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

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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 agro eco system. Highest reduction of root knot nematode adult females (73.2 per cent), egg masses (81.4 per cent) and eggs (63.5 per cent) and nematode population in soil (65.5 per cent) were occurred with the application of *P. lilacinum* as soil application among all

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the treatments. The lowest gall index (1.0) was recorded in case of soil application of *P. lilacinum* followed by seed treatment (1.42) of the same whereas it was on bar (2.13) with each other while applying seed treatment and soil application of *T. asperellum*.

Keywords: Root knot nematode; Meloidogyne incognita; Purpureocilium lilacinum; Trichoderma asperellum; Pseudomonas fluorescens; Biological Control; Solanum lycopersicum.

1. INTRODUCTION

The root-knot nematodes (*Meloidogyne* spp.) are sedentary endoparasites and rank among the most damaging agricultural pests, attacking a wide range of crops [1], particularly vegetables. They cause dramatic yield losses mainly in tropical and sub-tropical agriculture [2]. The infection begins 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, with a total production of 1489.03 thousand tonnes Root knot nematode, *M. incognita*, pose a significant threat to tomato crops worldwide. Due to its short life span, high fecundity rate, polyphagus nature and wide spread distribution, it is considered a one of the most dangerous nematode genera affecting tomatoes. The tomatoes are susceptible to *M. incognita* infestation and reported to cause 30 per cent yield loss [3]. 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 association with disease causing pathogens may leads to plant death. Several control measures were employed to control root-knot nematodes in infested areas. Neem based products are used to control plant parasitic nematodes. Due to the potency and efficacy of synthetic nematicides upon destructive nematode pests, the application of plant-based products slowly diminished. The traditional method of nematode control is based mainly on chemical nematicides. The granular form such as Carbofuran 3G and Aldicarb was used by the farmers. However, the potential negative impact on environment and ineffectiveness after prolonged use have led to a total ban or restricted use of these chemical nematicides. Hence, it is 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 root architecture, and altering rhizosphere interactions, these methods reduce the damage caused by parasitic nematodes. Antagonistic fungi/ bacteria can colonize the root zone and produce enzymes with nematicidal properties. They also have the ability to induce systemic resistance in plants, making them more resistant to nematode attack. By utilizing these nematode antagonist 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 agro ecosystem. Hence, the technology based on antagonistic fungi/ bacteria, aimed at managing *M. incognita* in tomatoes was investigated.

2. MATERIALS AND METHODS

2.1 Nematode Inoculum

The inoculum of root-knot nematode, *M. incognita* was extracted from the rhiosphere soil of naturally infected tomatoes was attained by a single egg mass. The nematode population was maintained on susceptible cultivar of PKM 1 tomato in greenhouse [4].

The experiment was conducted in the greenhouse at the Department of Nematology, Anbil Dharmalingam Agricultural College and Research Institute, Navalurkuttapattu, Trichy during the period 2022-2023. The average temperature of the greenhouse is 75° F during the day and 70° F at night. The study followed Complete Randomized Design (CRD) with eight treatments, 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 steam sterilized soil mixture containing 2 parts red soil, 1 part sand, and 1 part well decomposed farm yard manure. One week after planting, the seedlings were thinned to one per pot. The pots were watered periodically, and after seven days, 5000 newly second stage juveniles from egg masses of *M. incognita* (extracted from tomato plants) were inoculated at 7.5 cm depth around the root zone of each plant in every pot.

The talc- based formulation of *Purpureocillium lilacinum* was obtained from the Department of Nematology, while *Trichoderma asperellum* and *Pseudomonas fluorescens* were obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India. The spore load of the fungal antagonist is 2x 10^7 cfu/g of talc and for *Pseudomonas fluorescens* it was 2x 10^8 cfu/g of talc. The treatments were imposed as follows: T1-Seed treatment of *P. lilacinum* at 10g/kg of seeds, T2-Seed treatment of *T. asperellum* at 10g/kg of seeds, T3-Seed treatment of *P. 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 a 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 (*M. incognita* alone).

The experiment was terminated at eight weeks after transplanting and the effects of the treatments on tomato plant growth parameters and nematode population buildup were recorded by taking biometric measurements, including shoot length (cm), fresh shoot weight (g), dry shoot weight (g), fruit yield (g), root length (cm), fresh root weight (g), and dry root weight (g). Nematode population per 200 g of soil was estimated using Cobb's sieving and decanting method [5] and Modified Baermann funnel technique [6]. The number of adult female per g root, the number of egg mass per root, the number of eggs per egg mass and gall index were also recorded. The gall index was rated by visually for the number of galls using 0–5 scale galling index (GI) as follows: 0 = 0 galls; 1 = 1–2 galls; 2 = 3–10 galls; 3 = 11–30 galls; 4 = 31–100 galls; 5 > 100 galls [7]. The data were statistically analyzed as per the analysis of variance [8].

3. RESULTS AND DISCUSSION

Observations showed that the application of *P. lilacinum, T. asperellum* and *P. fluorescens* increased all evaluated parameters of tomato over control treatment (Table 1). The highest biometric measurements *viz*., shoot length (cm), fresh shoot weight (g), dry shoot weight (g), fruit yield (g), root length (cm), fresh root weight (g), and dry root weight (g) were recorded with the soil application of *P. lilacinum* and the seed treatment of *P. lilacinum* followed by soil application of carbofuran. The application of *P. lilacinum* in soil was found to be the most effective treatment among all. The highest reduction of *M. incognita* adult females (73.2 per cent), egg masses (81.4 per cent), eggs (63.5 per cent), and nematode population in soil (65.5 per cent) was achieved through the application of *P. lilacinum* in soil, among all the treatments. Smallest average of nematode population was also recorded as 110.26, 117.72 in the case of seed treatment with *P. lilacinum* and soil application of carbofuran, respectively. The lowest root knot index (1.00) was recorded with the soil application of *P. lilacinum*, followed by seed treatment of *P. lilacinum* (1.42) and soil application of carbofuran (1.73). The root knot index was similar between the seed treatment 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 [9]. *P. lilacinum* is capable of colonizing the gelatinous matrix [10]. Eggs in earlier embryonic stages are reported to be more successfully infected by nematophagus fungi [11]. The specie *T. asperellum* is a ubiquitous soil fungus that colonizes root surfaces and root cortices providing excellent control of root-knot nematodes [7]. The highly branched conidiophores of *Trichoderma* produce conidia that can attach to different nematode stages. The application of *Trichoderma* species resulted in reduced nematode galling and improved plant growth and tolerance. *Pseudomonas fluorescens*

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 *Purpureocillium lilacinum* 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. lilacinum* at 50mg/ pot with 5kg capacity	38.17	18.60	7.08	265.34	32.10	10.50	4.39
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 *Purpureocillium lilacinum* at 10g/kg of seeds	15.31 (69.45)	7.21 (77.21)	109.21 (61.58)	110.26 (63.64)	1.42
T_2-Seed treatment of *Trichoderma asperellum* at 10g/kg of seeds	20.22 (59.66)	11.32 (64.23)	119.29 (58.04)	124.17 (59.06)	2.13
T_3-Seed treatment of *Pseudomonas fluorescens* at 10g/kg of seeds	26.19 (47.75)	16.44 (48.05)	134.81 (52.58)	148.63 (50.99)	3.42
T_4-Soil application of *P. lilacinum* at 50mg/ pot with 5kg capacity	13.42 (73.22)	5.87 (81.45)	103.72 (63.51)	104.51 (65.54)	1.0
T_5-Soil application of *T. asperellum* at 50mg/ pot with 5kg capacity	21.08 (57.94)	12.22 (61.39)	120.17 (57.73)	127.24 (58.05)	2.13
T_6-Soil application of *P. fluorescens* at 50mg/ pot with 5kg capacity	22.03 (56.05)	13.72 (56.65)	125.17 (55.97)	130.19 (57.07)	2.24
T_7-Soil application of Carbofuran 3G at 300mg/ pot with 5kg capacity	17.13 (65.82)	9.86 (68.84)	115.13 (59.50)	117.72 (61.18)	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

The figures in the parenthesis denotes the per cent decrease over control

improve the plant growth promotion of tomatoes either by increasing the phosphorus content of the soil or produced more indol acetic acid (IAA) compared to the untreated control [12,13].

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

4. CONCLUSION

The present study demonstrates that treating tomato plants with different fungal/ bacterial antagonist agents against *M. incognita* reveals that soil application of *P. lilacinum* effectively suppresses this nematode infestation in tomatoes. This method not only protects the tomato crop but also promote the overall health and resilience of the agro ecosystem. Therefore, our results conclude 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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