uninoculated control. The immobile nematodes observed under a microscope (Olympus CX31 RFS) at 20X-40X showed the infection of fungal hyphae into the nematode body (Figure 5) as well as the formation of a sticky trap structure (Figure 6). Studies indicated that *Penicillium* sp. effectively controlled root-knot nematodes such as *P. chrysogenum* (snef1216). Snef1216 could be used as a biological control agent against *M. incognita*, with J2 mortality up to 97.8% after 72 h at 100% concentration (Sikandar et al., 2020). Snef 1216 was also introduced as a biomass enhancer and has potential as a biocontrol agent against *M. incognita* in cucumbers by significantly reducing the density and growth of nematode, reducing fertility rate, interfering with gall formation, and improving seed germination (Sikandar et al., 2019). A compound produced from *P. commune* KACC 45973 was reported for the first time to kill second-stage larvae of *M. incognita, M. hapla*, and *M. arearia*. It also significantly inhibited egg

hatching of *M. incognita* and *M. hapla* after 28 days of treatment with concentrations > 25 µg/mL (Nguyen et al., 2021). *Paecilomyces* spp. was immobilized in J2 of *Meloidogyne* spp. from 28.5% to 60%. Dávila & Hío (2005) noted that *Paecilomyces* sp. can parasitize > 50% of *M. javanica* populations at 72 h under controlled conditions. The mechanism of action of the nematophagus may be that the spores adhere and adhere to the epidermis, mouth, excretory pore, anus, or sensory organs before germination and infection of the nematode. After the production of germ tubes, the nematode cuticle is penetrated and the growth of hyphae occurs, which remain alive until the hyphae reach the vital organs (Devi, 2018). We recorded the image of hyphae growing out of the second-stage juveniles *Meloidogyne* sp. clearly in Figure 5a. In recent years, biological control of nematodes has received attention from researchers in many countries (Larsen, 2002). Current research results demonstrate that some environmentally dispersed saprophytic fungi can effectively reduce the number of second-stage juveniles *Meloidogyne* sp.

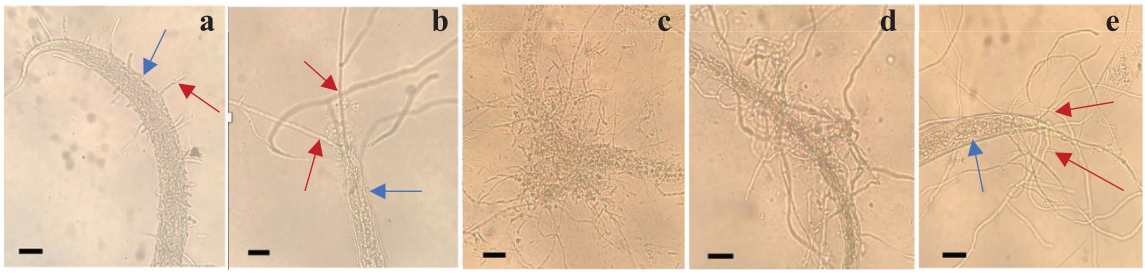

Figure 5. The hypae infecting J2 *Meloidogyne* spp. a, b, c, d: Strains belonging to *Paecilomyces* sp., e: Strains belonging to *Penicillium* sp. Blue arrow: second stage juveniles, red arrow: hypae. Scale bar = 20 µM.

Pramer & Stoll (1959) reported that nematophagous fungi use specialized trapping devices to attack nematodes; they do not produce traps constitutively but rather initiate trap formation in response to their prey. In this study, we noted the characteristics of trap plugs formed in the presence of nematodes; under culture conditions on a PDA medium, the fungus did not form trap structures. Tunlid et al. (1992) reported that sticky traps were triggered by various biotic and abiotic factors, of which direct contact between mycelium and living nematodes was the most important key for nematode-trapping fungi. Nematodes were attracted to fungal traps, and once they touched the trap, they became stuck to the sticky trap. The nematode was paralyzed before the mycelium developed a structure that penetrated the cuticle, allowing the fungus to grow inside the body and eventually completely digest it (Lopez-Llorca et al., 2008). This entire process could take less than 24 h for many nematode-trapping fungi. Li et al. (2000) reported that more than 200 species of fungi belonging to Basidiomycota, Ascomycota, and Zygomycota use sticky trap structures to trap nematodes in soil, indicating that sticky traps are a common type of trap among filamentous fungi. Olivares and Lopez-Llorca (2002) pointed out that the ability to adapt to a variety of environments and the production of extracellular enzymes are two characteristics that make filamentous fungi suitable for use as biocontrol agents.

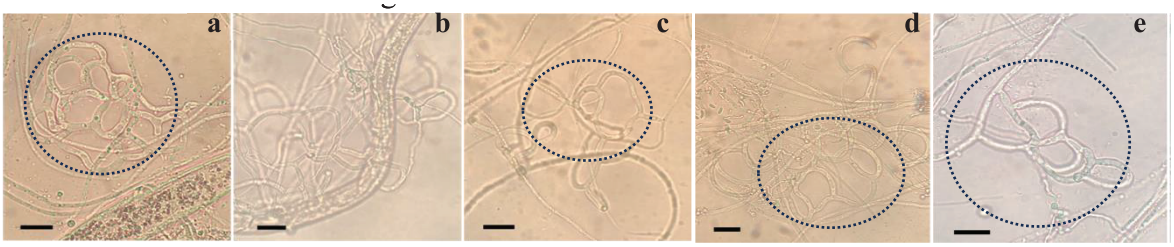

Figure 6. An adhesive network trap structure of five fungi strain. a, b, c, d: *Paecilomyces* sp., e: *Penicillium* sp. Scale bar = 20 µM.

3.4. Application of nematode-trapping fungi to *Meloidogyne* spp. on Malabar spinach in a nethouse

Table 3. Effect of applying nematode-trapping fungi on Malabar spinach in a pot trial with *Meloidogyne* spp.

Treatment	Root length (cm) (± SE)	Root weight (g) (± SE)	Galls/root (± SE) (gall)	Egg masses/ root (egg)	Juveniles J2/50 g soil (± SE)
T0 - control (uninocutaed nematodes)	$18.5^{c} \pm 1.0$	$0.3^{c} \pm 0.15$	0^{e}	0	0^{d}
T1 - control (inoculated nematodes)	$11.2^{e} \pm 1.4$	$0.2^{d} \pm 0$	$6.7^{a} \pm 2.5$	72	$160.0^{a} \pm 9.0$
Paecilomyces sp.	$18.2^{c} \pm 1.6$	$0.3^{c} \pm 0.05$	0^{e}	0	0^{d}
Mariannaea sp.	$20.5^{b} \pm 1.4$	$0.3^{c} \pm 0.1$	0^{e}	0	0^{d}
Penicillium sp.	$26.5^{a} \pm 3.9$	$0.5^{a} \pm 0.1$	0^{e}	0	0^{d}
Meloidogyne spp. + *Paecilomyces sp.*	$14.7^{d} \pm 0.8$	$0.2^{d} \pm 0.05$	$0.3^{d} \pm 0.5$	0	$13.7^{c} \pm 1.5$
Meloidogyne spp. + *Mariannaea* sp.	$25.3^{c} \pm 0.7$	$0.4^{b} \pm 0.05$	$1.7^{c} \pm 0.5$	2	$15.7^{c} \pm 2.5$
Meloidogyne spp. + *Penicillium* sp.	$25.8^{bc} \pm 1.9$	$0.3^{c} \pm 0.05$	$2.0^{b} \pm 1.0$	3	$55.0^{b} \pm 3.0$

$^{a-d}$Different letters indicate significant differences within a column at $P < 0.05$; Inoculated with 1000 Meloidogyne spp. eggs and juveniles per 100 cm^3 soil.

The fungi in the study were isolated from the rhizosphere soil, so it will be monitored for up to 40 days to evaluate the effects of the fungi on plant growth. Table 3 and Figure 7 showed that the root length in the treatments had significant differences after 40 days of injection. The *Penicillium* sp. treatment resulted in longer root lengths than the uninoculated control (26.5 cm compared with 18.5 cm) and the nematode-inoculated control (11.2 cm) on Malabar spinach. Recently, some reports have demonstrated the application of *Penicillium* as a PGPF in crops such as soybean (Bilal et al., 2019), wheat (Elgharably & Nafady, 2021), and corn (Zhao et al., 2021). In addition, several studies have shown the effects of *Penicillium* on plant growth and increased tolerance to salt stress in various plant species. Jin et al. (2022) reported that the phosphate solubilizing strain *P. funiculosum* P1 improved the development of quinoa plants

under salt-stress conditions, with an increase in the antioxidant system by producing organic acids. Miao et al. (2019) showed that *Penicillium brefeldianumon* isolated from melons effectively against *M. incognita*. Gouli et al. (2013) multiplied the biomass of entomopathogenic fungi in the genera *Beauveria, Metarhizium, Mariannaea*, and *Tilachlidium*, which commonly exist in nature as hemisaprophytes in soil and controlling entomopathogenic. In Korea, *Mariannaea elegans* was first published in 2004 as the anamorph stage of *Cordyceps pruinosa*, an insect pathogenic fungus (Shin et al., 2004). Maehara & Futai (2000) studied the change in the nematode population of the pinewood when applying nine species of fungi isolated from pine trees, in which *Mariannaea elegans* strain was tested, however, there was no significant difference in nematode population in the *M. elegans* inoculated treatment compared to the control treatment. Only a few published reports on the control of nematodes by *M. elegans*.

Additionally, the results reported the number of galls/root and the number of juveniles/50 g of soil were both lower than the control, especially the *Paecilomyces* sp. strain treatment, which had the best nematode control effect with 0.3 galls/root compared to the control of 6.7 galls/root. The number of juveniles/50 g of soil was 13.7 J2 compared to the control of 160 J2. Combined *P. lilacinus* and *Monacrosporium lysipagum* reduced 62% of galls and 94% of *M. javanica* juveniles on tomatoes compared to the experiment with no fungi added. Sixty-five percent of *H. avenae* cysts were reduced on barley by combined application of fungi (Khan et al., 2006). As a result, all three native fungal strains (*Penicillium* sp., *Paecilomyces* sp., and *Mariannaea* sp.) affected root and controlled nematode growth on Malabar spinach in the nethouse.

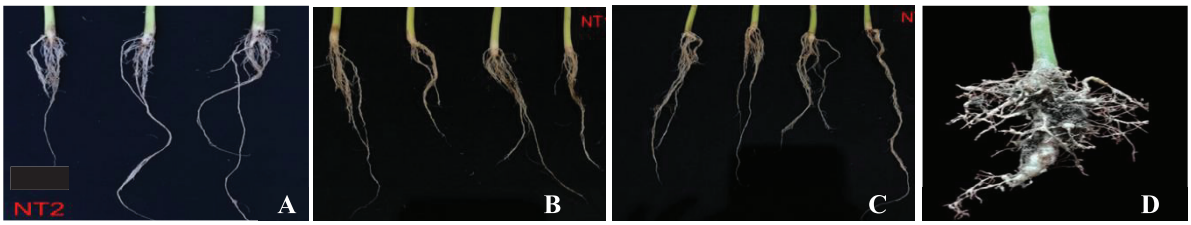

Figure 7. Root length of Malabar spinach at 40 days inoculated nematodes and at 30 days innocuted fungi. A. T0 - control (uninocutaed nematodes), B. T1 - control (inoculated nematodes), C. *Meloidogyne* spp. + *Mariannaea* sp. D. Galls on Malaber spinach root caused by *Meloidogyne* sp. after 60 days in the garden maintaining the nematode source.

4. Conclusions

The study isolated four fungi groups (*Aspergillus* sp., *Paecilomyces* sp., *Mariannaea* sp., and *Penicillium* sp.) from 35 samples collected in soil and roots on carrots, spinach, tomatoes, amaranth, and chilies plants at Lam Dong province (Da Lat city), Ho Chi Minh City (Cu Chi district), and Dong Nai province (Cam My district). *Paecilomyces* sp., *Mariannaea* sp., and *Penicillium* sp. effectively inactivated second-stage juveniles (J2) after 72 h. Specifically, *Paecilomyces* sp. immobilized 64.5% of the nematodes, *Mariannaea* sp. 72%, and *Penicillium* sp. 70%, compared to only 13.5% in the control without inoculation. *Paecilomyces* sp. controlled the hatching of

nematode eggs, with the rate of unhatched eggs ranging from 68.33 to 90.67%. LD-CC 15.01 (*Paecilomyces* sp.) showed the percentage of eggs not hatching to 90.67%. The experiment investigating the influence of fungal strains on J2 in Malabar spinach (*Basella alba* L.) in a net house showed that the *Mariannae* sp. strain inoculated on plants at a density of 10^7 cfu/mL produced the most expected results in terms of root length (25.3 cm) and root weight (0.4 g) compared to the control without fungus inoculation (18.5 cm; 0.3 g) and the control with only the nematode (11.2 cm; 0.2 g). Additionally, the *Mariannaea* sp. significantly reduced the number of juveniles/50 g of soil (13.7 J2) compared to the control (160 J2) in Malabar spinach (*Basella alba* L.) in a net house.

Conflict of interest

The authors have no conflict of interest to declare.

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