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# Biological control of root-knot nematode, *Meloidogyne incognita* infecting tomato (*Solanum lycopersicum*)

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**Abstract**
Tomato (*Solanum lycopersicum*) is one of the major vegetable crop grown in India. Root-knot nematode, *Meloidogyne incognita* is widely distributed in India and causes severe damage in tomato. Various nematicides have been so far used to control root-knot nematode are not only expensive but also hazardous to human and soil health. In view to reduce dependence on nematicides, alternative eco-friendly solutions have been tried. Present investigation was carried out to test the efficacy of fungal biocontrol agents (Trichoderma viride and *Trichoderma asperellum*) against root-knot nematode, *M. incognita* on tomato. Efficacy of fungal bio control agent's *viz*., *T. viride* and *T. asperellum* has been evaluated to control the root-knot nematode, *M. incognita*. Results showed that the soil application of *T. viride* at 15 ml/kg soil was found best treatment in improving plant growth of tomato and significantly reduced reproduction of root-knot nematode on tomato, closely followed by *T. viride* at 10 ml/kg soil. *T. viride* at 10 ml/kg soil and *T. asperellum* at 15 ml/kg soil which also had almost similar impact in plant growth and nematode reproduction parameters.

**Keywords:** Solanum lycopersicum, meloidogyne incognita, trichoderma viride, trichoderma asperellum

## Introduction
Tomato is one of the most important vegetable crop in the world. It is a self-pollinated crop, belongs to family Solanaceae. Tomato has originated from Peru, Ecuador and Bolivia on the basis of availability of numerous wild and cultivated relatives existing in this region. It is cultivated in both temperate and tropical regions of the world. It is consumed fresh in salad and sandwiches, cooked or processed in ketchup, sauce, juice or dried powder. Tomato plays an important role in nutrition by providing essential amino acids and rich source of minerals. (Bose and Som, 1990) [7]. It contains lycopene, which is very important antioxidant and can prevent cancer (Agarwal and Rao, 2000) [1]. Various factors have been recognized for low yield of tomato such as its poor quality of seed and incidence of disease and pests including plant-parasitic nematodes. Nematodes are regarded as important economical pests of horticultural crops including tomato. In Rajasthan, Root-knot nematode on tomato was first reported from Jodhpur (Arya, 1957) [3]. Tomato is severely affected by root-knot nematode, *Meloidogyne* spp. (Bhardwaj, 1972) [4]. Root-knot nematode has been considered as a major nematode problem in India and received greater attention due to its polyphagous nature, short life span and adaptability to adverse conditions. It causes severe losses in vegetables, fruits, pulses, oilseeds and other ornamental crops. Estimation of crop losses due to nematodes up to 46.0% in Haryana state only (Bhatti and Jain, 1977) [6]. Estimation of losses on tomato in Karnataka was recorded 39.77 % at 20 larvae/g soil (Reddy, 1985) [16]. Heavy infestation of nematodes can lead to yield losses of over 30% in highly susceptible vegetable crops (Sikora and Fernandez, 2005) [17]. Tomato yield loss is very high 27.21% due to nematodes. Monetary loss was calculated up to Rs. 2204 million (Jain *et al.*, 2007) [11]. 40 % yield losses in tomato was recorded due to root-knot nematode, *M. incognita* (Singh and Kumar, 2015) [19]. Past investigations that were conducted for the management of root-knot nematode involved change in physical, chemical and biological properties of soil through sanitation, fertilizers amendment both organic and inorganic (Collange *et al*., 2011) [8]. Most effective alternative found till date for the management of root-knot nematode is biological control. Application of antagonistic microbes not any competes with (Singh and Mathur, 2010) [18]. Bio control agents are host specific and are potential candidates for integrated pest management (Arora *et al*., 2000) [2]. Therefore, in this prospect the present research emphasized on the application of these antagonistic microbes for biological control of root-knot nematode infecting tomato.

## Materials and Methods

A pot experiment was carried out to test the efficacy of fungal biocontrol agents (*T. viride* and *T. asperellum*) against root-knot nematode, *M. incognita* infecting tomato in the Department of Nematology, Rajasthan College of Agriculture, Udaipur during 2018-19. During the period of investigation, various materials used and methods were followed.

All the culture media and distilled water were sterilized in an autoclave at 121.6 ˚C, 15 PSI pressure for 20 minutes, polythene bags were sterilized with 5% formalin, binocular stereoscopic microscope, laminar air flow, electric balance, earthen pots autoclave.

### Maintenance of pure culturing of *M. incognita*:

Egg masses of *M. incognita* was collected from tomato roots and the population was multiplied on a susceptible tomato variety (Nav Uday) grown in pots containing sterilized soil.

### Preparation of stock solution of biocontrol agents by using mother culture

One ml of sterilized double distilled water was added on fully grown fresh mother culture of *T. viride* and *T. asperellum* and than scraped with a spade to produce slurry and then transferred to 99 ml of distilled water to prepare a suspension that was referred as stock solution. From this stock solution 10 ml suspension was transferred into 90 ml distilled water that was referred as 2nd dilution suspension. Subsequent dilutions were made repeating the same process till 6th dilution suspension *i.e.* spore load of $2x10^6$ CFU/ml was obtained. Different doses of 6th dilution suspension were used for pot inoculums.

### Treatments and experimental layout

The experiment was laid out in a Complete Randomized Design with seven treatments *viz.*, *T. viride* at 5 ml/kg soil, *T. viride* at 10 ml/kg soil, *T. viride* at 15 ml/kg soil, *T. asperellum* at 5 ml/kg soil, *T. asperellum* at 10 ml/kg soil, *T. asperellum* at 15 ml/kg soil untreated check. Each treatment was replicated thrice. Data was analyzed using analysis of variance (ANOVA) as per standard method of CRD.

### Methodology

Field soil was sterilized in an autoclave for 2 hrs at 15 psi and 121.6˚C temperature. Sterilized soil was left in cage house for atleast 10 days for maturation. Three tomato seedlings (*cv.* Nav Uday) were transplanted in each pot containing sterilized soil. After 20 days of transplanting *T. viride* and *T. asperellum* at 5, 10, 15 ml/kg soil were added to all pots. At 25th day of transplanting 5000 larvae of *M. incognita* at 2-3 nematodes per g of soil (10 ml stock suspension containing 1000 larvae of *M. incognita*) were applied to all pots. The experiment was harvested 45 days after nematode inoculation. Observation on various plant growth parameters *viz.*, shoot and root weight, shoot and root length, were recorded. Observations on nematode reproduction *viz.*, number of galls/plant, number of egg masses/plant, number of egg and larvae/egg mass and final nematode population/100 cc soil were also recorded. For studying the nematode infection, the roots were stained with 0.1% acid fuchsin lacto-phenol at 80˚C for 2-3 minute (Mc Beth *et al.*, 1941) [13].

## Results and Discussion

The result of experiment showed that the biocontrol agents *T. viride* at 15 ml/kg soil had significantly reduced the number of galls/plant, number of egg masses/plant, number of eggs and larvae/egg mass and final nematode population/100 cc soil and considerably increased the shoot and root weight, shoot and root length of tomato as compared to untreated check. Among both biocontrol agents, *T. viride* was better as compared to *T. asperellum* against root-knot nematode, *M. incognita* on tomato. Recorded observations are presented in Table 1.

**Table 1:** Efficacy of *T. viride* and *T. asperellum* as soil application against root-knot nematode, *M. incognita* on tomato

| Treatment | Shoot length (cm)* | Root length (cm)* | Shoot weight (g)* | Root weight (g)* | No. of galls/ Plant** | No. of egg masses/ Plant** | No. of eggs & larvae/ egg mass** | Final nematode population/ 200 cc soil** |
|---|---|---|---|---|---|---|---|---|
| $T_1$ = *T. viride* at 5 ml/kg soil | 40.33 (81.99) | 14.90 (129.23) | 30.00 (198.21) | 8.32 (51.54) | 15.00 (48.27) | 12.33 (47.88) | 145.00 (27.01) | 264.00 (51.55) |
| $T_2$ = *T. viride* at 10 ml/kg soil | 53.66 (142.14) | 20.16 (210.15) | 38.50 (282.70) | 15.58 (183.78) | 8.33 (71.27) | 7.66 (67.62) | 118 .33 (40.43) | 181.00 (66.78) |
| $T_3$ = *T. viride* at 15 ml/g soil | 55.50 (150.45) | 23.83 (266.61) | 42.00 (317.49) | 18.39 (234.97) | 6.66 (77.03) | 6.33 (73.24) | 113.66 (42.78) | 165.00 (69.72) |
| $T_4$ = *T. asperellum* at 5 ml/kg soil | 38.33 (72.96) | 13.83 (112.76) | 27.80 (176.34) | 6.50 (18.39) | 19.33 (33.34) | 16.33 (30.98) | 166.00 (16.44) | 294.00 (46.05) |
| $T_5$ = *T. asperellum* at 10 ml/kg soil | 44.50 (105.32) | 17.66(171.69) | 33.53 (233.30) | 10.96 (99.63) | 13.00 (55.17) | 10.33 (56.33) | 134.66 (32.21) | 226.00 (58.53) |
| $T_6$ = *T. asperellum* at 15 = ml/kg soil | 50.50 (127.88) | 20.16 (210.15) | 35.50 (252.88) | 13.38 (143.71) | 9.66 (66.68) | 8.33 (64.79) | 124.66 (37.24) | 204.00 (62.56) |
| $T_7$ = Control | 22.16 | 6.50 | 10.06 | 5.49 | 29.00 | 23.66 | 198.66 | 545.00 |
| SEm± | 1.036 | 0.380 | 0.746 | 0.253 | 0.441 | 0.313 | 3.831 | 6.823 |
| CD 5% | 3.142 | 1.152 | 2.262 | 0.767 | 1.337 | 0.950 | 11.620 | 20.696 |

Initial Nematode Population 250 juveniles / 100 cc Soil
*****Increased Plant Parameter over Control (%),
****** Reduction Nematode Reproduction over Control (%)

### Plant growth characters

Data presented in Table1 and Fig. 1(a) revealed that the soil application of *T. viride* at 15 ml/kg soil significantly increased shoot length (150.45%), root length (266.61%), shoot weight (317.49%) and root weight (234.97%) as compared to control.

### Nematode reproduction

Data presented in Table1 and Fig.1(b, c, d and e) revealed that the soil application of *T. viride* at 15 ml/kg soil significantly reduced the number of galls/plant (77.03%), number of egg masses/plant (73.24%), number of eggs and larvae/egg mass (42.78%) and final nematode population/100 cc soil (69.72%) as compared to control. These results are in agreement with the findings of Bhatt *et al.*, (2002) [5] who reported that the root-knot index and the number of egg masses were significantly reduced upon three applications of *T. viride*. Another findings of Goswami and Mittal (2004) [10] who reported that *Trichoderma viride* and *Paecilomyces lilacinus*

both fungi were applied in combination at half the dose (1g/500g soil) reduced nematode population (80 larvar/500 gm soil) as compared to *P. lilacinus* (190 larvae/500 g soil) or *T. viride* (230 larvae/500 g soil) alone and plant growth significantly increased in *T. viride* alone and in combination of *T. viride* + *P. lilaceous* (24.5cm and 25.1cm respectively) as compared to *P. lilaceous* alone (21.5cm). Muthulakshmi *et al.*, (2010) [15] reported that the soil application of *T. viride* was able to control the nematode population and improve the mulberry leaves yield and nutritional values. Results of present investigation were also found similar with Kamau *et al.*, (2010) [12] who reported that the greenhouse experiments showed that treatment with *T. asperellum* reduced the galling in French beans significantly. Same trends were found with Mukhtar (2018) [14] who reported that the indigenous isolates of *T. harzianum* and *T. viride* have the potential to control *M. incognita* and Devi *et al.*, (2002) [9] who reported that *T. viride* or *T. harzianum* when mixed in soil at 1 g/kg soil improved plant height, shoot weight, root length and weight and reduced *M. incognita* population.

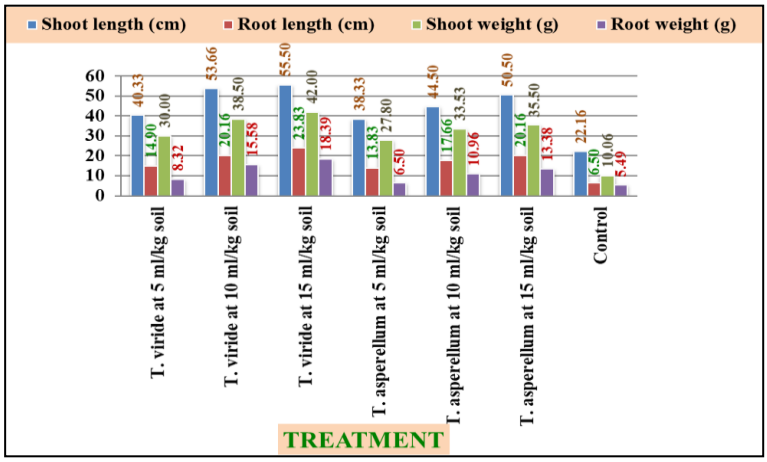


**Fig 1(a):** Effect of *T. viride* and *T. asperellum* as soil application against root-knot nematode, *M. incognita* on plant growth parameters of tomato (On X – axis- Details of treatment, Y – axis - plant growth parameters)

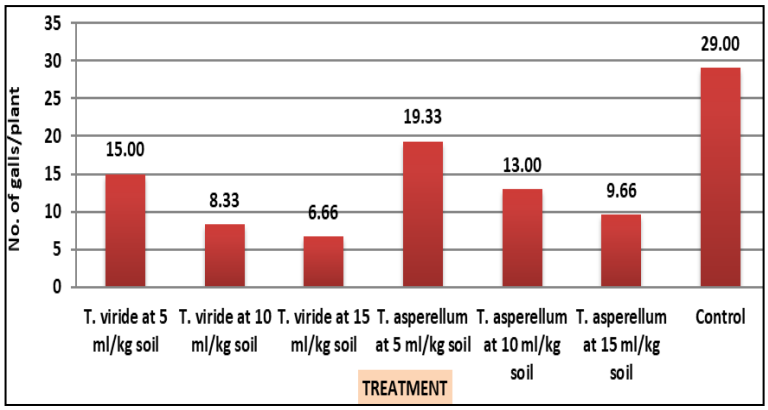


**Fig. 1(b):** Effect of *T. viride* and *T. asperellum* as soil application against reproduction parameters of root-knot nematode, *M. incognita* on tomato (On X – axis- Details of treatment, Y – axis – No. of galls/plant

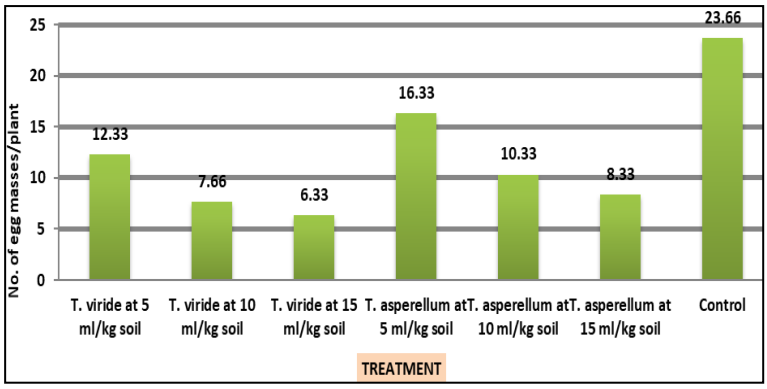


**Fig 1(c):** Effect of *T. viride* and *T. asperellum* as soil application against reproduction parameters of root-knot nematode, *M. incognita* on tomato (On X – axis- Details of treatment, Y – axis – No. of egg masses/plant)

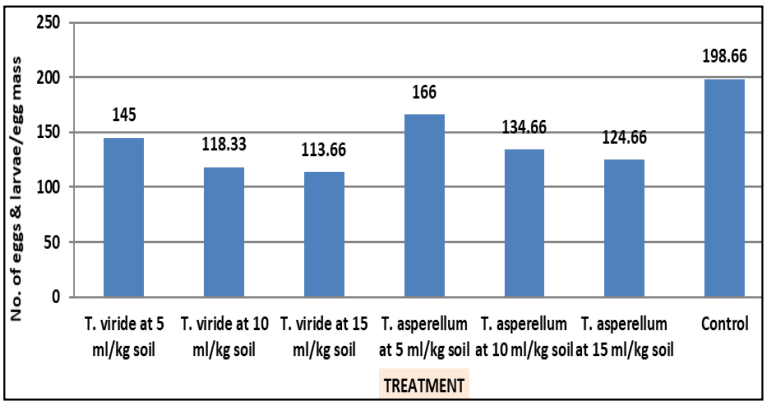


**Fig 1(d):** Effect of *T. viride* and *T. asperellum* as soil application against reproduction parameters of root-knot nematode, *M. incognita* on tomato (On X – axis- Details of treatment, Y – axis – No. of eggs & larvae/egg mass)

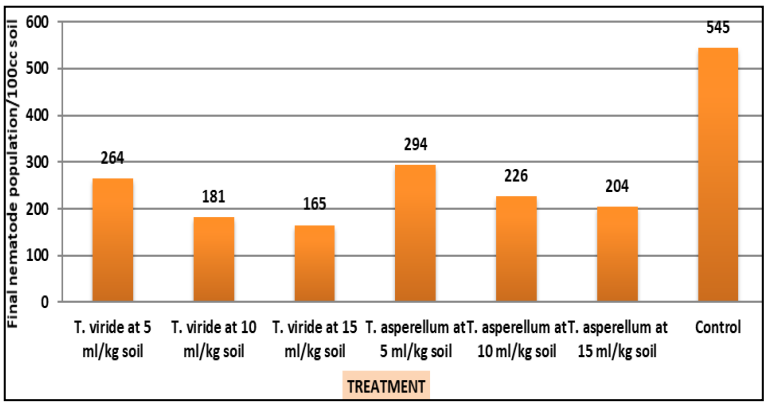


**Fig 1(e):** Effect of *T. viride* and *T. asperellum* as soil application against reproduction parameters of root-knot nematode, *M. incognita* on tomato (On X – axis- Details of treatment, Y – axis – Final nematode population/100 cc soil)

## Conclusion

The present investigation reveals that there is a potential scope for the utilization of different fungal biocontrol agents for nematode control. However, further research should be carried out to testing of local biocontrol agents under protected and field condition for controlling plant parasitic nematodes.

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