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RESEARCH ARTICLE

Enhancing tomato crop protection: Utilizing microbial and botanical bioproducts to control *Meloidogyne incognita* population

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Abstract

The cultivation of tomatoes (*Solanum lycopersicum* L.) is seriously threatened by root-knot nematodes (*Meloidogyne* spp.), which can result in significant yield losses and decreased fruit quality. This study explores the efficacy of biological agents *Trichoderma harzianum*, *Photorhabdus luminescens* and neem cake as an alternative strategy for managing *M. incognita*. During the study period *T. harzianum* caused maximum mortality 91.60 % followed by neem cake 84.8 % and 79.2 % by *P. luminescens* against the infective second stage juveniles (IJ2) after 72 hr exposure period under laboratory conditions. The LC_{50} value and associated 95 % confidence limits were used to express the data on the *in vitro* nematicidal activity of biological agents. The pot experiment showed that the *T. harzianum* at 2×10^6 spores/10 mL suppressive effect was superior to all other treatments in terms of root gall index (RGI) (1.27), followed by neem cake (2.07). *P. luminescens* at 4×10^4/10 mL/pot was found less effective in preventing *M. incognita* with the highest root gall index (RGI) (2.60). Similarly, *T. harzianum* had lowest number of females and egg masses (33.33, 27.00), followed by neem cake (42.33, 48.33) and *P. luminescens* (58.0, 61.67). It also showed the most significant ($P<0.05$) decline in the nematode population (70.00) and reproduction factor (0.14) followed by neem cake and *P. luminescens* in field efficacy. *T. harzianum* also achieved the lowest nematode egg masses and galling index. The study found that *T. harzianum*, neem cake and *P. luminescens* treatments improved plant growth, with maximum shoot length, root length and fruit yield respectively. Nematode infection suppressed chlorophyll, carotenoid and sugar content in plants, with *T. harzianum* causing the most significant increase in chlorophyll and carotenoid content, followed by neem cake and *P. luminescens*, with minimal increases in these parameters. These findings suggest that integrating these biological agents into nematode management strategies can offer an effective and sustainable approach for controlling root knot nematodes in tomato crop.

Keywords: biological control agents; management; *M. incognita*; neem cake; *P. luminescens*; *T. harzianum*

Introduction

All around the world, root-knot nematodes (RKNs) are a common soil-borne pathogen and are obligate, ubiquitous, biotrophic endoparasites that belong to the genus *Meloidogyne*. Nearly 100 species of genus *Meloidogyne* are recognized to attack >3000 plants species that causing multibillion-dollar losses yearly (1-3). They are sedentary endoparasites that induce permanent feeding sites inside the roots of their host plant, allowing the phytonematodes to develop further by growing in interaction with the host (4, 5). These nematodes are responsible for the formation of giant cells in roots which are metabolically active and serve as a constant food source for the growing nematode stages (6). *Meloidogyne incognita, M. hapla*, *M. javanica* and *M. arenaria* are the four main species of genus *Meloidogyne* which are responsible for the reduction in yields in many crops (7, 8). Among these four species, *M. incognita* is most devastating species of plant parasitic nematodes (PPNs) and shown key concern by the scientist or agriculturist due to its ability to reproduce on the several crops worldwide and also causing a severe damage to infested plant (2). The injury caused by *M. incognita* typically goes unnoticed as it infests the below-ground parts of host plants, simultaneously making plants more susceptible to fungal and other bacterial diseases. This attack causes yield suppression, wilting, chlorosis, nutritional deficiencies and root galling (9-11). Finally, they interfere with the movement of water and nutrients in infested plants and ultimately, crop failure and yield reduction are observed (12,

13). Throughout the subtropical and tropical regions of the world, this southern RKN is a key pest of many vegetables, including tomato, carnation, ginger, okra, capsicum, cucumber, melon, carrot, gourds, lettuce and peppers (14-19).

Among many vegetable crops the more susceptible host is tomato (*Solanum lycopersicum* L.) in the biosphere. In the field conditions, *M. incognita* hinders the growth as well as development and cause huge economic loss worldwide (20, 21). Different studies revealed that the yield loss in tomato due to the infestation of *M. incognita* in many tomato cultivars ranges between 25-100 % (21). Farmers use primarily the chemical nematicides to control the population of RKNs in their fields to protect crops from damage (22, 23). Considering the high economic importance of this particular nematode pest, various researchers have developed different strategies for managing this nematode species. Chemical nematicides are effective against nematodes, but their application requires cautious consideration of different factors, such as human health risks, environmental impact, effects on non-target organisms and regulatory requirements (24, 25). The continuous use and high-dose application of chemical nematicides can cause ineffectiveness after prolonged use and contaminate the groundwater (26). Therefore, restrictions are implemented in their use to manage the population of nematodes in the soil (27).

The most effective and cost-effective alternative to manage nematode populations is biological control, which involves the use of microbes (28, 29). Biocontrol is an environmental friendly alternative against many pests. Numerous bio-agents are studied to estimate their effectiveness against many species of nematodes but a bit of them are successfully commercialized worldwide (30). In this context, several species of filamentous ascomyceteous fungus belonging to genus *Trichoderma* have been used and have pesticidal potential against nematodes and other soil borne and foliar pests (31, 32). Different species of *Trichoderma* antagonize the parasitic nematodes through direct egg and juvenile's parasitism and through the enzymatic actions. It faces competition for nutrients and available space in the rhizospheric soil, due to the generation of hazardous byproducts. Also boosting the plant defense responses and promote plant growth by the production of indole-3-acetic acid (IAA) and phosphate solubilization (33-35). *T. harzianum*, *T. viride*, *T. asperellum*, *T. koningiopsis* and *T. virens* are the commercially formulated products used to control nematodes (36).

Soil-inhabiting entomopathogenic nematodes (EPNs), associated bacteria *Xenorhabdus* spp. and *Photorhabdus* spp is secreting toxins eradicate many insect pests. This nematode associated bacteria cause death in host cells by producing chemicals that induce necrosis or apoptosis, by the secreation of hemolysin and cytolysin toxins (37, 38). These associated bacteria are used against diverse nematode species, such as *Meloidogyne* spp. *Belonolaimus longicaudatus*, *Globodera rostochiensis*, *Rotylenchulus reniformis* and *Criconemoides* spp. (39). Neem cake, a byproduct of compressing neem seeds to extract oil, is considered a valuable source of organic fertilizer that protects plant roots from nematodes, termites and soil grubs through the release of residual limonoids (40, 41). When neem cake is added to the soil, the production of root galls, as well as the population of 2nd-stage juveniles of *M. incognita*, is reduced (42, 43). In this study, bacteria (*P. luminescens*), fungus (*T. harzianum*) and neem cake approaches are used against *M. incognita* under laboratory, polyhouse and field conditions.

Materials and Methods

Laboratory experiment

Maintaining a pure culture of the nematode *M. incognita* throughout the experiment on a hybrid tomato variety Abhinav, was grown in a protected environment. After chopping the galled roots into 1-2 cm long pieces and vigorously shaking them in a 1 % NaOCl solution for three to five min, the eggs were removed. Eggs were collected using a mesh sieve (20 µm) and carefully rinsed with water before storing in a refrigerator. To separate freshly hatched IJ2, rinsed eggs were placed on an Oostenbrink dish and incubated at room temperature for hatching. Over the course of a week, the recently hatched IJ2s were gathered. *In-vitro* nematode bioassays were demonstrated on J2 suspension of *M. incognita* isolated from the tomato plants. Commercially available formulation of *T. harzianum* Neemoderma-H (1 % WP, $2x10^8$ CFU/gram, International Panaacea Limited, New Delhi, India), neem cake 100 % natural and organic fertilizer and *P. luminescens* EUPT-S (Accession no. OP268192) culture used in this study was already maintained in the Department of Zoology (44).

Bio-efficacy of *P. luminescens*, *T. harzianum* and neem cake

Bio-efficacy of biocontrol agents such as *P. luminescens*, *T. harzianum* and neem cake were assessed against IJ2 stage of *M. incognita* in the laboratory. Three different concentrations were applied against the 50 juveniles in addition to the complete control of the Petri dish. A growing media that facilitates the development of microorganisms was necessary for pure culture investigations. *P. luminescens* bacterial inoculation concentration was adjusted 2×10^2, 3×10^3 and 4×10^4 CFU/mL from the prepared broth and was used against the J2 stage (IJs) of *M. incognita*. The concentration of *T. harzianum* fungus was adjusted to 2×10^2, 2×10^4 and 2×10^6 spores/mL and was employed against 2nd stage juveniles of *M. incognita*. The concentrations of the botanical bio-control agent neem cake were employed 0.5, 1.0 and 2.0 %. The petri plates were incubated at 27 °C in the dark and data on the percent mortality of juveniles were recorded after three exposure periods 24, 48 and 72 hr (45). This study used a completely randomized design (CRD) and was conducted twice and recorded data were pooled of two consecutive years for statistical analysis. The suspensions of J2 were gently shaken to facilitate aeration and track any mortality recovery. Under a stereomicroscope (Leica Si9), dead J2 stages were observed every 24 hr. Juveniles were considered dead when in physical stimulation with a small needle they showed no movement and assumed a straight shape (46).

Evaluation under *in vitro* condition

The experiments were laid out in Entomology & Nematology polyhouse, fields of Agriculture, Eternal University, Baru Sahib, Himachal Pradesh, India. Tomato variety "Abhinav" seeds were bought from the local market. In this experiment the uniform tomato seed were surface sterilized with 1.0 % NaOCl solution for 1-2 min and washed 2-3 times with sterile water

(47). Tomato seeds were directly sown in earthen pots using autoclaved soil mix (clay:sand:loam at 1:1:1) at ≥ 25 ˚C temperature and 14 ± 2 hr of daytime under aseptic conditions of polyhouse. The three to four seeds were placed about 1.5 inches deep in a 30 cm wide pot filled with 4.0 kg soil mix. When the seedlings developed into 2-3 sets of leaves, the plants were trimmed. Two healthiest seedlings were carefully chosen and the remaining was eliminated. Every 2-4 days the plants were irrigated and applied fertilizers for productiveness. Wooden stakes were used to hold the plants and instil the branches to wrap around them. For inoculation, eggs of *M. incognita* were extracted from freshly uprooted galled tomato roots and used for the experiment (48). The extracted infected juveniles (J2) suspension was adjusted to a final volume at 500 /10 mL of water using a stereozoom microscope. Seedlings of tomato were inoculated with infected juveniles suspension of *M. incognita* using sterile micropipette following standard inoculation procedures (45). *P. luminescens* EUPT-S at 4×10^6 CFU/10mL, *T. harzianum* at 2×10^6 spores/10mL and neem cake at 2.0 %/10 mL were dispensed into three holes made around tomato seedlings using sterile micropipette (49). After that, sterile sand was used to fill the holes surrounding the seedlings and 45 mL of tap water was used to irrigate each pot. The control seedlings were given just 55 mL of pure water. In a polyhouse, seedlings were irrigated as needed and treated with slow-release fertilizer every 15 to 20 days. Experiments were conducted with 5 replicates in using a CRD (completely randomized design).

Chlorophyll and carotenoids contents

The chlorophyll content was determined by the method given by Mackinney (50). 10 mL of 80 % acetone was added to 1 g of fresh tomato leaves, which were then crushed into a fine pulp using a crusher and pestle. After incubating the resulting mixture for 25 to 30 min, the supernatant was collected in a volumetric flask. Three times, the residue was cleaned with 80 % acetone, collected in the same flask and the final volume was adjusted. On a spectrophotometer, the absorbance of chlorophyll was measured at wavelengths of 645, 663 and 480 nm for carotenoid and 80 % acetone as a blank (51). The chlorophyll and carotenoid content present in the extract (mg g^{-1} tissue) was calculated by using formula:

Chlorophyll

$$\text{Total chlorophyll content} = 20.2(A_{645}) + 8.02(A_{663}) \times \frac{\text{Final volume of extract (V)}}{1000} \quad \text{(Eqn. 1)}$$

Carotenoids

$$\text{Carotenoid content} = 7.6(A_{480}) - 1.49(A_{510}) \times \frac{V}{1000\ D\ W} \quad \text{(Eqn. 2)}$$

Whereas;

A_{480}, A_{510}, A_{645}, A_{663} = Absorbance of extract at given wavelength

W= Fresh weight of leaf sample

D= Length of path of life

Evaluation under *in vivo* condition

Experiments were conducted to test the effects of biological control filtrates on, *M. incognita* in tomato cultivar' Abhinav; under natural field infestations in the spring, 2022 and 2023. Fields were selected on the basis of previous crop history. The soil population density of *M. incognita* was determined by taking random soil cores (200 cc soil form ten different sites) from nematodes infested tomato field (52). The tomato seeds was sown for nursery preparation in the polyhouse. RKNs infected site was sub-divided into twenty different plots of 9 m^2 areas with plot to plot gap of 45 cm. Plant to plant and row to row spacing is 30 and 70 cm, respectively, for every bed. Each trial consisted of ten seedlings per crop per replication transplanted from nursery to respective plots. Five replications of each field study were conducted using a Randomized Complete Block (RCB) design. After the growing season ended, the experiments were terminated and data on nematode and plant growth metrics were recorded. At the time of harvesting, information was recorded on fresh and dry shoot weights, fresh root weights per plant and yield. In order to evaluate nematodes, some metrics were recorded as previously mentioned, including the galling index, the quantity of egg masses and the adult females per 10 g of roots. Under natural field conditions, *P. luminescens* 4×10^6 CFU/10 mL, *T. harzianum* at 10 g/m.sq and neem cake at 100 g/m.sq were the treatments evaluated for *M. incognita* in tomato. These newly transplanted seedlings were managed carefully according to the guidelines, package of practices for vegetable crops, regarding a proper thinning, weeding, hoeing and earthling up and stacking given by the Agricultural and Horticultural Universities of the State for growing a healthy crop.

Statistical analysis

Experimental data were analyzed using one-way analysis of variance (ANOVA) with SPSS 25.0 statistical software (SPSS Inc., Chicago, IL, USA); differences between treatments were determined by Duncan's Multiple Range Test (DMRT). Probit analysis was performed and the median lethal concentration (LC50) was calculated (53). The corrected per cent mortality was calculated using Abbott's formula (54). Means were considered significant at $P<0.05$.

Results

Bio-efficacy of *P. luminescens*, *T. harzianum* and neem cake against *M. incognita*

Results from *in vitro* experiments revealed significant effect on J2 mortality of *M. incognita* by bio-control agents of different concentrations at 24, 48 and 72 hr of incubation. *T. harzianum* showed the highest mortality followed by neem cake and *P. luminescense* respectively. In this bioassay experiment, highest mortality 91.60 % was observed in *T. harzianum* at highest dose followed by neem cake (84.8 %) and *P. luminescense* (79.2 %) respectively after 72 hr (Table 1). The statistical analysis revealed that there was a significant difference ($P<0.05$) between the treatments and the control group. A significant differences were also recorded in the mortality percentage by *T. harzianum* ($F=4.84$, df=2,). In neem cake significant differences were recorded ($F=4.13$, df=2). In treatment with *P. luminescens* significant differences were

Table 1. Effect of *P. luminescens T. harzianum* and neem cake on infective juvenile mortality of *Meloidogyne incognita* under laboratory bioassay

Treatments	Concentration	The number of dead juveniles (Mean ± SE) at varying concentrations with corrected mortality rate %		
		Exposure time (hr)		
		24 hr	48 hr	72 hr
P. luminescens	2× 10^2 CFU/m	5.8±0.87(11.6)	15.4±0.89(30.8)	25.6±0.89(51.20)
	3× 10^4 CFU/mL	11.4 ±1.01(22.8)	22.8±1.08(45.6)	31.8±1.46(63.60)
	4× 10^6 CFU/mL	17.0±1.19(34.0)	28.0±1.61(56.6)	39.6±1.55(79.20)
	Control	0.00±0.00	0.00±0.00	0.00 ± 0.00
T. harzianum	2× 10^2 spores/mL	8.4±0.70(16.80)	18.0±1.19(36.00)	33.00±0.95(66.00)
	2× 10^4 spores/mL	13.4±1.01(26.80)	27.4±1.16(54.80)	38.2±0.87(79.60)
	2× 10^6 spores/mL	20.0±1.19(40.00)	33.4±1.01(66.80)	45.6±1.08 (91.60)
	Control	0.00±0.00	0.00±0.00	0.00± 0.00
Neem Cake	0.5 %	7.2±0.87(14.40)	15.4±1.01(30.80)	31.0±1.19(62.00)
	1 %	12.0±0.80(24.00)	24.8±1.08(49.60)	36.2±1.08(72.40)
	2 %	17.8±1.15(35.60)	30.2±1.21(60.40)	42.4±1.58(84.80)
	Control	0.00 ± 0.00	0.00±0.00	0.00 ± 0.00

Data is Mean±Standard error of five replicates; Arc sine transformation ($p<0.05$) statistically significant (df 2); CFU- Colony forming unit

F=3.21,df=2. The information about the nematicidal activity of biological agents *in vitro* was presented as an LC_{50} value together with the associated 95 % confidence limits. The LC_{50} of each of the four treatments was computed as a probit result. The mortality of IJ2 increased in a gradual manner in tandem with the concentration increase. After 24, 48 and 72 hr of exposure, *T. harzianum* had LC_{50} values of 3234.35, 3794.57 and 1306.38 ppm, indicating that it was highly harmful to juveniles. In same context, neem cake 41,667.59, 11,589.86 and 2,876.72 ppm after 24, 48 and 72 hr followed by *P. luminescens* with LC_{50} values values 62,505.58, 45,390.79 and 3,1280.70 ppm respectively also have nematicidal properties against *M. incognita*. The findings showed that after 72 hr bioassay intervals, *T. harzianum* was the most effective followed by neem cake and *P. luminescens* respectively against juveniles (Table 2).

Table 2. Nematicidal action of all biological agents on *M. incognita* juveniles

Treatment	Susceptibility hr	LC_{50} at 95 % CL in ppm
P. luminescens	24	62,505.58
	48	15,39.79
	72	1,1280.70
T. harzenium	24	3,234.35
	48	3,794.57
	72	1306.38
Neem cake	24	41,667.59
	48	11,589.86
	72	2,876.72

LC_{50}: 50 % of the population died after 24, 48 and 72 hr at 95 % confidence levels (CL) due to the lethal concentration

Evaluation under *in vitro* condition

Effect of *P. luminescens*, *T. harzianum* and neem cake on *M. incognita*

In a polyhouse setting, the impact of microbial and botanical bioproducts chemicals on *M. incognita* in tomatoes was investigated in the spring. By incorporating biological control agents, the development and reproduction of *M. incognita* was significantly reduced ($P<0.05$) in terms of the root gall index, number of egg masses/root system, number of females/root system and nematode population in the soil. Consequently, the growth of tomato plants was enhanced ($P<0.05$). In terms of root gall index (1.27), *T. harzianum* at 2x10^6 spores/10 mL had a nematode-suppressive impact that was superior to all other treatments, followed by neem cake (2.07). Following control, *P. luminescens* at 4×10^4/10 mL/pot was shown to be less efficient against *M. incognita*, with a maximum RGI of 2.60. Likewise, *T. harzianum* had the fewest egg masses and females (33.33, 27.00) followed by neem cake (42.33, 48.33) and *P. luminescens* (58.0, 61.67). Within the same framework, the application of *T. harzianum* demonstrated the greatest significant ($P<0.05$) decrease in nematode population and reproduction factor (70.00, 0.14), trailed by neem cake (93.33, 0.21), while *P. luminescen* (111.67, 0.26), after control (224.67, 0.45), exhibited the least significant reduction (Table 3).

After applying *T. harzianum* at a rate of 2x10^6 spores/10 mL, the plant growth was much higher ($P<0.05$). Out of all the growth parameters that were tested, the plants showed the greatest rise in root length (21.58 cm), shoot length (73.91 cm), shoot fresh weight (69.90 g) and dry weight (18.60 g); and the greatest decrease in root fresh weight (16.56 g) and dry weight (2.4 g). In addition, neem cake promotes reduced fresh weight (19.11), dry weight (3.0), shoot fresh weight (59.30), shoot dry weight (15.70) and root length (15.78 cm), shoot length (64.40 cm) and shoot fresh weight (59.30). As a result, *P. luminescens* showed the least amount of growth development in terms of root length, shoot length, shoot fresh weight, shoot dry weight and root fresh weight and root dry weight following control (Table 4). The control roots showed obvious signs of galling, withering and twisting, which are indications of the root-knot disease. Reduced growth metrics were the outcome of these nematode-caused damages to the tomato roots.

Chlorophyll and carotenoids contents

In a way similar to that observed in the case of plant growth characteristics, nematode infection also reduced the levels of sucrose, carotenoid and chlorophyll. However, the application of *T. harzianum* produced the most significant increase in the amounts of chlorophyll (Chl a 1.543 mg g^{-1} and Chl b 2.769 mg g^{-1}) and carotenoid (2.30 mg g^{-1}), respectively, followed by the amounts of chlorophyll (Chl a 1.360 mg g^{-1} and Chl b 2.226 mg g^{-1}) and carotenoid (2.263 mg g^{-1}) in neem cake. *P. luminescens* gave a minimum increase in these parameters Chl a (1.249 mg g^{-1}), Chl b (2.205 mg g^{-1}) and carotenoids 2.404 mg g^{-1} were detected in plants after control (Chl a 0.810, Chl b 0.344 mg g^{-1} and carotenoid 1.248 mg g^{-1}) (Table 3).

Table 3. The effectiveness of *T. harzianum, P. luminescens* and neem cake against *M. incognita* in relation to pathological and biochemical parameters

Treatments	Final nematode population	Egg mass	RGI	Adult female	Rf	Chlorophyll (mg g^{-1})		Carotenoid (mg g^{-1}) (470nm)
						Chl a (645nm)	Chl b (633nm)	
P. luminescens	111.67±1.12^{b}	58.00±1.57^{b}	2.60±0.29^{b}	61.67±1.12^{b}	0.26	1.249^{abc}	2.205^{b}	2.263^{b}
T. harzianum	70.00±1.47^{c}	33.33±1.43^{c}	1.27±0.22^{bc}	27.00±0.28^{c}	0.14	1.543^{a}	2.769^{a}	2.404^{a}
Neem cake	93.33±0.97^{bc}	42.33±1.30^{bc}	2.07±0.88^{b}	48.33±1.43^{bc}	0.21	1.360a^{b}	2.226^{b}	2.303^{ab}
Control	224.67±1.92^{a}	103.33±1.12^{a}	5.00±0.00^{a}	97.00±1.70^{a}	0.45	0.810^{c}	0.344^{c}	1.248^{c}

Each treatment's methods comprise five replicated plants. Means are compared using the Duncan Multiple Range Test, which indicates a significant difference (P < 0.05)

Table 4. The effectiveness of *P. luminescens, T. harzianum* and neem cake on plant growth parameters of tomato under *in vitro* conditions

Treatments	Shoot length	Root length	Fresh shoot weight	Dry shoot weight	Fresh root weight	Dry root weight
P. luminescens	56.24±2.81^{b}	14.53±1.37^{ab}	51.70±2.63^{b}	17.20±1.17 ^{a}	20.00±1.56^{b}	3.2±1.06^{a}
T. harzianum	73.91±3.99^{a}	21.58±1.53^{a}	69.90±2.42^{a}	18.60±0.89^{a}	16.56±1.63^{b}	2.4±0.82^{a}
Neem cake	64.40±2.93^{ab}	15.78±1.66^{ab}	59.30±1.89^{ab}	15.70±1.10^{a}	19.11±1.72^{ab}	3.0±0.90^{a}
Control	49.25±2.05^{c}	9.98±1.34^{c}	33.60±2.51^{c}	14.70±1.23^{a}	28.11±2.39^{a}	6.6±1.37^{a}

Each treatment's methods comprise five replicated plants. Means are compared using the Duncan Multiple Range Test, which indicates a significant difference (P < 0.05)

Evaluation under *in vivo* condition

The impact of bio-control agents

The application of bio agents effectively suppressed RKN, *M. incognita*, in tomato fields despite natural field infestation. *T. harzianum* attained the lowest nematode galling index (1.12), egg masses per 10 g of root (31.33), adult female per 10 g of root (35.67) and reproduction factor (0.30). Neem cake also resulted in a minimum reduction in RGI (2.80), egg mass/10 g root (47.33), number of females/10g root (48.33) and reproduction factor (0.43), followed by *P. luminescens*, respectively. The control treatment showed the highest galling index (5.00), egg masses (89.00) and adult female per 10 g of root (109.00), as well as the highest reproduction factor (1.05) among all treatments (Table 5). In comparison to the control, the nematode population was significantly decreased by all treatments.

Chlorophyll and carotenoids contents

In a way similar to that observed in the case of plant growth characteristics, nematode infection also reduced the levels of sucrose, carotenoid and chlorophyll. However, the application of *T. harzianum* produced the greatest increase in the amount of chlorophyll (Chl a 1.466 mg g^{-1} and Chl b 2.573 mg g^{-1}) and carotenoid content (2.726 mg g^{-1}) followed by neem cake Chl a 1.448 mg g^{-1} and Chl b 2.515 mg g^{-1} and carotenoid content (2.467 mg g^{-1}) respectively. *P. luminescens* gave minimum increase in Chl a 1.336 mg g^{-1}, Chl b 2.317 mg g^{-1} and carotenoids 2.372 mg g^{-1}. In control the amount of chlorophyll (Chl a 0.938, Chl b 1.500 mg g^{-1} and carotenoid (1.591 mg g^{-1}) was recorded (Table 5).

The impact of biocontrol agents on plant growth

Under naturally infested field conditions, treatments with biocontrol agents significantly increased tomato plant growth and yield. In line with greenhouse outcomes, the treatment in this case the highest shoot length (130.75 cm), root length (27.76 cm), fresh shoot weight/plant (116.33 g), dry shoot weight/plant (55.67 g), fresh root weight/plant (8.75 g) and fruit yield/kg/ha (454.34) were all achieved by *T. harzianum*. Neem cake produced the second-best results in terms of enhancing plant growth, with shoot length (101.05 cm), root length (18.62 cm), fresh shoot weight/plant (82.50 g), fresh shoot weight/ plant (42.00 g), fresh root weight/plant (22.50 g) and fruit yield/ kg/ha (310.40 kg), respectively. Likewise, *P. luminescens* contributes to the growth of shoot length (96.00 cm), root length (16.02 cm), fresh shoot weight/plant (77.67 g), dry shoot weight/plant (36.67 g), fresh root weight/plant (25.83 g) and fruit yield/kg/ha (288.08 kg). The values for the dry root weight (18.80 g), fruit yield/kg/ha (179.65 kg), fresh shoot weight/plant (44.33 g), dry shoot weight/plant (28.00 g), fresh root weight/ plant (29.67 g) and shoot length (68.67 cm) were all lowest for the control treatment (Table 6).

Table 5. The effectiveness of *P. luminescens, T. harzianum* and neem cake against *M. incognita* in relation to pathological and biochemical parameters

Treatments	Nematode soil population (200 cc)						Chlorophyll (mg g^{-1})		Carotenoid (mg g^{-1})
	Initial	Final	Egg masses	RGI	Adult female	Rf	Chl a	Chl b	
P. luminescens	264.00±0.71^{a}	126.67±1.00^{b}	53.33±1.12^{b}	3.00±0.29^{a}	65.0±0.90^{b}	0.48	1.336^{b}	2.317^{b}	2.372^{ab}
T. harzianum	270.67±1.41^{a}	81.67±0.71^{c}	31.33±1.58^{b}	1.12±0.3c	35.67±1.82^{c}	0.30	1.466^{a}	2.573^{a}	2.726^{a}
Neem Cake	262.33±1.22^{a}	111.67±1.22^{b}	47.33±1.30^{b}	2.80±0.41^{ab}	48.33±1.58^{bc}	0.43	1.448^{a}	2.515^{a}	2.467^{ab}
Control	278.33±1.73^{a}	291.33±1.73^{a}	89.00±0.90^{a}	5.00±0.00^{a}	109.00±1.30^{b}	1.05	0.938^{c}	1.500^{c}	1.591^{c}

Each treatment's methods comprise five replicated plants. Means are compared using the Duncan Multiple Range Test, which indicates a significant difference (P < 0.05)

Table 6. The effectiveness of *P. luminescens, T. harzianum* and neem cake on plant growth parameters of tomato under *in vivo* conditions

Treatments	Shoot length	Root length	Fresh shoot weight	Dry shoot weight	Fresh root weight	Dry root weight	Yield per hectare (kg/ha)
P. luminescens	96.00±1.29^{b}	16.02±1.61^{b}	77.67±3.87^{bc}	36.67±1.73^{a}	25.83±2.20^{a} $_{b}$	10.50±0.49^{ab}	288.08±2.06^{ab}
T. harzianum	130.75±2.69^{a}	27.76±1.89^{a}	116.33±4.65^{a}	55.67±2.08^{a}	18.75±1.26^{b}	2.67±0.69^{c}	454.34±2.72 $_{a}$
Neem cake	101.05±1.84^{ab}	18.62±1.02^{b}	82.50±3.85^{ab}	42.00±1.29^{a}	22.50±2.12^{b}	9.67±0.69^{ab}	310.40±2.18^{ab}
Control	68.67±1.44^{b}	11.66±0.80^{c}	44.33±0.98^{c}	28.00±0.47^{a}	29.67±2.58^{a}	18.80±0.50^{a}	179.65±2.67^{b}

Each treatment's methods comprise five replicated plants. Means are compared using the Duncan Multiple Range Test, which indicates a significant difference ($P < 0.05$)

Discussion

Meloidogyne species are reported as the prevalent and most destructive nematode pests in the tomato crop, responsible for causing high levels of economic losses, especially in tropical and sub-tropical agricultural systems (55, 56). In this present investigation, nematicidal potential of *P. luminescens*, *T. harzianum* and neem cake was evaluated against J2 stage of *M. incognita* under laboratory conditions, polyhouse and field conditions. The present results revealed that *T. harzianum* showed the highest juvenile mortality at the highest concentration under laboratory, pot and field conditions. Similarly, it resulted in the maximum reduction in gall index, egg mass and the number of females in both pot and field experiments, while also enhancing plant growth parameters such as root and shoot length, shoot weight and reducing root weight, thereby contributing to increased yield. *Trichoderma* spp. have branched conidiophores that produce conidia, which infest different stages of nematode pests (57). They inhibit the reproduction and growth of parasitic nematodes through secreting some antagonistic substances (58). Generally they produces secondary metabolites, like antibacterial peptide, butyrolactones, protease, lipase, chitinase, gliotoxin, β-1, 3-glucanase, heptadecarboxylic acid, sesquiterpene terpenes, trichomycin, viridin, polypeptides, polyketones and a number of volatile substances as aldehydes, alcohols, alkanes, olefins, furans, esters, hydrocarbons, heterocyclic and aromatic compounds and many other terpenoids which help in development of plant growth (59). Research indicates that *T. harzianum*, an effective fungus that suppress *M. incognita* infestation with reduction in population under greenhouse conditions in okra, mungbean, cucumber and tomato (60, 61).

A study conducted in polyhouse and laboratory experiments evaluated the effects of different concentrations of *T. harzianum* and *T. viride* against *M. javanica* on tomato, observing suppressed nematode pest reproduction compared to controls (62, 63). Their result was also in line with the present investigation, in which the mortality percentage increased when inoculum densities of *T. harzianum* increased, enhancing the plant growth parameters. Previous studies revealed *T. harzanium*, *T. viride, T. atroviride and T. asperellum* as tremendous biocontrol agents against RKNs that promotes plant growth and tolerance (64, 65). A study use different concentrations of neem extract, Furadan and *T. harzianum* against *M. incognita*. They reported that *M. incognita* significantly suppressed tomato plant growth by developing a large number of galls (66). Neem cake and *T. harzianum* have been effective in reducing disease development in tomato plants at higher doses and improving plant growth (67).

Two antagonistic fungi *T. viride* and *T. harzianum* were evaluated against the *M. incognita* infesting tomato. The outcome demonstrates that, in a dose-dependent manner, these two applications dramatically increased the shoot weight and decreased the root weight of the tomato. The most significant dose resulted in a considerable reduction in the number of galls, egg masses, eggs per egg mass and reproductive variables in addition to a maximum rise in shoot weight and a decrease in root weight. The greatest reductions in these parameters are caused by both treatments at the two highest dosages (8×10^3 and 1×10^4 CFU/g) (57). The potency of *T. viride* as an antagonist was assessed against *M. incognita* within the tomato plant. At 6 gm/kg soil and 9×10^8 CFU/mL bare root dip treatment, *T. viride* dramatically boosted shoot weight and lowered root weight. Additionally, it decreased galls, egg masses and reproductive factors, suggesting that it may be able to manage the *M. incognita* (68, 69). The biocontrol ability of *T. harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescensm* was recorded earlier against *M. incognita* (70). According to research on the interactions between biocontrol agents and diseases, *T. harzianum, B. subtilis* and *P. fluorescens* prevented 75 % of *M. incognita* eggs from hatching. When exposed to a fresh culture of *T. harzianum, M. incognita* eggs were also found to be 89 % infected.

Similarly, neem cake caused the highest mortality at the highest concentration (84.60 %) after 72 hr of exposure in the laboratory assay. It also helps in enhancing plant growth and gives a minimum reduction in gall index, egg masses and the number of adult females, while reducing reproduction and minimizing the soil nematode population in both pot and field experiments. Neem cake is a rich source of naturally active compounds such as azadirachtin, an insecticide with nematicidal and biopesticidal properties (71). The results of the present study is in accordance with a previous study (72). In that study it was observed that the extract of neem cake is most effective against the juveniles of *M. incognita* due to the presence of different biological substances, including azadirachtin, nimbin, salannin and derivative compounds such as triterpenoids and limonoids, which also promote plant growth. Javed and co-wokers evaluated the aqueous extracts as seedling dip treatment prepared from seed, leaves and cakes of the Neem and observed a significant reduction in

nematodes population (73). Neem is one of the most important botanical pesticides, possessing fungicidal, nematicidal, bactericidal, molluscicidal, diuretic and antiarthritic properties (74). Research indicates that oilcakes can improve tomato plant development and lower the number of nematodes (75). It enhances microbial activity in amended soil, which in turn promotes the conversion of nitrogen to the nitrate form. This increases plant growth and metabolic activity. The application of Neem seed or cake improved crop growth and plant yields by 53 % thereby enhancing crop growth and farmer income (76). An experiment was conducted to compare the efficacy of neem cake, Mahua, Karanja, Mustard, Groundnut, Polango and Kusum oil cake for managing *M. incognita* in tomato plants. Every treatment considerably improved the plant growth metrics, chlorophyll content and decreased the number of gall formations. The applications of Neem oil cake and Mahua cake outperformed the others in terms of shoot plant weight, plant height, root length and plant chlorophyll content (77). Research indicates that the increase in chlorophyll content in leaves in the presence of decomposed organic wastes is due to an increase in N uptake (78). The addition of organic compounds resulted in increased photosynthetic efficiency, translocation of nutrients and other metabolites toward the formation of fruits, as well as the efficacy of different oil cakes in managing RKNs infecting tomato (79). A study reported that neem cake was significantly superior among the five different oil cakes against the juveniles of *M. incognita* under laboratory conditions (80). Research indicates that the induction of defence enzymes polyphenol oxidase (PPO), peroxidase (PO), superoxide dismutase (SOD) and phenylalanine ammonia lyase by oil cakes in a chilli. Mustard and neem cake when added to soil found as best treatment to upsurge the catalytic activity against the J2 juveniles of *M. incognita* (81).

Entomopathogenic bacteria *P. luminescens* caused 79.20% mortality at higher concentrations over 72 hr of exposure. Additionally, it was reported to reduce gall formation, the number of females and the reproduction rate, while promoting growth parameters in polyhouse and field experiments as well. A similar finding was reported, indicating that the nematicidal effect is more pronounced at higher concentrations over time against the J2 stage of *M. incognita* (82). Most research on the use of entomopathogenic bacteria and their secondary metabolites indicates their insecticidal, nematicidal and fungicidal activity (83). A study evaluated the high virulence of *Photorhabdus* and *Xenorhabdus* bacteria and their toxins at higher doses against the juveniles of *M. incognita* in the laboratory (84). A study assessed the efficacy of EPNs and the supernatants of their mutualistic bacteria under *in vitro* conditions against *M. incognita* and *M. arenaria* (79). Earlier studies reported the usefulness of *Steinernema* spp. and *Heterorhabditis* spp. in controlling the population of nematode pests (84). A study was conducted to evaluate the effectiveness of bio-agents and soil amendments in controlling *M. incognita* in cucumbers (49). According to the findings, *P. luminescens* not only promoted plant growth in terms of fresh and dry weight, shoot and root length and leaf, flower, fruit and fruit weight, but it also dramatically decreased the production of galls. *Photorhabdus*-derived secondary metabolites (4E)-5-phenylpent-4-enoic acid and Trans-cinnamic acid (t-CA) lead to the reduction in *M. incognita* infection in cowpea without impairing the plant growth and chlorophyll content (85, 86).

Conclusion

The findings of this study suggest that bio-agents may be good options for the biological control of *M. incognita*. This will effectively reduce pesticide consumption and contribute to the development of safer, more sustainable agricultural practices. *T. harzianum* and neem cake are the most effective treatments in laboratory, polyhouse and field experiments, keeping the level of root-knot nematode below the economic threshold level. More significantly, these substances can reduce nematode populations, which is essential for boosting yield characteristics and plant health. The discovery of effective biocontrol agents, such as *T. harzianum*, neem cake and *P. luminescens*, presents a sustainable and innovative path forward in nematode management, paving the way for more environmentally friendly agricultural practices. It is suggested that these biological agents be used to support organic farming and environmentally sound, long-term nematode control initiatives.

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Authors' contributions

SJ conceived the study, carried out the formal analysis and investigation and drafted the original manuscript. NT conceived of the study and participated in its design and coordination. SS, NY, AKR and ANY reviewed and edited the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflicts of Interest: There is no conflict of interest

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