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Reaction of locally isolated bio-agents on hatching and mortality of root-knot nematode, *Meloidogyne incognita*

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Abstract
Investigations were carried out *in vitro* to evaluate the antagonistic effect of fungal bio-control agents (local isolates) *i.e., Trichoderma viride* and *Trichoderma asperellum* on hatching and larval mortality of root-knot nematode, *Meloidogyne incognita*. Bio-control agents were tested at 10^6, 10^7 and 10^8 dilutions on hatching and juvenile mortality of *M. incognita* after 24, 48, 72 and 96 hrs exposure period as compared to control. *T. viride* and *T. asperellum* were found at par and significantly effective on hatching inhibition and larval mortality of *M. incognita*. Among different dilutions, *T. viride* at 10^6 dilutions gave maximum hatching inhibition and larval mortality followed by *T. asperellum* at 10^6 dilutions and *T. viride* at 10^7 dilutions after 96 hrs. *T. asperellum* at 10^8 dilutions was found least effective at different period of exposure.

Keywords: *Meloidogyne incognita*, *Trichoderma viride*, *Trichoderma asperellum*, hatching, mortality

Introduction
Root-knot nematode, *Meloidogyne incognita* is a polyphagous, short life span, sedentary endoparasites and adaptability to adverse conditions to crops. Root-knot nematode causes severe losses in vegetables, fruits, pulses, oilseeds and other ornamental crops. Bhatti and Jain (1977) [7] estimated the crop losses up to 46.0% in Haryana state only. Reddy (1985) [19] estimated a loss of 39.77 % in the tomato field in Karnataka. Sharma and Baheti (1992) [21] reported heavy losses (47.8 %) caused by root-knot nematode, *M. incognita* and *M. incognita* on tomato in light soil of Rajasthan. Heavy infestation of nematodes can lead to yield losses of over 30% in highly susceptible vegetable crops (Sikora and Fernandez, 2005) [22]. In tomato, yield loss is very high (27.21%) due to nematodes. Monetary loss was calculated up to Rs. 2204 million in India (Jain *et al*., 2007) [14]. Yield losses (40 %) in tomatoes were recorded due to root-knot nematode, *M. incognita* (Singh and Kumar, 2015) [23]. Baheti and Bhati (2017) [5] reported the avoidable yield caused by to root-knot nematode, *M. incognita* on okra to the extent of 41.30-45.50, 37.50-41.52 and 22.45-25.38 % in light, medium and heavy texture soil, respectively. The traditional method of nematode management is mainly based on chemical nematicides. However, the negative impact on the environment, costly and ineffectiveness after prolonged use have led to a total ban or restricted use of most chemical nematicides (Zukerman and Esnard, 1994) [26]. Researchers all over the world are engaged in standardizing the root-knot nematode management strategies by following non-chemical and eco-friendly alternatives such as sanitation, soil management, organic amendments, fertilization, biological control and heat-based methods to stabilize and enhance vegetable production (Collange *et al*., 2011) [10].

Biological control promises to be one of the good alternatives. Application of microorganisms antagonistic to nematodes or compounds produced by these microbes could provide an additional option for managing the damage caused by nematodes. Biocontrol agents are host specific and are potential candidates for integrated pest management of nematodes (Arora *et al*., 2000) [3]. The use of bioagents is found an increase in attention and use of such bioagents offer an effective, safe, persistent and natural durable protection against crop pest (Anita and Samiyappan, 2012) [1]. Therefore, in the present investigation, isolation and identification of around Udaipur was done, thereafter *in vitro* experiment was conducted to test the antagonistic effect of fungal biocontrol agents on hatching and larval life of root-knot nematode.

Materials and Methods

I. Isolation, purification and identification of biocontrol agents:

1. Collection of soil samples

Soil samples (100 g) were collected randomly from the rhizosphere of tomato plants with 3-4 reference points.

2. Isolation and purification of fungi through serial dilutions and single spore technique

Isolation of fungal biocontrol agents was attempted by using Potato dextrose agar (PDA) by dilution plate method (Warcup, 1955) [25]. PDA amended with 25 ppm chloramphenicol and 2 ml Triton X- 100/litre (Budge and Whipps, 1991) [8] was used for isolation of *Trichoderma*. Thereafter, a single spore culture isolation technique was used for fungus purification.

3. Identification of fungal bio-agents

Fungal isolates were identified based on morphological characteristics under light microscope and sequence data. Fungal isolates were grown on PDA for 3-7 days. Then fungal isolates were identified to species level by the Department of plant pathology, Rajasthan College of Agriculture, Udaipur and Indian Type Culture Collection (ITCC), New Delhi.

II. Antagonistic effect of fungal biocontrol agents on hatching and larval life of root-knot nematode, *M. incognita in vitro* conditions:

1. Preparation of spore suspension:

Nematode antagonistic fungi sub cultured on PDA and incubated at 25 ºC for 7 days. Collect aerial conidia of fungi by dislodging from culture. Suspend conidia in 2 ml of sterile distilled water with 0.01% Tween. Mixed the conidial spores in sterile water thoroughly. The spore suspension filtered through 500 µm mesh or Whatmann filter paper no.1 to remove mycelial particles. Spore load per ml was determined by counting a number of spores/ml using haemocytometer and different concentrations of spore suspension (10^6, 10^7 and 10^8) were prepared by adding sterilized water.

2. Collection of egg masses of *M. incognita*

Egg masses of *M. incognita* were collected from tomato roots maintained as a pure culture. Egg masses was handpicked up from the galled root with help of sterilized forceps. The picked egg masses were kept in sterilized cavity block containing 2ml sterilized water.

3. Hatching Test

Five ml of spore suspension of biocontrol agents (*T. viride* and *T. asperellum* dilution 10^6, 10^7 and 10^8) in each sterile cavity block was taken. Surface sterilization of *M. incognita* egg masses were done with 0.01% mercuric chloride and rinsing was done three times in sterile water these surface sterilized *M. incognita* egg masses were transferred into cavity blocks containing spore suspension (one egg mass/cavity block). Cavity blocks were incubated for 96 hours and the numbers of hatched juveniles were recorded out for every 24 hours interval (*i.e.* After 24, 48, 72 and 96 hours).

The percent inhibition in egg hatching was calculated by using the formula:

Percent inhibition of egg hatching = $(C-T/C) \times 100$ %

Where,

C = number of hatched juveniles in control.

T = number of hatched juveniles in each concentration of extract.

4. Mortality Test

Freshly hatched second stage juveniles (J_2) of *M. incognita* were collected and sterilized with 0.5% sodium hypochlorite (NaOCl) for 2 minutes and rinsed five times with sterilized distilled water. 50 juveniles were transferred to glass petri dishes containing 5 ml of spore suspension of biocontrol agents. Incubation was done at 28 ºC for 4 days and numbers of juveniles parasitized were recorded.

The percent mortality was calculated by using the formula:

Percent mortality = $(C/T) \times 100\%$

Where,

C = number of parasitized nematodes after 24, 48, 72 and 96 hrs exposure.

T = total number of nematodes in a cavity block.

Plate 1: Mother Culture of *Trichoderma viride*

Plate 2: Mother Culture of Trichoderma aspereilum

Result and Discussion

Egg Hatching

The effect of different dilutions (*i.e.*, 10^6, 10^7 and 10^8) of isolated bio-agents *T. viride* and *T. asperellum* were tested for their ability to inhibit the egg hatching of *M. incognita*. Observations were recorded after 24, 48, 72 and 96 hours.

I. Hatching after 24 hours

The minimum number of hatched juveniles (31.33) was observed with the *T. viride* at 10^6 dilutions followed by *T. asperellum* at 10^6 dilutions (36.33), *T. viride* at 10^7 dilutions (40.33) and a maximum number of hatched juveniles (97.00) was observed in untreated check. (Table:1)

II. Hatching after 48 hours

The minimum number of hatched juveniles (44.66) was observed with the *T. viride* at 10^6 dilutions followed by *T. asperellum* at 10^6 dilutions (54.33), *T. viride* at 10^7 dilutions (61.66) and a maximum number of hatched juveniles (167.66) was observed in untreated check. (Table:1)

III. Hatching after 72 hours

The minimum number of hatched juveniles (51.00) was observed with the *T. viride* at 10^6 dilutions followed by *T. asperellum* at 10^6 dilutions (62.00), *T. viride* at 10^7 dilutions (70.66) and a maximum number of hatched juveniles (232.00) was observed in untreated check. (Table:1)

IV. Hatching after 96 hours

The minimum number of hatched juveniles (54.00) was observed with the *T. viride* at 10^6 dilutions followed by *T. asperellum* at 10^6 dilutions (67.00), *T. viride* at 10^7 dilutions (75.66) and a maximum number of hatched juveniles (305.00) was observed in untreated. (Table:1)

Table 1: Antagonistic effect of *Trichoderma viride* and *Trichoderma asperellum* on hatching of root-knot nematode, *M. incognita in vitro* conditions.

Spore suspension dilution	No. of hatched juveniles after an exposure period			
	24 hrs	48 hrs	72 hrs	96 hrs
T. viride at10^6	31.33	44.66	51.00	54.00
T. viride at 10^7	40.33	61.66	70.66	75.66
T. viride at 10^8	57.00	83.66	98.66	111.33
T. asperellum at 10^6	36.66	54.33	62.00	67.00
T. asperellum at 10^7	48.66	74.66	90.00	99.66
T. asperellum at 10^8	60.66	94.00	115.66	133.66
Control	97.00	167.66	232.00	305.00
SEm ±	0.728	0.746	0.933	0.969
CD at 5 %	2.207	2.263	2.831	2.939

One egg mass of *M. incognita* per cavity block. Data are average values of three replications.

Mortality of J_2 of *M. incognita*

I. Mortality of juveniles after 24 hours

Data presented in Table 2 showed that *T. viride* at 10^6 dilutions resulted in highest mortality of 18.66 % followed by *T. asperellum* at 10^6 dilutions 18.00 % and *T. viride* at 10^7 dilutions of 15.33 %. While, lowest mortality 6.00 % was observed in control.

II. Mortality of juveniles after 48 hours

Data presented in Table 2 showed that *T. viride* at 10^6 dilutions resulted in highest mortality 24.00 % followed by *T. asperellum* at 10^6 dilutions 22.66 %, *T. viride* at 10^7 dilutions 19.33 %, *T. asperellum* at 10^7 dilutions 17.33 % and lowest mortality 7.33 % was observed in control.

III. Mortality of juveniles after 72 hours

Data presented in Table 2 showed that *T. viride* at 10^6 dilutions resulted in highest mortality 35.33 % followed by *T. asperellum* at 10^6 dilutions of 34.66 % and *T. viride* at 10^7 dilutions of 27.33 %. While, lowest mortality 9.33 % was observed in control.

IV. Mortality of juveniles after 96 hours

Mortality percentage increased with an increase in the exposure period. Data presented in Table 2 showed that *T. viride* at 10^6 dilutions showed the highest mortality 66.66 % followed by *T. asperellum* at 10^6 dilutions of 65.33 %. While, the lowest mortality 13.33 % was observed in control.

Table 2: Effect of *Trichoderma viride and Trichoderma asperellum* onmortality of root-knot nematode, *M. incognita in vitro* conditions

Spore suspension dilution	Per cent mortality of juveniles after an exposure period			
	24 hours	48 hours	72 hours	96 hours
T. viride at 10^6	18.66	24.00	35.33	66.66
T. viride at 10^7	15.33	19.33	27.33	51.33
T. viride at 10^8	13.33	16.66	21.33	38.00
T. asperellum at 10^6	18.00	22.66	34.66	65.33
T. asperellum at 10^7	14.00	17.33	26.00	48.66
T. asperellum at 10^8	12.66	16.00	22.66	37.33
Control	6.00	7.33	9.33	13.33
SEm ±	0.354	0.405	0.663	1.095
CD at 5 %	1.073	1.230	2.011	3.320

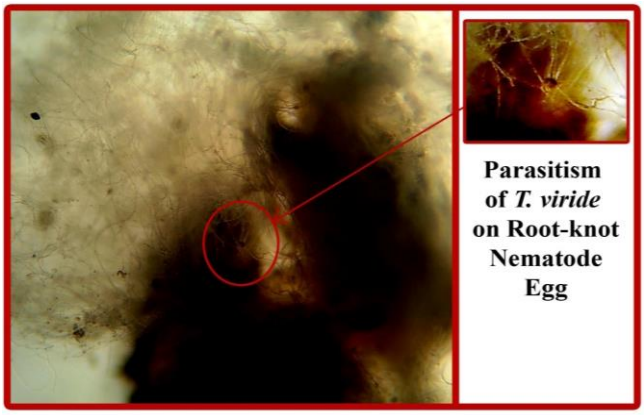
Note: 50 juveniles per cavity block. Data are per cent of the average values of three replications.

Inhibitory effects on hatching were observed after 24, 48, 72 and 96 hrs exposure period as compared to control. All dilutions (10^6, 10^7 and 10^8) of *T. viride* and *T. asperellum* were found significantly effective on hatching of *M. incognita* over control. Among all dilutions, *T. viride* at 10^6 dilution (82.29%) was found most effective followed by *T. asperellum* at 10^6 (78.03%) and *T. viride* at 10^7 dilution (75.19%) whereas *T. asperellum* at 10^8 dilution (56.17%) was found least effective after 96 hrs. These findings are in agreement with the results of Rompalli *et al.,* (2016) [20] who reported 92.72% inhibition in the hatching of root-knot nematode, *M. incognita* by *T. viride* after 120 hrs. Naserinasab *et al.,* (2011) [18] showed parasitism of *M. javanica* eggs by *T. harzianum* BI ranged from 21% in control to 84% in antagonistic fungi.

T. harzianum BI reduced nematode damage to tomato. Mehta *et al* (2015) [17] reported the efficacy of *Trichoderma viride* against *Heterodera zeae* infecting maize.

All dilutions (10^6, 10^7 and 10^8) of *T. viride* and *T. asperellum* were found significantly effective on mortality of *M. incognita* juveniles. Among all dilutions, *T. viride* at 10^6 dilution (66.66%) of was found most effective followed by *T. asperellum* at 10^6 dilution (65.33%) and *T. viride* at 10^7 dilution (51.33%) whereas *T. asperellum* at 10^8 dilution (37.33%) was found least effective after 96 hrs. These findings are in agreement with the results of Rompalli *et al.*, (2016) [20] who reported 89.12% juvenile mortality of root-knot nematode by *T. viride* after 120 hrs. Results of present findings are in accordance with Goswami and Mittal (2004) [12] who reported 60% toxicity in culture filtrates of *T. viride* as compared to *P. lilacinus* (25%) against juveniles of *M. incognita*. They also observed 65% inhibition of hatching of *M. incognita* eggs by culture filtrates of *T. viride* and 40% by *P. lilacinus*. Results of the present study showed similarity with Hashem *et al.*, (2011) [13] who found 45% and 30% juvenile mortality after 48 hours of exposure through treatment with *Pseudomonas fluorescens* and *Purpureocillium lilacinus* as compared to control. Devi and Bora (2018) [11] reported that the highest inhibition of egg hatching and juvenile mortality in *T. harzianum* followed by *T. viride*. Similar results were also investigated by Channppa *et al* (2008) [9] with *P. fluorescens*. Singh and Mathur (2010) [24] with *Acremonium strictum, Aspergillus terreus, A. nidulans, A. niger, Chetomium aubense, Chladosporium oxysporum, Fusarium chlamydosporium, F. dimarum, F. oxysporum, F. solani, Paecilomyces lilacinus, Pochonia chlamydosporia, Trichoderma viride* and *T. harzianum*. Ashoub and Amara (2010) [4] with *Bacillus thuringiensis* and *Pseudomonas fluorescens* besides, *Rhizobium leguminosarum*. Kavitha *et al.* (2012) [15] tested nematicidal activity of six antagonistic entophytic strains of *B. subtilis* (*viz.*, Bs N 1, Bs N 3, Bs N 4, Bs N 7, Bs 5 and Bs N 11) against *M. incognit*a. Sharma *et al.* (2014) with *Paecilomyces lilacinus*, Annapurna *et al.* (2018) [2] with *Trichoderma viride, T. harzianum, Pochonia chlamydosporia* and *Purpureocillium lilacinum*, Basyony and Zaid (2018) [6] with *Bacillus subtilis*, Khan *et al.* (2020) [16] with ten *Trichoderma* spp.

These studies clearly indicated that fungal biocontrol agents were effective on hatching and larval life of root-knot nematode.



Mycelial growth of *Trichoderma viride* on root-knot nematode egg mass

Mycelial growth of *Trichoderma asperellum* on root-knot nematode egg mass

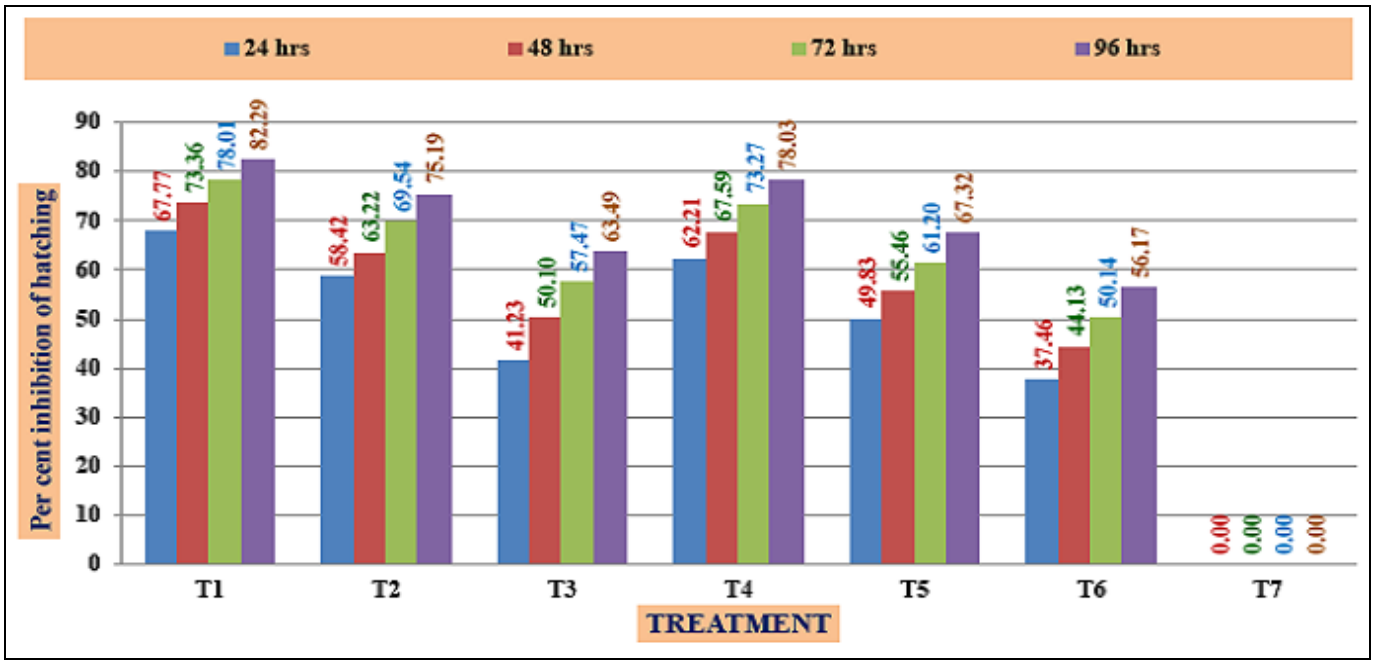


Fig 1: Effect of *Trichoderma viride* and *Trichoderma asperellum* on hatching inhibition of root-knot nematode, *M Incognita* at different exposure periods

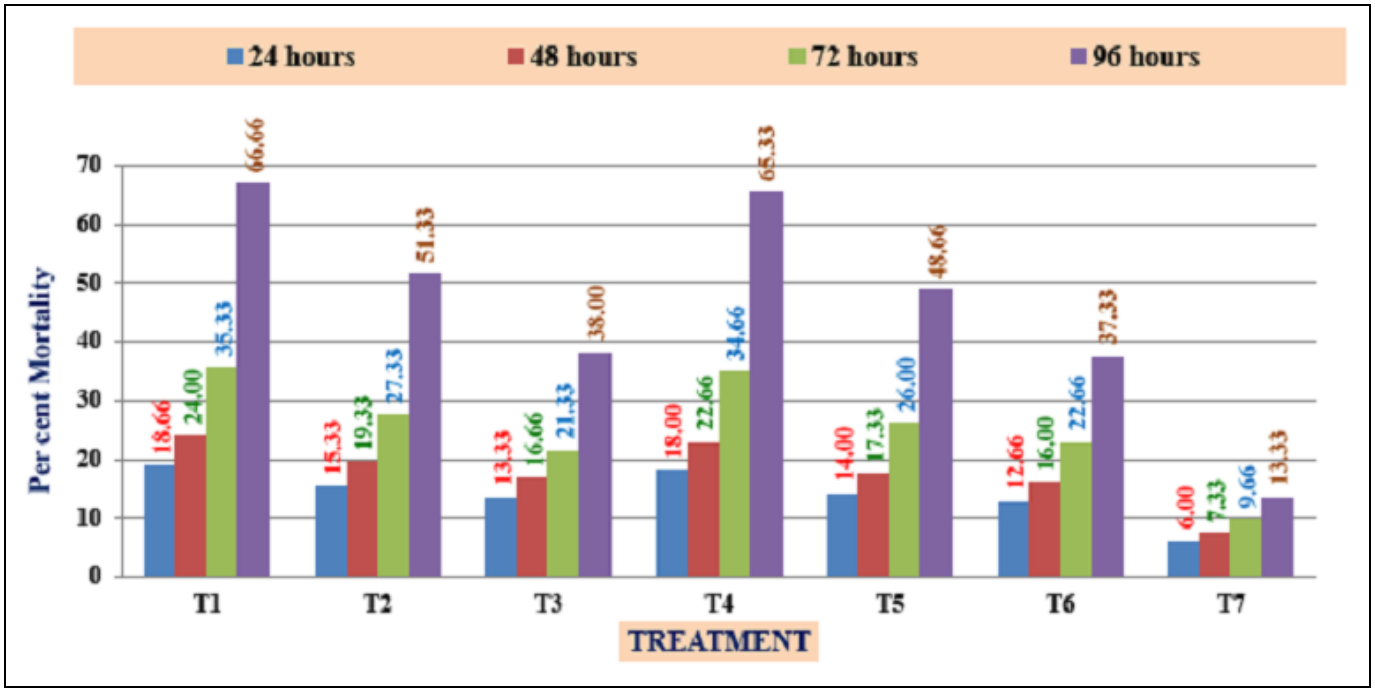


Fig 2: Effect of Trichoderma viride and Trichoderma asperellum on mortality of root-knot nematode, M. incognita at different exposure periods

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