

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 agro eco system. 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 par with each other in case of seed treatment of *T. asperellum* and soil application of *T. asperellum*

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

1. INTRODUCTION

The root-knot nematodes (*Meloidogyne* spp.) are sedentary endoparasites and are among the most damaging agricultural pests attacking a wide range of crops (1), in particular vegetables, causing dramatic yield losses mainly in tropical and sub-tropical agriculture (2). The infection starts with root penetration of second-stage juveniles (J2) hatched in soil from eggs encapsulated in egg masses laid by females on the infected roots. Tomato (*S. 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 ha with a total production of 1489.03 thousand tonnes. Root knot nematode, *M. incognita*, pose a significant threat to tomato crops

worldwide. Since, it is having short life span, high fecundity rate, polyphagous and wide spread is 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 sometimes it associate with disease causing pathogen 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 and reduce the damage caused by parasitic nematodes. The antagonistic fungi/ bacteria can colonize the root zone and produce enzymes that have nematicidal properties. They also have the ability to induce systemic resistance in plant, making it more resistant to nematode attack. By utilizing these nematode antagonist farmers can effectively manage and reduce the damage caused by root knot nematodes in tomato. These methods of control not only protect the tomato crop but also promote the overall health and resilience of the agro ecosystem. Hence, the antagonistic fungi/ bacteria based technology involving for the management of *M. incognita* in tomato was investigated.

2. MATERIAL AND METHODS

2.1 Nematode inoculum

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

The experiment was conducted in the greenhouse of Department of Nematology, AnbilDharmalingam Agricultural College and Research Institute, Navalurkuttapattu, Trichy

during 2022-2023 following Complete Randomized Design (CRD) with eight treatments and each replicated three times.

One month old seedlings of tomato var. CO5 were planted @ three seedlings /pot filled with 5 kg steam sterilized soil mixture containing 2 parts red soil, 1 part sand and 1 part well decomposed farm yard manure. One week after planting, 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 into 7.5 cm depth around the root zone of the plant in each pot.

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

The effect of the treatments on plant growth parameters of tomato and population buildup of nematode were recorded by taking biometric measurements of 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/200 g soil was estimated by Cobb's sieving and decanting method (3) and Modified Baermann funnel technique (4). Number of adult female/g root, number of egg mass/ root, number of eggs/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.

3. RESULTS AND DISCUSSION

Observations showed that application of *P. lilacinum*, *T. asperellum* and *P. fluorescens* increased shoot length, shoot weight, dry shoot weight, fruit yield, root length, root weight and dry root weight of tomato over control in a treatment dependent manner (Table 1). Maximum plant growth parameters were recorded in soil application of *P. lilacinum* and seed treatment of *P. lilacinum* followed by soil application of carbofuran.

Application of *P. lilacinum* in soil found to be the most effective among all treatments.

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. Minimum number of nematode population was also recorded as 110.26, 117.72 in case of seed treatment of *P. lilacinum* and soil application of carbofuran respectively. Lowest root knot index (1.00) was recorded in case of soil application of *P. lilacinum* followed by seed treatment of *P. lilacinum*(1.42) and soil application of carbofuran (1.73). Root knot index was on par with each other in case of seed treatment of *T. asperellum* and soil application of *T. asperellum*(Table 2).

Using fungal/ bacterial antagonist to 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 nematophagous fungi (7). *T. asperellum* is a ubiquitous soil fungus which colonizes root surfaces and root cortices and provided excellent control of root-knot nematodes (8). The highly branched conidiophores of *Trichoderma* produce conidia that can attach to different nematode stages. Application of *Trichoderma* species resulted in reduced nematode galling and improved plant growth and tolerance. *P. fluorescens* improve the plant growth promotion of tomato because of it increase the phosphorus content of the soil or produced more indol acetic acid (IAA) as compared to untreated control (9).

In the present study, soil application of *P. lilacinum* has effectively suppressed root knot nematode infestation in tomato than that seed treatment of *P. lilacinum*. This might be due to

good establishment of the biocontrol agent in plant rhizosphere. The reason for increased plant growth, yield and other parameters observed here could be attributed to the release of growth promoting substances by bio-agents or by producing toxic metabolites which inhibit nematodes and exclude other deleterious microorganisms. Reduction in nematode galls and egg masses might be due to high rhizosphere competency of bio-agents as they can easily colonize roots and may reduce feeding sites for nematodes. The reduction of root gall number may be due to failure of majority of the juveniles to penetrate the host root. Furthermore, the use of fungal antagonist, such as *Purpureocillium* and *Trichoderma* has shown promising for the management of *M. incognita* in tomato.

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 <i>Purpureocilliumlilacinum</i> @ 10g/kg of seeds	35.40	17.71	6.77	246.94	29.6	9.95	4.15
T ₂ -Seed treatment of <i>Trichoderma asperellum</i> @ 10g/kg of seeds	28.75	15.90	6.12	205.74	25.45	8.92	3.69
T ₃ -Seed treatment of <i>Pseudomonas fluorescens</i> @ 10g/kg of seeds	21.90	14.10	5.65	165.34	19.50	7.89	3.12
T ₄ -Soil application of <i>P. lilacinum</i> @ 50mg/ pot with 5kg capacity	38.17	18.60	7.08	265.34	32.10	10.50	4.39
T ₅ -Soil application of <i>T. asperellum</i> @ 50mg/ pot with 5kg capacity	27.17	15.46	6.01	195.34	23.98	8.62	3.49
T ₆ -Soil application of <i>P. fluorescens</i> @ 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 @ 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 <i>Purpureocillium lilacinum</i> @ 10g/kg of seeds	15.31	7.21	109.21	110.26	1.42
T ₂ -Seed treatment of <i>Trichoderma asperellum</i> @ 10g/kg of seeds	20.22	11.32	119.29	124.17	2.13
T ₃ -Seed treatment of <i>Pseudomonas fluorescens</i> @ 10g/kg of seeds	26.19	16.44	134.81	148.63	3.42
T ₄ -Soil application of <i>P. lilacinum</i> @ 50mg/ pot with 5kg capacity	13.42	5.87	103.72	104.51	1.0
T ₅ -Soil application of <i>T. asperellum</i> @ 50mg/ pot with 5kg capacity	21.08	12.22	120.17	127.24	2.13
T ₆ -Soil application of <i>P. fluorescens</i> @ 50mg/ pot with 5kg capacity	22.03	13.72	125.17	130.19	2.24
T ₇ -Soil application of Carbofuran 3G @ 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

4. CONCLUSION

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

REFERENCES

1. N. Sahebani and Hadavi., Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum* Soil Biology and Biochemistry, 2008; 40(8):2016-2020
2. Kiewnick, S and Sikora, R.A. Biological control of root knot nematode, *Meloidogyne incognita* by *Paecilomyces lilacinum* strain 251. Biological control, 2006; 38(2):179-187.
3. Cobb, N.A. Estimating the nematode population of soil. United States Department of Agriculture, 1918; Circular No.1-48.
4. Schindler, A.F. A simple substitute for a Baermann funnel. Plant Disease Reporter. 1961; 45: 747-748.
5. Chen, SY., F.J. Chen. Fungal parasitism of *Heterodera glycines* eggs as influenced by egg age and pre-colonization of cysts by other fungi. Journal of Nematology, 2003; 35:271– 277.
6. Meyer, S. L. F., and W. P. Wergin. Colonization of soybean cyst nematode females, cysts, and gelatinous matrices by the fungus *Verticillium lecanii*. Journal of Nematology, 1998; 30(4): 436–450.
7. Khan, A., Williams, K. L., and Nevalainen, H. K. M. Infection of plant-parasitic nematodes by *Paecilomyces lilacinus* and *Monacrosporium lysipagum*. BioControl, 2006; 51:659–678.
8. Sharon, E., I. Chet, A. Viterbo, M. Bar-Eyal, H. Nagan, G.J. Samuels and Y. Spiegel. Improved attachment and parasitism of *Trichoderma* on *Meloidogyne javanica* in vitro. Eur. J. of Plant Pathol. 2009; 123 (3): 291 - 299.
9. Khan, M. Z., Akhtar, M. E., Mahmood-ul-Hassan, M., Mahmood, M. M., and M.N. Safdar. Potato tuber yield and quality as affected by rates and sources of potassium fertilizer. <http://dx.doi.org/10.1080/01904167.2012.653072>, 2012; 35, 664–677.