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Effect of *Trichoderma* spp. against Root-Knot Nematode (*Meloidogyne incognita*) on Tomato (*Lycopersicon esculentum* L. Mill)

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A B S T R A C T

The root-knot nematode (*Meloidogyne incognita*) is one of the important pathogen of Tomato plant in India. Pot experiments conducted for the evaluation of effect of *Trichoderma* species against root knot Nematode (*Meloidogyne incognita*) in Tomato. The results revealed that the Treatments T4proved to be the most effective treatment that showed better larval mortality (54.55% @24 hrs and 65.12% @48 hrs) and reduction in nematode population (113.33 %) and root gall/ root system(74.89%. The treatment of the soil with the antagonistic fungus improved nematode control as the isolates significantly reduced the nematode populations. The fungus enhanced plant growth and yield in all the treated pots.

Introduction

"Tomato (*Lycopersicon esculentum* L. MILL) is a major crop of world commerce and one of the most widely grown vegetables. It belongs to the family of Solanaceae, which contains about 85 genera and 2,300 species. Tomato is mostly affected by root- knot nematode (*Meloidogyne* spp) Bhardwaj (1972). Common species causing root- knot in tomato are *Meloidogyne incognita*, *Meloidogyne javanica*. Root-knot infection causes 24-26% loss in Tomato Sasser., (1979).

Root-knot nematodes (RKNs, *Meloidogyne* spp.) are sedentary, polyphagous root endoparasites and among the most damaging agricultural pests, attacking a wide range of crops. Their infection starts with root penetration of second-stage juvenies (J2), hatched in soil from eggs stored in egg masses that have been laid by the females on the infected roots Barker *et al.*, (1985).

The plants infected with root-knot nematodes have an unthrifty appearance and often show symptoms of yellowing, rotting, wilting and

premature shedding of the foliage with severe stunting that result in huge losses to the infected crops Saifullah *et al*., (1990).

Trichoderma isolates have been used successfully to control the damage caused by soil-borne pathogens in greenhouses and under opened-field conditions. *Trichoderma harzianum* and *Trichoderma viride* were tested for their capacity to reduce the incidence and pathogenicity of the root-knot nematode *Meloidogyne incognita* on tomato. In vitro studies demonstrated that all tested isolates were effective in causing second-stage juvenile (J2) mortality compared with the control. *Trichoderma* slightly reduced nematode damage to tomato in vivo Papavizas (1985). Devi *et al*., (2002) reported that *T. viride* or *T. harzianum* when mixed in soil @ 1 g/kg soil improved plant height, shoot weight, root length and weight and reduced M. incognita population. Goswami and Mittal (2004) reported that the culture filtrates of *T. viride* showed 60% toxicity compared to *P. lilacinus* (25%) against J2s of *M. incognita* infecting tomato and showed that the inhibition of hatching of *M. incognita* eggs was 65% by culture filtrates of *T. viride* compared to 40% by *P. lilacinus.* Pathak *et al.,* (2005) reported that application of *T. harzianum* or *T. virens* one week prior to nematode inoculation @4 and 8 g/kg soil significantly reduced the number of galls, number of j2s penetrated/plant root system and final *Meloidogyne spp*. population in soil compared to control. Zhang *et al.,* (2012) reported an isolate of *T. longibrachiatum* that had a strong lethal and parasitic effect on the J2s of *Meloidogyne incognita* at a concentration of 1.5 x 107 conidia/ml, inhibited and parasitized more than 88% J2 s in cucumber seedlings after 14 days. Sokhandani *et al*., (2016) showed that *T. longibrachiatum* concentrations at 108 conidia/mL suspension produced the best plant growth and reduced the reproduction of *M. javanica.* Several control measures like cultural practices, chemical and biological control are used to reduce the population of the nematode but unfortunately there are many problem associated with the use of chemical control. Biological control is a favourable alternative for the management of root knot nematodes, as it is economical, sustainable and environment friendly, thus, maintaining soil biodiversity and health. *Trichoderma* species are free living fungi, common in soil and root ecosystem. They are being widely investigated for their capacity to produce antibiotics, parasitize other fungi and compete or antagonize deleterious plant microorganisms and nematode pests Harman *et al.,* (2004).

Keeping in view of the above points the present titled " Effect of *Trichoderma* spp. against Root-Knot nematode*(Meloidogyne incognita*) on Tomato (*Lycopersicon esculentum* L. MILL*)"* was conducted with the following objectives:-

To observe the effect of *Trichoderma* spp. on larvae emergence of *Meloidogyne incognita* at 24 and 48 hr after exposure.

To count the number of root- knot infestation on tomato roots at 90 days after transplanting.

Materials and Methods

The nematode inoculation: The tomato plants were infected with root-knot nematode eggs which isolated from the infested roots of the eggplant (*Solanum melongena L.)* that obtained from Central Research Field, SHUATS, Allahabad. Sodium hypochlorite (NaOCl) was utilized for isolation of nematode eggs from root galls according to Hussey and Barker (1973). Moreover, the roots were stained for 15 minutes in an aqueous solution of Phloxine B stain to detect the presence of nematode egg masses (Holbrook *et al*., 1983).

The Pots experiment: The pot experiment was carried out using tomato plants, the Pots were 15 cm in diameter and 20 cm in depth and each pot filled with 1kg of autoclaved artificial mixture soil. The isolated eggs of root-knot nematode were applied at the rate of 1000 eggs / pot. Six treatments were applied, next to untreated check and chemical check (carbofuron @3g per pot) and each treatment was replicated three times, and each replicate contains three plantlet. 90 days after planting, the seedlings were uprooted and root systems were assessed for galling (number of galls/root system), and egg masses/root system, in addition to the mortality rate and root weight.

Application of *Trichoderma* spp: 10ml of each species of *Trichoderma* solution were applied to the respective pots near the root zone. The treatments were as follows; T_0 Control, $T_1$1000 larvae/plant + Tr 1 (2 ×105 spores per cm3), $T_2$1000 larvae/plant +Tr 2 (2.2 × 105spores per cm3), $T_3$1000larvae/plant +Tr 3 (2.3 ×105spores per cm3),$T_4$1000larvae/plant +Tr 4 (2.8 ×105spores per cm3), $T_5$1000larvae/plant +Tr 5 (2.4 ×105spores per cm3),$T_6$1000larvae/plant +Tr 6 (2.1 × 105spores per cm3), $T_7$1000larvae/ plant + Carbofuron @ 3g/pot. All treatments were applied two days after infection.

Effects of Trichoderma isolates on Mortality of *M. incognita.* The effect of Trichoderma isolates on juvenile mortality was studied under in vitro conditions. The juveniles (J2) of M. incognita used in this experiment were obtained from the nematode maintained on tomato roots. Approximately one hundred number of second stage juveniles (J2) were placed in 10 ml of suspension of Trichoderma isolates contained in sterilize petri plates and incubated at room temperature. The plates were examined after 24 and 48 hours and the number of dead juveniles were counted. Three replications were maintained. The juveniles inoculated in carbofuran 3G served as check and the juveniles inoculated in sterile distilled water were taken as control.

Evaluation of Trichoderma spp. on root gall / root system of Tomato plant. After 90 DAT (days after transplanting), the plants were uprooted, thoroughly washed and then fresh weight of roots and number of galls/ plant were recorded. Fresh galling damage was assessed per plant of pot in the greenhouse at harvest. The root galls of each plant were counted and scored for number of galls and indexing was done using 0-5 scale as follows: (Taylor and Sasser, 1978).

Statistical analysis

Data of the present study were analyzed using variance test (ANOVA). The experimental design was a complete randomized design. The least significant differences (LSD) at the 5% level of probability were determined.

Results and Discussion

Table 1 shows that all the treatments significantly reduces the root gall/ root system of Tomato when compared with inoculated control. Among the treatments T_4 shows significant decreased of root galls compare to all the other treatments.

Table 2 shows the effect of *Trichoderma* isolates on the larval population of *Meloidogyne incognita*. All the treatments significantly reduce the larval population compare to the inoculated control. Among the treatments T_4 shows maximum reduction of larval population compare to all the other treatments.

Table 3 shows the mortality rate of *Meloidogyne incognita* recorded after 24 and 48 hrs. All the treatments shows significant increase in percentage of mortality compared to the inoculated control. Among the *Trichoderma* isolates maximum larval mortality was observed in T_4.

Table.1 Root- knot index of *M. incognita*

Grade	Description	Reaction
0	No galls	Highly resistant
1	1-2 galls/ root system	Resistant
2	3-10 galls/ root system	Moderately resistant
3	11-30 galls/ root system	
4	31-100 galls/ root system	Susceptible
5	>100 galls/ root system	Highly susceptible

Table.2 Effect of *Trichoderma* spp. on root gall/ root system of Tomato plant at 90 DAT

Symbol	Name of Treatments	*mean	Galling index (0-5 scale)
	Control	232.44	5
T_1	*Trichoderma* isolate 1	163.15	5
T_2	*Trichoderma* isolate 2	113.33	5
T_3	*Trichoderma* isolate 3	95.53	4
T_4	*Trichoderma* isolate 4	74.89	4
T_5	*Trichoderma* isolate 5	90.74	4
T_6	Trichoderma isolate 6	130.29	5
T_7	Carbofuron 3G	65.30	4

*Mean of 3 replicates

Table.3 Effect of *Trichoderma* spp. on larval population / g of roots on Tomato plant at 90 DAI

Symbol	Name of treatment	*Mean
T_0	Control	373.86
T_1	*Trichoderma* isolate 1	263.49
T_2	*Trichoderma* isolate 2	222.55
T_3	*Trichoderma* isolate 3	167
T_4	*Trichoderma* isolate 4	113.33
T_5	*Trichoderma* isolate 5	133.78
T_6	*Trichoderma* isolate 6	237.19
T_7	Carbofuron 3G	88.95

*Mean of 3 replicates

Table.4 Effect of *Trichoderma* spp. on mortality of second stage larvae of *M.incognita* after 24 hrs an d 48 hrs

Symbol	Name of Treatments	*Mean of larval mortality	
		24 hrs	48 hrs
T_0	Control	1.11	4.95
T_1	*Trichoderma* isolate 1	40.33	47.23
T_2	*Trichoderma* isolate 2	47.22	56.22
T_3	*Trichoderma* isolate 3	43.45	53.86
T_4	*Trichoderma* isolate 4	54.55	65.12
T_5	*Trichoderma* isolate 5	52.89	57.75
T_6	*Trichoderma* isolate 6	41.55	45.94
T_7	Carbofuron 3G	63.74	71.38

*Mean of 3 replicates

Root-knot nematodes (*Meloidogyne* sp.) are sedentary endoparasites and are among the most destructive pests of agricultural crops. They are worldwide in distribution having a very wide host range. *Trichoderma* isolates have been used successfully to control the damage caused by soil-borne pathogens in greenhouses and under opened-field conditions (Papavizas, 1985). *Trichoderma* species also have been shown to have activity toward root-knot nematodes (Windham *et al*., 1989; Sharon *et al*., 2001). This experiment has therefore shown that *Trichoderma* isolates can reduce the number of *Meloidogyne incognita* juveniles' counts, as the counts were much lower in the treated plots than the control plots. After 90 DAT the tomato plant was uprooted and the number of root gall/root system was observed (Table 4).

The maximum root gall/ root system was recorded in T0 (Control) and maximum reduction of root galls/ root system was observed in T7 (Carbofuron 3G).Among the treatments T4 shows the maximum reduction of root gall/root system followed by T5 while minimum reduction of root gall/ root system was observed in T1.

The highest fresh root weight was recorded on pots with inoculated control (T0) and the lowest root weight was observed in T7 (Carbofuron 3G). Among the treatments Trichoderma isolate 4 showed significantly decreased from all the other treatments followed by Trichoderma isolates 5, Trichoderma isolate 3, Trichoderma isolate 2, Trichoderma isolate 6 and Trichoderma isolate 1.

The infected roots collected were cut and grinded and the total root population was determined with the number of larvae in 1g root and multiplying it with total weight of root. The highest larval population was recorded in T0. The lowest larval population was observed in T7 (Carbofuron 3G). Among the treatments T4 (Trichoderma isolate 4) show significant decrease in larval population followed by T5 (Trichoderma isolate 5),T3 (Trichoderma isolate 3),T2 (Trichoderma isolate 2),T6 (Trichoderma isolate 6) and T1 (Trichoderma isolate 1). The effect of Trichoderma isolates on juvenile mortality was studied under in vitro conditions. Approximately one hundred number of second stage juveniles (J2) were placed in 10

ml of suspension of Trichoderma isolates contained in sterilize petri plates and incubated at room temperature. The plates were examined after 24 and 48 hours and the number of dead juveniles were counted. Among the Trichoderma isolates the maximum larval mortality was observed in T4 (Trichoderma isolates 4) followed by T5 (Trichoderma isolate 5), T2 (Trichoderma isolate 2), T3 (Trichoderma isolate 3), T6 (Trichoderma isolate 6), and T1 (Trichoderma isolate 1).

It is concluded that use of bio- agents such as *Trichoderma* spp can significantly enhance our lives, the environment and our productivity. Trichoderma species are well known to cause antagonistic effects on plant parasitic nematodes. The fungus enhanced plant growth and yield in all the treated pots. In India, various species of Trichoderma are reported as nematicidal. Use of *Trichoderma* spp against phytonematodes and as a growth enhancer has received much attention by many researchers. All the treatments significantly control the root knot of Tomato when compared with inoculated control. Among the treatments, T4 (1000larvae/plant +Tr4) showed better larval mortality and reduction in nematode population, root weight and root gall followed by T5 (1000larvae/plant +Tr5), T3 (1000larvae/plant +Tr5), T2 (1000larvae/ plant +Tr5),T6 (1000larvae/plant +Tr5) and T1 (1000larvae/plant +Tr5) in Tomato. Thus, the treatment of the soil with the antagonistic fungus improved nematode control as the isolates significantly reduced the nematode populations.

From all these results we can conclude that *Trichoderma* spp can be effectively used as soil treatment in pots on Tomato crop for control of *M. incognita* and is eco- friendly in management of the root- knot avoiding pollution and residual hazards to human beings and animals. There were more *M. incognita* juveniles in the control plots that were not treated with *Trichoderma* isolates after harvest. The fungus provided gave some level of nematode suppression as much as synthetic nematicides. Tomato plants that were treated with *Trichoderma* isolates were less attacked by the root-knot nematodes and also shows significant reduction in root gall/ root system of Tomato plants (Neog *et al*., 2014). The untreated plants had high nematodes population. The *Trichoderma* isolates shows maximum larval mortality of *Meloidogyne incognita* when recorded after 24 and 48 hrs of exposure as compared with untreated control. There was significant increase in growth and yield of tomato plants treated with *Trichoderma* isolates compared to those not treated with *Trichoderma* isolates. This is in agreement with Sasser, (1980) reported that root-knot nematodes (*Meloidogyne* sp) are capable of causing reduced growth rate. This observation also agrees with those of earlier researchers; Papavizas, 1985; Windham *et al.,* 1989; Sharon *et al*., 2001, as well as Meyer *et al*., 2001 who reported the importance of *Trichoderma* isolates in enhancing plant growth, increasing crop yield and reducing root-knot nematode population build up in the soil as well as their damage.

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