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In vitro Evaluation of Bio-agents on Hatching and Mortality of Root-knot Nematode, *Meloidogyne javanica*

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ABSTRACT: Investigations were carried out *in vitro* to evaluate the antagonistic effect of bio-control agents (*viz., Trichoderma asperellum, Trichoderma harzianum, Verticillium lecanii, Metarhizium anisopliae* and *Bacillus subtilis*) on hatching and larval mortality of root-knot nematode, *Meloidogyne javanica*. Bio-against were tested at 5, 10 and 20 per cent concentrate on hatching and larval mortality of *M. javanica* after 24, 48 and 72 hrs exposure period as compared to control in laboratory). Experimental results showed that all the bio-agents significantly reduced the per cent hatched juveniles and increased the per cent mortality of juveniles Among the tested bio-agents *T. harzianum* was found most effective treatment with minimum per cent hatched juveniles and maximum per cent mortality of juveniles @ 20 per cent concentration after 72 hours followed by *Bacillus subtilis* and *Metarhizium anisopliae*.

Keywords: Root-knot nematode, *Meloidogyne* spp., Hatching, Mortality and Bio-agents.

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) are one of the most destructive pests and causes severe economic losses (Trudgill and Blok 2001; Kiewnick and Sikora 2006; Kalele, *et al*., 2010; Collange *et al*., 2011). Various species of *Meloidogyne* induce major morphological and physiological changes within roots, resulted reduced yield and quality (Khan, *et al*., 2005). The plant infected with root-knot nematode have an unthrifty appearance and often show symptoms of yellowing, rotting, wilting and premature shedding of the foliage with sever stunting that result in huge losses to the infected crops (Saifullah *et al*., 1990).

The damage caused by root knot nematode has resulted 36% yield loss in the country (Senthamarai *et al*., 2006). In the context of tomato cultivation, nematode infestations result in a considerable yield reduction of approximately 27.21%. This loss translates to a substantial economic impact, reaching as high as Rs. 2204 million in India (Jain *et al*., 2007). A significant yield decline of 40% in tomato crops can be attributed to the presence of root-knot nematodes (Singh and Kumar 2015). The all over estimated yield loss in major crops due to plant parasitic nematodes is12.37 reported by Das *et al.* (2018). Biopesticides, which utilize living microorganisms, plant extracts, and other natural compounds, serve as environmentally friendly and non-chemical alternatives (Kumar *et al*., 2018). Biopesticides, particularly soil-dwelling microorganisms such as bacteria and fungi, have shown considerable effectiveness as bioagents in combatting plant-parasitic nematodes. This approach holds significant promise as an alternative strategy for managing root-knot nematode infestations (Hussain *et al*., 2017). The management of root-knot nematode, uses of nematicides are effective, rapid, more reliable, economical and widely in use at present time. But most of the plant parasitic nematodes are found in the soil in some part of their life cycles. Soil itself act as a major barrier for the nematicide to reach at the target site in a lethal dose. In soil there are several biological organisms which may degrade or inactivate the nematicide before reaching in sufficient quantity to kill the nematodes so a very high dose is applied to achieve the desired results. Phytonematodes spend some of their life stages in plants either endoparasitically or ectoparasitically. The effectiveness of nematicides is generally greater when targeting nematodes in the soil phase compared to when they have already invaded the plants. To achieve effective control, nematicides are frequently administered at higher concentrations, but this approach can prove expensive, economically impractical, and potentially harmful to plants

(phytotoxic). Moreover, the use of high doses may lead to residue issues, creating ecological disruptions in the natural environment. Consequently, the pursuit of a high dosage for desired control might not be viable due to various negative effects associated with nematicide application.

With this background view, the present investigations were under taken to assess the potential of bio-agents against root-knot nematode *in vitro*.

MATERIAL AND METHODS

The experiment on management of root-knot nematode in tomato through bio-agents was conducted in laboratory.

Preparation and maintenance of pure culture of *M. javanica*. Tomato plants infected with *M. javanica* were uprooted from the pure culture plots and brought to the laboratory. The roots were first rinsed carefully in water to remove adhering soil particles. Egg masses, collected from the infected roots were kept in distilled water in watch glasses at room temperature for hatching. Freshly hatched J_2 were inoculated on one-month old tomato plants already grown and maintained in earthen clay pots filled with steam sterilized soil to obtained adequate pure population of *M. javanica* on the plants and in soil to carry out further experiments.

Preparation of culture filtrates of fungal and bacterial bio-agents. The potato dextrose agar (PDA) for fungal agents and nutrient broth (NB) for bacterial agents were prepared, inoculated with respective bio-agents in 100 ml conical flasks followed by incubation at 30°C in a shaker for 48 hours. The cultures were centrifuged at 6000 rpm for 20-30 minutes. The supernatant was kept as a stock solution of cent percent concentration. Next grade of 5, 10, and 20 percent concentration were made by dilution with distilled water.

One ml of sterilized double distilled water was added on fully grown fresh mother culture of bio-agents *Trichoderma asperellum, Trichoderma harzianum, Verticillium lecanii, Metarhizium anisopliae* and *Bacillus subtilis* 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.

Testing of different bio-agents on hatching of *M. javanica.* The experiment on tests the efficacy of bio-agent on hatching of *M. javanica* was laid out in completely randomized design (CRD) with four replications. One healthy average sized and freshly egg mass were collected from infected roots of tomato. The egg mass was kept in glass cavity block (1 egg mass/ cavity block) containing 3 ml of bio-agents 5, 10 and 20 % (*Trichoderma asperellum, Trichoderma harzianum, Verticillium lecanii, Metarhizium anisopliae*and *Bacillus subtilis*] concentrations respectively. A distilled water control was maintained simultaneously. Number of juveniles hatched after 24, 48 and 72hours were recorded under binocular microscope.

The per cent of egg hatching was calculated by using formula:

Hatching per cent = (C/T) × 100 %

Where,

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

T = total number of nematodes in a cavity block.

Different bio-agents testing on mortality of *M. javanica.* Freshly hatched ten second juveniles of *M. javanica* were transferred to different cavity blocks containing 3 ml of bio-agents formulation 5, 10 and 20 % (*Trichoderma asperellum, Trichoderma harzianum, Verticillium lecanii, Metarhizium anisopliae* and *Bacillus subtilis)* concentrations respectively. A distilled water control was maintained simultaneously. The experiment was laid out in completely randomized design (CRD) with four replicates of each set was maintained. Per cent larval mortality rate was counted at intervals of 24, 48 and 72 hours. The dead juveniles attained the shape of straight line and the mortality was ensured by touching the juvenile with a fine needle.

The per cent mortality was calculated by using formula:

Per cent mortality = (C/T) × 100

Where,

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

T = total number of nematodes in a cavity block.

RESULTS AND DISCUSSION

Culture filtrates of bio-agents were diluted in different concentration (5,10 and 20 per cent) and tested the effect on hatching and mortality of root-knot nematode, *M. javanica*on different time interval (24, 48 and 72 hours). Observations on per cent hatching of root-knot nematode showed that among the tested bio-agents minimum number of hatched juveniles (7.42 per cent) were observed with the *Trichoderma harzianum* at 20 per cent concentration followed *Bacillus subtilis* (10.42 per cent) and *Metarhizium anisopliae* (11.75 per cent) at 20 per cent concentration after 24 hours while after 48 hours it increased, which was 9.92 per cent were observed with *Trichoderma harzianum,* 13.33 per cent with *Bacillus subtilis* and 14.33 per cent with *Metarhizium anisopliae* at 20 per cent concentration. Whereas, after 72 hours increasing trained was observed 11.67 per cent, 15.92 per cent 16.75 per cent respectively. The per cent hatching of root-knot nematode juveniles were regularly increased in among tested bio-agents but the rate of hatching was minimum as compared with untreated check *i.e.*, 30.75 per cent, 52.83 per cent and 75.75 per cent after 24, 48 and 72 hours 1).

Effect of bio-agents also tested on per cent mortality of root-knot nematode after 24, 48 and 72 hours. Results showed that all the bio-agents significantly increased the per cent mortality of juveniles as compared to untreated check. Among the tested culture filtrate (5,10 and 20 per cent) of bio-agents on different time interval (24, 48 and 72 hours), 20 per cent concentrations after 72 hours was found most effective for maximum per cent mortality of juveniles. Results showed that among the bio-agents *Trichoderma harzianum* was observed

best treatment with maximum per cent mortality (87.50, 92.50 and 95.00 per cent) after 24, 48 and 72 hours at 20 per cent concentration followed by *Bacillus subtilis*(82.50, 85.00 and 92.50 per cent) and *Metarhizium anisopliae* (67.50, 70.00 and 77.50 per cent). However, juvenile's mortality was not observed in untreated check (Table 2).

Table 1: Effect of bio-agents on per cent hatching of eggs of root-knot nematode, *M. javanica* in vitro.

Concentration →	5%			10%			20%		
Treatment	**24 h**	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**
T. asperellum	17.42	20.17	22.33	14.92	18.75	21.17	12.83	15.67	18.00
T. harzianum	11.33	13.08	14.75	10.08	11.50	13.17	7.42	9.92	11.67
V. lecanii	19.00	21.17	23.92	16.58	20.50	21.75	15.17	17.75	20.17
M. anisopliae	16.50	19.42	21.33	14.08	17.83	20.33	11.75	14.33	16.75
B. subtilis	15.58	17.83	20.25	12.92	16.67	19.92	10.42	13.33	15.92
Control	30.75	52.83	75.75	30.75	52.83	75.75	30.75	52.83	75.75
SEm±	0.49	1.020	0.869	0.44	1.062	0.413	0.439	1.150	0.721
CD 5%	1.46	3.07	2.620	1.32	3.202	1.245	1.32	3.468	2.173
CV	4.56	7.33	5.07	4.59	7.99	2.49	5.16	9.65	4.73
* Average of four replications									

Table 2: Effect of bio-agents on per-cent larval mortality of root-knot nematode, *M. javanicain vitro*.

Concentration	5%			10%			20%		
Treatment	**24 h**	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**
T. asperellum	42.50	47.50	52.50	47.50	57.50	65.00	52.50	55.00	60.00
T. harzianum	82.50	85.00	87.50	85.00	87.50	92.50	87.50	92.50	95.00
V. lecanii	25.00	27.50	32.50	27.50	30.00	32.50	30.00	35.00	37.50
M. anisopliae	60.00	65.00	70.00	62.50	67.50	70.00	67.50	70.00	77.50
B. subtilis	72.50	77.50	85.00	77.50	82.50	87.50	82.50	85.00	92.50
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SEm±	3.15	2.491	3.086	2.27	2.227	2.077	2.121	2.357	2.206
CD 5%	9.49	7.51	9.169	6.52	6.407	5.977	6.10	6.782	6.347
CV	11.58	8.56	13.52	14.40	13.05	11.39	12.63	13.31	11.59
* Average of four replications									

The bio-agents significantly suppressed the egg hatching, Bio-agents, *T. harzianum* showed highest egg hatch inhibition and juvenile mortality of *M. incognita* (Annapurna *et al* 2018). Maximum inhibition of egg hatching and larval mortality of root-knot nematode recorded with *T. harzianum* after 72 hours of incubation. *T. harzianum* and *T. viride* were able to colonize *M. incognita* eggs and second stage juveniles and female. *In vitro* studies demonstrated that both tested isolates were effective in causing nematode mortality compared with the control (Jegathambai, 2011; Naserinasab, 2011; Devi, 2018; Guru Prasad and Ravichandra 2018). All bioagent showed distortion of juveniles was observed in most of the eggs in the present study. The well-known observations suggested that the inhibitory effect of the bio-agents on hatching of the nematode larvae may be due to the nematotoxic metabolites like chitinase and other lytic enzymes like proteases and lipases that cause break down of egg shell and facilitate egg penetration for successful establishment (Kalele, 2010, Li B, 2005). Study reported that mortality of root-knot nematode increased with increase in exposure time as well as the concentration of culture filtrate. Mortality of second stage juveniles by these bio-agents might be due to release of lytic enzymes like chitinases, lipases and acetic acid in the filtrates that cause breakdown of nematode cuticle proteins (Annapurna *et al.*, 2018). The highest mortality of larvae was observed at 72 hours with 20 percent concentration of all bio-agents of tested plants while lowest was observed at low concentration *i.e.,* 10 percent. The percent mortality was recorded nil in control (water) *i.e.,* 0.00 per cent at 72 hours. *T. viride* and *T. asperellum* were found 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 (Kumari *et al.,* 2021).

Statistical Analysis. After completion of experiment, data were statistically analyzed for interpretation of finding. The critical deference was calculated for comparison of treatment for significant at 5 % level of significance.

CONCLUSIONS

Based on the findings, it can be inferred that all the fungal and bacterial bioagents that were tested demonstrated the ability to manage root knot nematodes by reducing egg hatching and causing the death of second stage juveniles in laboratory conditions. Additional research is necessary to confirm their efficacy in both pot and field environments. Moreover, more investigations are needed to identify and characterize the compounds produced by these bioagents that are responsible for their nematicides properties.

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Conflict of Interest. None.

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