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Evaluation of microbial antagonists as eco-friendly alternatives against root-knot nematode, *Meloidogyne incognita* management in tomato

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Abstract
Tomato productivity is severely constrained by root-knot nematode (*Meloidogyne incognita*), prompting the need for sustainable alternatives to chemical nematicides. Microbial biocontrol agents represent an eco-friendly strategy through direct antagonism of nematodes and enhancement of plant growth. In the present study, the efficacy of *Trichoderma asperellum*, *Purpureocillium lilacinum*, and *Pseudomonas fluorescens* was evaluated against *M. incognita* under *in vitro* and pot conditions. Cell-free culture filtrates significantly affected juvenile survival, with *P. lilacinum* inducing the highest mortality of second-stage juveniles (66%), followed by *T. asperellum* (52.6%) and *P. fluorescens* (45%). Pot experiments showed significant reductions in nematode infestation, with gall numbers reduced to 87.6 and 115.8 per plant in *P. lilacinum* and *T. asperellum* treatments, respectively, compared with 237.4 galls in the untreated control. Correspondingly, egg mass production declined markedly, reaching as low as 10.8 egg masses per plant under *P. lilacinum*. Biocontrol treatments also resulted in significant improvement in plant growth, with *T. asperellum* recording the highest shoot length (53.2 cm) and shoot biomass (141.96 g), compared with 21.96 cm and 57.72 g in the untreated control. Overall, the results demonstrate that these microbial biocontrol agents exert significant nematicidal effects while enhancing tomato growth, indicating their potential as sustainable components of integrated root-knot nematode management.

Keywords: *Meloidogyne incognita*, biological control, *Trichoderma asperellum*, *Purpureocillium lilacinum*, *Pseudomonas fluorescens*, nematode management, plant growth promotion

Introduction
Tomato (*Solanum lycopersicum* L.) is most grown and important vegetable crop globally and plays a crucial role in food security and farm income generation [1]. However, its productivity is severely impeded by the attack of various kinds of plant-parasitic nematodes, among which the root-knot nematode (*Meloidogyne incognita*) is the most destructive[2]. The nematode infection causes galls on the roots that impair water and nutrient uptake, disrupting normal root physiology, and ultimately leading to significant yield and quality losses [3,4]. In tropical regions, yield losses due to root-knot nematodes in tomato have been reported to range from moderate to severe, depending on multitute of factors such as inoculum density, crop stage, and environmental conditions [5].

Management of root-knot nematodes has traditionally depended on chemical nematicides because of their fast nature of efficacy. Nevertheless, growing concerns regarding environmental hazards, human health risks, non-target effects, and the restriction of several effective nematicides have urged the development of alternatives that are sustainable [6]. In this context, biological control has emerged as a promising and eco-friendly strategy for nematode management, aligning with the principles of integrated nematode management [6].

Among biological control agents, most attention have been given to antagonistic fungi and plant growth-promoting rhizobacteria due to their different mechanisms of mode of action against nematodes. *Trichoderma asperellum* is well known for its mycoparasitic ability, with rapid rhizosphere colonization, and production of secondary metabolites such as lytic enzymes that can adversely affect nematode eggs and juveniles [7]. *Purpureocillium lilacinum* is important egg-parasitic fungus that penetrates nematode egg shells and suppress

nematode multiplication, thereby reducing soil inoculum potential[8]. Similarly, *Pseudomonas fluorescens*, a rhizosphere- bacterium, shows nematicidal effects through the production of antibiotics, hydrogen cyanide, siderophores, and induced systemic resistance in host plants [9].

Although these microbial agents have individually demonstrated potential against root-knot nematodes, systematic evaluation under controlled *in vitro* and pot conditions is essential to elucidate their direct antagonistic effects on nematode mortality and their overall efficacy in reducing nematode infestation. Therefore, the present investigation was undertaken to evaluate the efficacy of *Trichoderma asperellum*, *Purpureocillium lilacinum*, and *Pseudomonas fluorescens* against the root-knot nematode (*Meloidogyne incognita*) infecting tomato through *in vitro* bioassays and pot culture experiments.

Materials and Methods

Culture and maintenance of root-knot nematode

The nematode, *M. incognita*, used in the studies was obtained from a single egg mass and multiplied on a susceptible eggplant cultivar. Four-week-old tomato seedlings were transferred in 6-inch earthen pots containing and inoculated with about 3000 freshly hatched second stage juveniles (J2s) (2 J2/cc soil) of *M. incognita* maintained on tomato cv. Ruby red one week after transplantation. The inoculated plants were kept on the bench of the screen house of the department at 25 ± 2 °C and watered as needed. Egg masses were handpicked using a sterilized forceps from the galled roots. Identification of the nematode was further done by preparing perenial patterns of ten adult female nematodes [10]. The egg masses were thoroughly rinsed with distilled water and transferred onto a sieve lined with tissue paper for hatching under ambient laboratory conditions (28°C). The freshly hatched juveniles were collected and utilised for further experiments.

Biocontrol agents

Pure cultures of fungus, *T. asperellum*, and *P. lilacinum*, bacteria. *P. fluorescens* were maintained on their potato dextrose agar and nutrient agar, respectively. For the pot culture experiment, talc-based formulations of *P. fluorescens* and *T. asperellum* were obtained from the College of Agriculture, Vellayani, while the formulation of *P. lilacinum* was sourced from the Cardamom Research Station, Kerala, and used for the study.

Preparation of cell free culture filtrates

Liquid cultures of above biocontrol agents were established in their respective broth in 500 mL conical flasks. Each flask was inoculated with the biocontrol agents and incubated at 25 ± 2 °C for a period of 14 days. Following incubation, the cultures were sequentially filtered through Whatman No. 1 and Whatman No. 42 filter papers and subsequently sterilized by passage through a 0.22 µm syringe filter. The resulting cell-free filtrates were collected and utilized for subsequent experiments [11].

Juvenile mortality bioassay

To assess the effect of biocontrol agents on the mortality of *M. incognita* second-stage juveniles (J2), J_2 mortality bioassay was conducted in 12 well tissue culture plates. 1 mL of undiluted cell free culture filtrate were separately dispensed into each well. Subsequently, 10 µl of suspension containing 100 freshly hatched *M. incognita* (J2) was introduced into each well. Sterilized distilled water and potato dextrose broth (PDB) were maintained as controls. Each treatment was replicated five times. After 24 h of incubation, the number of dead (immobile) juveniles was recorded. Mortality was confirmed by transferring the immobile juveniles to distilled water for a 24 h recovery period, and the percentage juvenile mortality was subsequently calculated [12].

Pot evaluation of biocontrol agents

Certified seeds of tomato (*Solanum lycopersicum*) cv. Ruby red were sown and the seedlings were raised in sterilized soil, and three-week-old seedlings were transplanted into 6-inch earthen pots containing 1 kg of autoclaved soil mixed with compost in a 3:1 ratio. One day prior to transplanting, each pot was inoculated by thoroughly mixing 10 mL of nematode suspension containing 2,000 second-stage juveniles (J2) of *Meloidogyne incognita*.

The treatments comprised *P. fluorescens* applied as a seed treatment at 10 g kg^{-1} seed; *T. asperellum* mass-cultured on a substrate consisting of cow dung and neem cake in a 9:1 ratio and incorporated into the soil at 20 g m^{-2}; and *P. lilacinum* enriched with farmyard manure (FYM) in a 1:100 ratio and applied at 20 g m^{-2}. These biological treatments were evaluated in comparison with a chemical control, fluopyram 400 SC, applied at 250 g a.i. ha^{-1} as a basal soil drench prior to transplanting.

Each treatment consisted of five replicates, each containing a single plant, and the experiment was laid out in a completely randomized block design in greenhouse. Plants were irrigated daily with tap water and maintained for a period of 45 days. At harvest, plants were uprooted carefully, and growth parameters including shoot and root length as well as shoot and root biomass were recorded. Root systems were gently washed under running water and examined visually for the enumeration of galls and egg masses [8].

Statistical analysis

All data were analysed using Origin Pro software. A one-way analysis of variance (ANOVA) to determine the significance of treatment effects on plant growth parameters, nematode infestation parameters and juvenile mortality of *Meloidogyne incognita*. Treatment means were compared using Tukey's HSD at 5% level of significance ($P < 0.05$). The results are presented as mean ± standard error.

Results and Discussion

One way ANOVA revealed that all plant growth, nematode infestation, and juvenile mortality parameters differed significantly among treatments ($P < 0.05$, Table. 1)

Effect Of Cell-free Filtrates Of Different Biocontrol Agents On Juvenile Mortality

Juvenile mortality differed significantly among treatments ($F = 1821.35$, $P < 0.05$). The highest mortality of *Meloidogyne incognita* J2 was observed in *P. lilacinum* (66.0%), followed by *T. asperellum* (52.6%) and *P. fluorescens* (45.0%). No mortality was recorded in VP (0.00%), while the untreated control showed minimal

juvenile mortality (1%). (Fig. 1). The pronounced nematicidal activity of *P. lilacinum* is consistent with its ability to produce extracellular enzymes and toxic secondary metabolites that compromise the integrity of nematode eggs and cuticle [13]. The moderate but significant mortality induced by *T. asperellum* and *P. fluorescens* suggests the involvement of nematicidal toxic metabolites, antibiotics, and enzyme-mediated antagonism indicating their direct inhibitory effects on infective juveniles under *in vitro* conditions [14].

Effect of Treatments on Plant Growth Parameters

Shoot length varied significantly among treatments ($F = 571.52$, $P < 0.05$). The maximum shoot length was recorded in *T. asperellum* (53.2 cm), followed by *P. fluorescens* (50.0 cm) and VP (47.26 cm), whereas the untreated inoculated control recorded the minimum shoot length (21.96 cm). Shoot weight also differed significantly across treatments ($F = 1672.11$, $P < 0.05$), with *T. asperellum* showing the highest shoot weight (141.96 g), followed by VP (133.66 g) and *P. fluorescens* (103.16 g). The untreated control recorded the lowest shoot weight (57.72 g). Root growth parameters were significantly influenced by the treatments. Root length showed significant variation ($F = 75.22$, $P < 0.05$), with *T. asperellum* (18.9 cm) and *P. fluorescens* (17.66 cm) recording higher values compared to *P. lilacinum* (13.86 cm) and VP (11.44 cm), while the untreated control showed the lowest root length (8.2 cm). Root weight also differed significantly among treatments ($F = 153.77$, $P < 0.05$), with *T. asperellum* (35.8 g) and *P. fluorescens* (34.04 g) recording higher root weights, whereas the untreated control recorded the minimum value (14.96 g).

Enhanced shoot length, shoot weight, root length, and root weight in treated plants indicate effective mitigation of nematode-induced stress and improved plant physiological performance. *Trichoderma asperellum* resulted in the greatest improvement in both shoot and root growth, followed by *Pseudomonas fluorescens*. The superior growth response in these treatments can be attributed to their combined antagonistic activity against *Meloidogyne incognita* and their well-documented plant growth-promoting traits, including enhanced rhizosphere colonization, nutrient mobilization, and production of bioactive metabolites [15,16]. In contrast, *Purpureocillium lilacinum* showed comparatively moderate effects on plant growth, suggesting that its primary contribution lies in nematode suppression rather than direct growth promotion. The untreated inoculated control consistently recorded the lowest growth values, reflecting severe impairment of root function due to extensive galling, which restricts water and nutrient uptake and disrupts normal plant metabolism [13]. Collectively, these results highlight the differential efficacy of microbial biocontrol agents and underscore their potential role in improving plant growth through both physiological stimulation and reduction of nematode pressure.

Effect of Treatments on Nematode infestation Parameters

Nematode infestation parameters showed significant variation among the treatments. The number of galls per plant differed significantly among treatments ($F = 650.76$, $P < 0.05$). The lowest gall numbers were recorded in VP (16.8), followed by *P. lilacinum* (87.6) and *T. asperellum* (115.8), whereas the untreated inoculated control recorded the highest galling intensity (237.4). Egg mass production also varied significantly across treatments ($F = 810.40$, $P < 0.05$), with VP recording the lowest number of egg masses (5.4), followed by *P. lilacinum* (10.8) and *T. asperellum* (13.6). The untreated control recorded the highest egg mass count (52.6). The untreated inoculated control exhibited the highest galling intensity and egg mass production, reflecting unrestricted nematode penetration, establishment, and multiplication. In contrast, all biocontrol treatments significantly reduced nematode infestation, demonstrating their effectiveness in suppressing nematode activity within the host root system.

Among the treatments, Velum Prime (Fluopyram) recorded the lowest number of galls and egg masses, followed by *Purpureocillium lilacinum* and *Trichoderma asperellum*. The strong suppressive effect of *P. lilacinum* is consistent with its egg-parasitic nature, which limits nematode multiplication and reduces inoculum potential. Similarly, reductions observed with *T. asperellum* and *Pseudomonas fluorescens* is due to their antagonistic metabolites and rhizosphere-mediated suppression of nematode establishment [13, 15, 16].

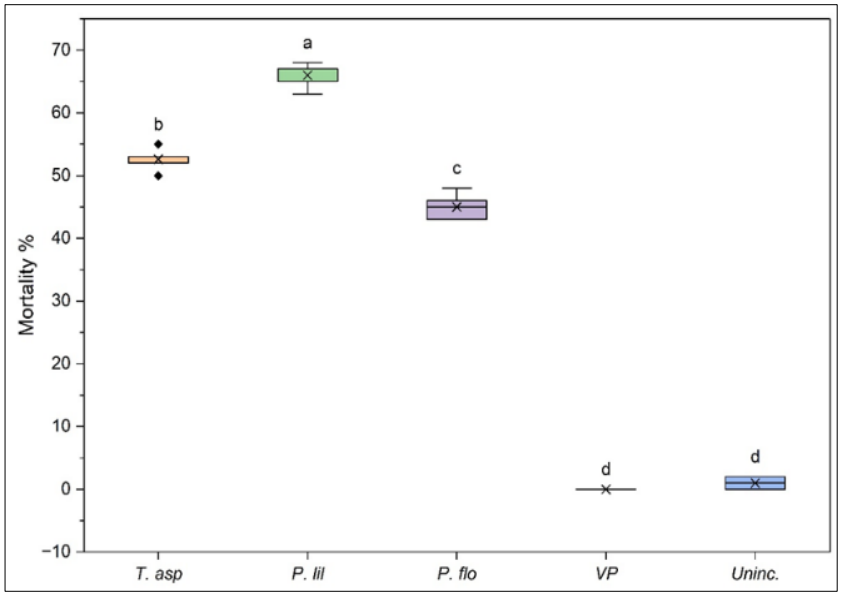
Table 1: Effect of different biocontrol treatments on plant growth and nematode infestation parameters of root-knot nematode *(Meloidogyne incognita*) in tomato under pot conditions

Treatment	SL	SW	RL	RW	Galls	EM
T. asperellum	53.2±0.583^{a}	141.96±1.059^{a}	18.9±0.319^{a}	35.8±0.323^{a}	115.8±1.562^{c}	13.6±0.51^{c}
P. lilacinum	39.2±0.374^{d}	77.14±0.357^{d}	13.86±0.399^{b}	20.68±0.193^{c}	87.6±1.327^{d}	10.8±0.374^{d}
P. fluorescens	50±0.316^{b}	103.16±1.042^{c}	17.66±0.392^{a}	34.04±0.319^{a}	152.4±3.076^{b}	21.2±0.583^{b}
Velum Prime	47.26±0.555^{c}	133.66±0.948^{b}	11.44±0.913^{c}	23.8±1.497^{b}	16.8±0.735^{e}	5.4±0.51^{e}
Uninc.	21.96±0.694^{e}	57.72±0.796^{e}	8.2±0.202^{d}	14.96±0.287^{d}	237.4±6.063^{a}	52.6±1.077^{a}
F value	571.52381	1672.11189	75.22298	153.76614	650.75942	810.39815

*Values represent mean ± SE. Means followed by different lowercase letters within a column are significantly different at $P < 0.05$ according to the Tukey's test

**SL = shoot length, SW = shoot weight, RL = root length, RW = root weight, EM = egg masses per plant

***F values indicate significant treatment effects for all parameters



*Box plots represent the percent mortality for each biocontrol agent (*Trichoderma asperellum* [T. asp], *Purpureocillium lilacinum* [P. lil], *Pseudomonas fluorescens* [P. flo], VelumPrime (Fluopyram) [VP], and uninoculated control).
**Different lowercase letters above the boxes indicate significant differences among treatments at $P < 0.05$.

Fig 1: Effect of cell-free filtrates of different biocontrol agents on juvenile mortality (%) of the root-knot nematode (*Meloidogyne incognita*) under *in-vitro* conditions

Conclusion

The study demonstrates that the tested microbial biocontrol agents are effective in suppressing *Meloidogyne incognita* and improving tomato growth under *in vitro* and pot conditions. *Purpureocillium lilacinum* exhibited the highest nematicidal activity against infective juveniles, while *Trichoderma asperellum* and *Pseudomonas fluorescens* significantly reduced nematode infestation and enhanced plant growth. The differential performance of these agents reflects their distinct modes of action, with combined nematode suppression and plant growth-promoting effects. These findings support the use of microbial biocontrol agents as sustainable alternatives to chemical nematicides. Future research should focus on field validation and optimization of application strategies to enhance efficacy and consistency under diverse agroecosystems.

References

1. Kumar A, Kumar V, Gull A, Nayik GA. Tomato (*Solanum lycopersicon*). In: Nayik GA, Gull A, editors. Antioxidants in vegetables and nuts - properties and health benefits. Singapore: Springer Singapore; 2020. p. 191-207.
2. Phani V, Khan M, Dutta T. Plant-parasitic nematodes as a potential threat to protected agriculture: Current status and management options. Crop Protection. 2021 Jun;144.
3. Seid A, Fininsa C, Mekete T, Decraemer W, Wesemael WM. Tomato (*Solanum lycopersicum*) and root-knot nematodes (Meloidogyne spp.)—a century-old battle. Nematology. 2015;17(9):995-1009.
4. Jones JT, Haegeman A, Danchin EGJ, Gaur HS, Helder J, Jones MGK, *et al*. Top 10 plant-parasitic nematodes in molecular plant pathology. Molecular Plant Pathology. 2013 Dec;14(9):946-961.
5. Rawal S. A review on root-knot nematode infestation and its management practices through different approaches in tomato. Tropical Agroecosystems TAEC. 2020;1(2):92-96.
6. Kiewnick S, Sikora R. Biological control of the root-knot nematode Meloidogyne incognita by Paecilomyces lilacinus strain 251. Biological Control. 2006;38(2):179-187.
7. Poveda J, Abril-Urias P, Escobar C. Biological control of plant-parasitic nematodes by filamentous fungi inducers of resistance: Trichoderma, mycorrhizal and endophytic fungi. Frontiers in Microbiology. 2020;11:992.
8. Khan Z, Kim SG, Jeon YH, Khan HU, Son SH, Kim YH. A plant growth promoting rhizobacterium, *Paenibacillus polymyxa* strain GBR-1, suppresses root-knot nematode. Bioresource Technology. 2008;99(8):3016-3023.
9. Tranier MS, Pognant-Gros J, Quiroz RD la C, González CNA, Mateille T, Roussos S. Commercial biological control agents targeted against plant-parasitic root-knot nematodes. Brazilian Archives of Biology and Technology. 2014;57(6):831-841.
10. Barker KR, Carter CC, Sasser JN. An advanced treatise on Meloidogyne: methodology. Vol. 2. Raleigh (NC): Department of Plant Pathology, North Carolina State University; 1985.
11. Vagelas I, Gowen SR. Control of *Fusarium oxysporum* and root-knot nematodes (Meloidogyne spp.) with *Pseudomonas oryzihabitans*. Pakistan Journal of Phytopathology. 2012;24(1):32-38.
12. Tadigiri S. In-vitro evaluation of potential bio agents on hatching and mortality of root knot nematode, Meloidogyne incognita. 2020.
13. Rigobelo EC, Nicodemo D, Babalola OO, Desoignies N. *Purpureocillium lilacinum* as an agent of nematode control and plant growth-promoting fungi. Agronomy. 2024;14(6):1225.

14. Baazeem A, Almanea A, Manikandan P, Alorabi M, Vijayaraghavan P, Abdel-Hadi A. *In vitro* antibacterial, antifungal, nematocidal and growth promoting activities of *Trichoderma hamatum* FB10 and its secondary metabolites. Journal of Fungi. 2021;7(5):331.
15. Tariq Javeed M, Farooq T, Al-Hazmi AS, Hussain MD, Rehman AU. Role of Trichoderma as a biocontrol agent (BCA) of phytoparasitic nematodes and plant growth inducer. Journal of Invertebrate Pathology. 2021;183:107626.
16. Meyer SLF, Everts KL, Gardener BM, Masler EP, Abdelnabby HME, Skantar AM. Assessment of DAPG-producing Pseudomonas fluorescens for management of Meloidogyne incognita and Fusarium oxysporum on watermelon. Journal of Nematology. 2016 Jan;48(1):43-53.