***Original Research Article***

# Biological Control of Root knot Nematode, *Meloidogyne incognita* Using Nematode Antagonist in Tomato




## ABSTRACT

Tomato (*Solanum lycopersicum* L.) is the world's largest vegetable crop after potato and sweet potato. Root knot nematodes, *Meloidogyne incognita*, pose a significant threat to tomato crops worldwide. To combat this nematode pest, biological control methods have been developed to reduce the reliance on chemical nematicide and promote sustainable agriculture practices.The antagonistic fungi/bacteria can colonize the root zone and produce enzymes that have nematicidal properties. These methods of control not only protect the tomato crop but also promote the overall health and resilience of the agroecosystem. Highest reduction of root knot nematode adult females (13.42), egg masses (5.87) and eggs (103.72) and nematode population in soil (104.51) were found by application of *P. lilacinum* as soil application among all the treatments. Root knot index was on bar with each other in case of seed treatment of *T. asperellum* and soil application of *T. asperellum*.


Keywords: Root knot nematode, *Meloidogyne incognita, Purpureociliumlilacinum, Trichoderma asperellum, Pseudomonas fluorescens*, Biological Control, Tomato

## 1. INTRODUCTION

The root-knot nematodes (*Meloidogyne* spp.) are sedentary endoparasites and rankare among the most damaging agricultural pests, attacking a wide range of crops (1), in particularly vegetables. They, causeing dramatic yield losses, mainly in tropical and sub-tropical agriculture (2). The infection beginsstarts with the root penetration of second-stage juveniles (J2) hatched in the soil from eggs encapsulated in egg masses laid by females on the infected roots. Tomato (*Solanum lycopersicum* L.) is the world's largest vegetable crop after potato and sweet potato. India is one of the largest producers of tomato in the world and the total area under tomato cultivation in Tamil Nadu is 45.82 thousand hectares,a with a total production of 1489.03 thousand tonnes. Root knot nematode, *M. incognita*, pose a

**Comment [A1]:** It's very vague, lacks an explanation of the methodology. It's not clear which antagonist organisms are being used.

**Comment [A2]:** Replace root knot nematodes with *M. incognita* for clarity, as root-knot nematodes is a very general term, giving the impression that various *Meloidogyne* species were used in this study.

**Comment [A3]:** Consider replace with 'occurred with the'

**Comment [A4]:** Consider changingto *Solanum lycopersicum*.

**Comment [A5]:**

significant threat to tomato crops worldwide. Due to itsSince, it is having short life span, high fecundity rate, polyphagous nature, and widespread distribution, it is considered a reason for being one of the most dangerous nematode genera affecting tomatoes. These microscopic nematodes invade the roots of tomato plants, causing swelling and formation of galls. This can lead to stunted growth, wilting, reduced yields, and, in some cases,sometimes it associatione with disease causing pathogens may leads to plant death. Several control measures were employed to control root-knot nematodes in infested areas. The traditional method of nematode control is based mainly on chemical nematicides. However, the potential negative impact on environment and ineffectiveness after prolonged use have led to a total ban or restricted use of most chemical nematicides and an urgent need for safe and more effective alternatives. To combat this nematode pest, biological control methods have been developed to reduce the reliance on chemical nematicide and promote sustainable agriculture practices. By generating lytic enzymes, antibiosis, paralysis and parasitism, they may directly decrease the damage caused by plant-parasitic nematodes. By enhancing the plant's ability to absorb nutrients and water, changing the root architecture, and altering rhizosphere interactions, these methods and reduce the damage caused by parasitic nematodes. AThe antagonistic fungi/bacteria can colonize the root zone and produce enzymes with that have nematicidal properties. They also have the ability to induce systemic resistance in plants, making themit more resistant to nematode attack. By utilizing these nematode antagonists, farmers can effectively manage and reduce the damage caused by root knot nematodes in tomatoes. These methods of control not only protect the tomato crop but also promote the overall health and resilience of the agroecosystem. Hence, the technology based onthe antagonistic fungi/bacteria, aimed at basedtechnology involving managingfor the management of*M. incognita* in tomatoes,was investigated.

## 2. MATERIAL AND METHODS

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### 2.1 Nematode inoculum

The inoculum of root-knot nematode, *M. incognita*, isolated from naturally infected tomatoes,was obtained from pure culture raised by a single egg mass. The nematode populationand wasmaintained on a susceptible cultivar of roots of tomato plants (*Solanum lycopersicum* L., nematode susceptible variety) in the greenhouse.

**Comment [A7]:** Please use another word instead of 'culture,' as this term might imply that the nematode can be cultivated in a culture medium.

**Comment [A8]:** Which cultivar was used in the experiment? Please specify

**Comment [A9]:** Please provide the average temperature of the greenhouse

The experiment was conducted in the greenhouse at the~~of~~ Department of Nematology, AnbilDharmalingam Agricultural College and Research Institute, Navalurkuttapattu, Trichy during the period 2022-2023. The study followed a~~ing~~ Complete Randomized Design (CRD) with eight treatments, ~~and~~ each replicated three times.

One-month-old seedlings of tomato var. CO5 were planted @at three seedlings per ~~/~~pot,each filled with 5 kg of steamsterilized soil mixture containing 2 parts red soil, 1 part sand, and 1 part well decomposed farmyard manure. One week after planting, the seedlings were thinned to one ~~number~~ per pot. The pots were watered periodically, and after seven days, 5000 newly hatched juveniles from egg masses of *M. incognita* (extracted from tomato plants) were inoculated at a~~into~~ 7.5 cm depth around the root zone of each~~the~~ plant in ~~each~~ everypot.

The talc-based formulation of *Purpureocilliumlilacinum*~~*P. lilacinum*~~was obtained from the Department of Nematology, while~~and~~ *Trichoderma*. *asperellum* and *Pseudomonas*~~.~~*fluorescens* were obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India. The treatments were imposed as follows: ~~as~~ T1 – -Seed treatment of *P.*~~*urpureocillium*~~*lilacinum*at~~@~~ 10g/kg of seeds, T2 – -Seed treatment of *T.*~~*richoderma*~~*asperellum* at ~~@~~ 10g/kg of seeds, T3 – -Seed treatment of *P*~~*seudomona.s*~~ *fluorescens*at~~@~~ 10g/kg of seeds,T4 – -Soil application of *P. lilacinum*at~~@~~ 50mg/pot with a 5kg capacity, T5 – -Soil application of *T. asperellum*at~~@~~ 50mg/pot with 5kg capacity,T6 – -Soil application of *P. fluorescens* at ~~@~~ 50mg/pot with a 5kg capacity, T7 – -Soil application of Carbofuran 3G at~~@~~ 300mg/pot with a 5kg capacity and T8 – Untreated control.

The effects of the treatments on tomato plant growth parameters ~~of tomato~~ and nematode population buildup ~~of nematode~~ were recorded by taking biometric measurements, including ~~of~~ shoot length (cm), fresh shoot weight (g), d~~D~~ry shoot weight (g), f~~F~~ruit yield (g), root length (cm), fresh root weight (g), and dry root weight(g).Nematode population per ~~/~~200 g ofsoil was estimatedusing~~by~~ Cobb's sieving and decanting method (3) and Modified Baermann funnel technique (4). The n~~N~~umber of adult female per ~~/~~gram of root, the number of egg mass per ~~/~~root, the number of eggs per ~~/~~egg mass, and root knot index were also recorded. The data were statistically analyzed as per the analysis of variance ~~test of completely randomized block design.~~

**Comment [A10]:** Did each experiment have only 3 repetitions?

**Comment [A11]:** You don't need to use this symbol @. Consider removing all the others from the text.

**Comment [A12]:** Provide the stage of development.

**Comment [A13]:** Please inform the concentration used for each biological agent applied in the treatments.

**Comment [A14]:** What does the control treatment consist of? Are these plants without inoculation and untreated with the biological agent, or are they inoculated plants without treatment?

**Comment [A15]:** What stages of Meloidogyne were quantified in the soil? The sentence suggests that other nematodes were also quantified. As it is a sedentary endoparasite, the population of root-knot nematodes in the soil is not usually quantified. And how was this quantification performed? Is Cobb's method (1918) recommended for quantifying Meloidogyne in the soil?

**Comment [A16]:** Indicate the reference for the severity rating scale used to determine the gall index.

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**Comment [A17]:** Explain in more detail how the statistical analysis of the data was conducted. Was any mean test applied? If so, which one? What software was used for the analyses?

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## 3. RESULTS AND DISCUSSION

Observations showed that the application of *P. lilacinum, T. asperellum*,and*P. fluorescens* increased all evaluated parameters~~shoot length, shoot weight, dry shoot weight, fruit yield, root length, root weight and dry root weight~~ of tomato over control treatment ~~in a treatment dependent manner~~ (Table 1). Maximum plant growth parameters were recordedwith the~~in~~ soil application of *P. lilacinum* and the seed treatment of *P. lilacinum*,followed by soil application of carbofuran. The a~~A~~pplication of *P. lilacinum* in soil was found to be the most effective treatment among all ~~treatments~~. The ~~—~~h~~H~~ighest reduction *of* ~~*root—knot nematode*~~*M. incognita* adult females (13.42), egg masses (5.87),~~and~~ eggs (103.72), and nematode population in soil (104.51) was~~erefound~~ achieved through the~~by~~ application of *P. lilacinum*in~~as~~ soil, ~~application~~ among all the treatments. Minimum number of nematode population was also recorded as 110.26adm~~,~~ 117.72 in the case of seed treatment with~~of~~*P. lilacinum* and soil application of carbofuran, respectively. The l~~L~~owest root knot index (1.00) was recorded in case of soil application of *P. lilacinum*, followed by seed treatment with~~of~~*P. lilacinum*(1.42) and soil application of carbofuran (1.73). The r~~R~~oot knot index was similar between the ~~on bar with each other in case of~~ seed treatment ~~of *T. asperellum*~~and soil application of *T. asperellum*(Table 2).

Using fungal/ bacterial antagonistto manage nematode problem in crop plants is a sustainable and eco friendly approach. It is important to note that biological control methods may need to be combined or integrated with each other, as well as with other management practices, to achieve optimal results. Regular monitoring and assessment of nematode populations are crucial for implementing appropriate control measures. By employing biological control strategies, growers can effectively manage the root knot nematode, *M. incognita* in tomato crops, reducing yield losses and minimizing the need for chemical nematicides.

Egg parasitism is the main mode of action of *P. lilacinum* against parasitic nematodes (5). *P. lilacinum* is capable of colonizing the gelatinous matrix (6). Eggs in earlier embryonic stages are reported to be more successfully infected by nematophagusfungi (7).The specie *T.asperellum* is a ubiquitous soil fungus ~~which~~ thatcolonizes root surfaces and root cortices, providing~~and provided~~ excellent control of root-knot nematodes (8). The highly branched

**Comment [A18]:** This section is where you should highlight your results and demonstrate the relevance of your study. Few citations were used, with most providing only generalized information. There is a wealth of literature on the biocontrol of gall-forming nematodes. Utilize as many as possible to compare whether the results are similar or different; this will enrich your work and underscore its importance to the scientific community.

**Comment [A19]:** Which parameter specifically? It's too vague.

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conidiophores of *Trichoderma* produce conidia that can attach to different nematode stages. The aApplication of *Trichoderma* species resulted in reduced nematode galling and improved plant growth and tolerance. *Pseudomonas. fluorescens* improve the plant growth promotion of tomatoes either bybecause of it increasinge the phosphorus content of the soil or produced more indole acetic acid (IAA) as compared to the untreated control (9).

In the present study, the soil application of *P. lilacinum* has effectively suppressed root knot nematode infestation in tomatoes compared to thethan that seed treatment of *P. lilacinum*. This might maybe due toattributed to the better good establishment of the biocontrol agent in the plant rhizosphere. The reason observed increase in for increased plant growth, yield, and other parameters observed here could be due to the attributed to the release of growth promoting substances by the bio-agents or theby production ofing toxic metabolites which thatinhibit nematodes and exclude other deleterious microorganisms. Reduction in nematode galls and egg masses might may result fromthe be due to high rhizosphere competency of bio-agents, enabling them to as they can easily colonize roots and may reduce potential feeding sites for nematodes. The reduction decrease in the numberof root gall number maycould be attributeddue to the failure of the majority of the juveniles to penetrate the host root. Furthermore, the use of fungal antagonist, such as *Purpureocilium and Trichoderma* has shown promise iningfor the management of *M. incognita* in tomatoes.

**Table 1. Effect of fungal/bacterial antagonist on plant growth characters of tomato inoculated with *M. incognita***

| Treatments | Shoot length (cm) | Fresh shoot weight (g) | Dry shoot weight (g) | Fruit yield (g/plant) | Root length (cm) | Fresh root weight (g) | Dry root weight (g) |
|---|---|---|---|---|---|---|---|
| $T_1$-Seed treatment of *Purpureocilliumlilacinum*at@ 10g/kg of seeds | 35.40 | 17.71 | 6.77 | 246.94 | 29.6 | 9.95 | 4.15 |
| $T_2$-Seed treatment of *Trichoderma asperellum*at@ 10g/kg of seeds | 28.75 | 15.90 | 6.12 | 205.74 | 25.45 | 8.92 | 3.69 |
| $T_3$-Seed treatment of *Pseudomonas fluorescens*at @ 10g/kg of seeds | 21.90 | 14.10 | 5.65 | 165.34 | 19.50 | 7.89 | 3.12 |
| $T_4$-Soil application of *P.* | 38.17 | 18.60 | 7.08 | 265.34 | 32.10 | 10.50 | 4.39 |

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|---|---|---|---|---|---|---|---|
| *lilacinum*at @ 50mg/pot with 5kg capacity | | | | | | | |
| $T_5$-Soil application of*T. asperellum*at@ 50mg/ pot with 5kg capacity | 27.17 | 15.46 | 6.01 | 195.34 | 23.98 | 8.62 | 3.49 |
| $T_6$-Soil application of *P. fluorescens*at@ 50mg/pot with 5kg capacity | 26.22 | 15.21 | 6.13 | 190.74 | 19.50 | 8.43 | 3.38 |
| $T_7$-Soil application of Carbofuran 3G at @ 300mg/pot with 5kg capacity | 31.90 | 16.83 | 6.46 | 230.84 | 27.60 | 9.47 | 3.93 |
| $T_8$- Untreated Control | 17.20 | 9.07 | 4.01 | 90.36 | 11.70 | 4.76 | 2.76 |
| CD (*P*=0.05) | 3.36 | 0.91 | 0.52 | 16.86 | 2.27 | 0.72 | 0.28 |

**Table 2. Effect of fungal/ bacterial antagonist on nematode population of tomato inoculated with *M. incognita***

| Treatments | No. of females/g of root | No.of egg masses/g of root | No. of eggs/egg mass | Nematode population /200g soil | Gall Index |
|---|---|---|---|---|---|
| $T_1$-Seed treatment of *Purpureocilliumlilacinum*at@ 10g/kg of seeds | 15.31 | 7.21 | 109.21 | 110.26 | 1.42 |
| $T_2$-Seed treatment of *Trichoderma asperellum*at@ 10g/kg of seeds | 20.22 | 11.32 | 119.29 | 124.17 | 2.13 |
| $T_3$-Seed treatment of *Pseudomonas fluorescens*at@ 10g/kg of seeds | 26.19 | 16.44 | 134.81 | 148.63 | 3.42 |
| $T_4$-Soil application of *P. lilacinum*at@ 50mg/ pot with 5kg capacity | 13.42 | 5.87 | 103.72 | 104.51 | 1.0 |
| $T_5$-Soil application of *T. asperellum*at@ 50mg/ pot with 5kg capacity | 21.08 | 12.22 | 120.17 | 127.24 | 2.13 |
| $T_6$-Soil application of *P. fluorescens*at@ 50mg/ pot with 5kg capacity | 22.03 | 13.72 | 125.17 | 130.19 | 2.24 |
| $T_7$-Soil application of Carbofuran 3G at@ 300mg/ pot with 5kg capacity | 17.13 | 9.86 | 115.13 | 117.72 | 1.73 |
| $T_8$- Untreated Control | 50.13 | 31.65 | 284.31 | 303.32 | 5.86 |
| CD (*P*=0.05) | 2.81 | 1.96 | 5.87 | 6.48 | 0.44 |

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Comment [A22]: Conduct the mean test (e.g., Tukey' test) and add the letters that statistically differentiate the treatments.

Comment [A23]: Pq utilizou cd e nao cv?

Comment [A24]: Why was CD used instead of CV? Why wasn't the mean test applied to compare the treatments?

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## 4. CONCLUSION

The present study demonstrates that ~~treatment~~ treating~~of~~ tomato plants with different fungal/bacterial antagonistagents against ~~root knot nematode,~~ *M. incognita* reveals ~~ed~~ that soil application of *P. lilacinum*~~has~~ effectively suppressesthis ~~root knot~~ nematode infestation in tomatoes. This method not only protects the tomato crop but also promote the overall health and resilience of the agroecosystem. Therefore, our results concluded that the application of *P.lilacinum* could be a sustainable and practical approach for managing the root knot nematode menace in tomato. However, more studies must be conducted under field conditions to confirm these results.

**Comment [A25]:** Without the mean test, it is not possible to assert that one treatment was superior to another, based solely on the mean value. The term 'suppressed' is incorrect in this context because *P. lilacinum* reduced the number of eggs and females, as well as the gall index, but there was still nematode reproduction. Additionally, the treatment with Carbofuran showed results close to that with *P. lilacinum*. Therefore, the mean test is important to accurately indicate the best treatment.

## REFERENCES

**Comment [A26]:** Review the references, as some contain minor errors in relation to the journal's standards.


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