Bioefficacy of different fungal bio-agents on juvenile mortality of root knot nematode, *Meloidogyne incognita* infecting tomato under laboratory conditions

ABSTRACT

Investigation were carried out *in vitro* to evaluate the antagonistic effect of fungal bio-control agents i.e., *Trichoderma harzianum*, *T. viride*, *Purpureocillium lilacinum*, *Metarhizium anisopliae* and *Beauveria bassiana* on J2 mortality of RKN at 10^6 and 10^7 dilutions on juvenile mortality of *M. incognita* after 24, 48, 72, 96 and 120 hrs exposure period as compared to untreated check. Among different dilutions, *T. viride* at 10^6 dilutions (86.00 per cent) gave maximum per cent juvenile mortality followed by *T. harzianum* at 10^6 dilutions (82.00 per cent), *P. lilacinum* at 10^6 dilutions (76.00 per cent) and *T. viride* at 10^7 dilutions (72.00 per cent) after 120 hrs. *M. anisopliae* at 10^7 dilutions (38.00 per cent) was found least effective at different period of exposure. Further studies to be conducted in pot and field conditions to evaluate the efficacy of these bio-agents against root knot nematode, *M. incognita*.

Key words: Fungal bio agents, Root knot nematode, Antagonistic*,* *In vitro*, Mortality.

Introduction

Plant-parasitic nematodes, particularly root-knot nematodes, are widely distributed and cause significant yield losses in a wide range of crops (1). *Meloidogyne incognita* is a limiting factor affecting production of vegetables including tomato in India. Overall, plant parasitic nematodes (PPNs) cause 21.3 per cent crop losses amounting to Rs. 102,039.79 million (1.58 billion USD) annually. *Meloidogyne incognita* was economically most important root-knot nematode (RKN) causing yield losses of Rs. 6035.2 million in tomato (2). The top most economically important obligate plant parasitic genus is *Meloidogyne* spp. distributed worldwide (3). *Meloidogyne* spp. are polyphagous in nature infecting more than 3000 host species including vegetables, fruits, oil, fiber, grains and leguminous crops (4, 5). The most frequently occurring species of root-knot nematodes include *Meloidogyne incognita*, *M. javanica, M. arenaria* and *M. hapla*. Among these, *M. incognita* and *M. javanica* are the most common and economically important species which are responsible for high economic yield losses in various crops (6). RKN was first noticed on tea roots in Kerala's Devala area (7). It

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was first observed on tomato crop in Jodhpur (8) and later it was found in the soils of Udaipur (9) infecting many economically significant crops. *Meloidogyne* spp. infect around 350 plant genera in India. *M. incognita* alone infecting about 250 taxa of plants. *M. incognita* infecting tomato crop, causing significant yield losses of 27.2 per cent (10).

Since use of chemicals is prohibitive as well as hazardous, attention has now been directed towards use of biopesticides, *Trichoderma harzianum*, *T. viride*, *Purpureocillium lilacinum*, *Metarhizium anisopliae* and *Beauveria bassiana* has a good potential as biocontrol agent to manage root-knot nematode, *M. incognita*. Nematode management through biocontrol agents is gaining importance in the new millennium as other measures have become less attractive to growers/farmers where economics demand specialization and intensification. In the last decade nematode management through biocontrol agents was in the forefront of research and development. Management of plant parasitic nematodes through ecofriendly means is the need of this era. In view of the increasing awareness about environment and demand of organic farming. The present study was initiated to investigate the boiefficacy of different fungal bio-agents on juvenile mortality of root-knot nematode, *M. incognita* on tomato under laboratory condition.

Materials and Methods

Treatments and experimental layout:

An experiment was conducted at the Department of Nematology, Rajasthan Agricultural of Agriculture, Udaipur, during 2022 to study the Bioefficacy of different fungal bioagents on juvenile mortality of root-knot nematode, *Meloidogyne incognita* under *in vitro* condition. The experiment was laid out in a complete randomized design (CRD) with eleven treatments *i.e., T. harzianum, T. viride, P. lilacinum, M. anisopliae* and *B. bassiana* at 2×10^6 and 2×10^7 spore/ml were tried and untreated control were also maintained for comparison purpose and replicated thrice.

Maintenance of pure culture of *Meloidogyne incognita*:

Egg-masses of *M. incognita* were collected from tomato roots and the population was multiplied on a susceptible tomato variety (SL-21) grown in pots containing sterilized soil.

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This was done six months prior to the start of the experiment. Other intercultural operations were applied as and when needed.

Source and maintenance of fungal bio-control agents:

Fungal biocontrol agents *i.e., Trichoderma harzianum* and *T. viride* was obtained from Department of Plant Pathology, RCA, Udaipur, *Purpureocillium lilacinum* was obtained from Department of Nematology, RCA, Udaipur and *Metarhizium anisopilae & Beauveria bassiana* was obtained from Department of Entomology, RCA, Udaipur. Pure culture of these fungal bio-agents were maintained on Potato Dextrose Agar media in laboratory for further studies.

Collection of egg-masses:

Egg masses were collected from the tomato plants maintained as pure culture. Roots were dissected with a sterilized dissecting needle and egg masses were hand picked up from the galled root with help of sterilized forceps. The picked egg masses were kept in sterilized cavity block containing 5ml sterilized water.

Surface sterilization of egg masses:

The collected egg masses were surface sterilized in 0.4 per cent sodium hypochlorite (NaOCl) for two minutes (11). Egg masses were washed thoroughly with sterile distilled water until the traces of NaOCl is removed and placed in cavity block for further use.

Extraction of juveniles (J2) from eggs

For extraction of juveniles (J2), the sterilized eggs collected as described above were placed on a double layer facial tissue paper supported on a course aluminum wire mesh. This was placed over a 10cm diameter petridish filled with required quantity of water at 24-26°C in BOD incubator for hatching. Several such assemblies were maintained. The juveniles collected from these were mixed together at the time of inoculation in pot experiment as also *in-vitro* studies. Counting of juveniles in the suspension was made by using Hawkshley counting dish.

Preparation of media

Potato Dextrose Agar (PDA)

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The ingredients used for preparation of PDA were Peeled potato - 200 g, Dextrose - 20 g, Agar - 20 g, Water - 1000 ml, pH – (6.0 to 6.5). Peeled potatoes were cut into slices and boiled in 500 ml of distilled water, till they are easily penetrated by a glass rod. Filter through double layer muslin cloth and measured amount of dextrose was added to the extract. In another flask, remaining 500ml distilled water was taken and allowed agar-agar to melt by boiling. The molten agar- agar was strained through double layer muslin cloth and mixed with potato extract solution. The volume was made up to 1000ml by adding distilled water. pH of the media was adjusted to 6.0-6.5. The media was poured into culture tubes and conical flask plugged by non-absorbent cotton and then sterilized in autoclave at 121℃ for 20 minutes. (12).

Potato Dextrose Broth (PDB)

The potato dextrose broth was also prepared following the same method as describe above except that no agar-agar was added.

Preparation of culture filtrates of bio-agents

For the preparation of fungal culture filtrates, 100ml potato dextrose broth was prepared in 250ml Erlenmeyer flasks as mentioned above and seeded with tested fungal bio-agents. The inoculated flasks were incubated at 25± 2°C for 15days in BOD incubator. Then fungal culture filtrates were obtained by filtering through Whatman filter paper no.1. The filtrates so obtained were further centrifuged at 2000 rpm to remove the extra spores and mycelia. Then supernatants were collected and used in the *in-vitro* studies.

Process of spore counting:

For estimation of spores, pure culture of isolated biocontrol agents diluted to 10^6 and 10^7 was used. Haemocytometer was cleaned with ethyl alcohol and left for few minutes to dry. One ml of spore suspension was placed at the centre of the slide and then covered with cover slip. Before counting, the preparation was allowed to slant for 2 minutes for setting of spores. At the bottom of the haemocytometer, ten small squares were selected at random and the spores were counted inside these squares. The bottom was cleaned again and the same procedure was repeated. Estimation of spores/10 squares of haemocytometer for calculation of spores per ml of suspension.

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Mortality Test:

Freshly hatched second stage juveniles (J2) of *M. incognita* were collected and sterilized with 0.5 per cent sodium hypochlorite (NaOCl) for two minutes and rinsed five times with sterilized distilled water. Fifty juveniles were transferred to glass Petri dishes containing five ml of spore suspension of biocontrol agents. Incubation was done at 28ºC for five days and numbers of juveniles parasitized were recorded. The per cent mortality was calculated by using formula:

$$\text{Per cent mortality} = (C \div T) \times 100$$

Where, C = Number of parasitized nematodes after 24, 48, 72, 96 and 120 hrs exposure.

T = Total number of nematodes in a cavity block.

Results and Discussion

Table: 1. Antagonistic effect of fungal bio control agents on larval life of root-knot nematode, *M. incognita* under *in vitro* conditions.

Spore suspension dilution	No. of parasitized juveniles after an exposure period				
	24 hours	**48 hours**	**72 hours**	**96 hours**	**120 hours**
T_1 – *T. harzianum* 2×10^6 spore/ml	10.33	12.33	15.66	30.66	41.00
T_2 – *T. harzianum* 2×10^7 spore/ml	7.00	7.33	11.00	24.33	34.00
T_3- *T. viride* 2×10^6 spore/ml	11.66	13.66	17.00	32.66	43.00
T_4 – *T. viride* 2×10^7 spore/ml	8.33	9.33	12.66	26.33	36.00
T_5 – *P. lilacinum* 2×10^6 spore/ml	9.00	10.66	15.00	28.66	38.00
T_6 – *P. lilacinum* 2×10^7 spore/ml	5.66	6.66	9.33	22.66	29.00
T_7 – *M. anisopliae* 2×10^6 spore/ml	3.66	3.66	5.66	16.66	20.33
T_8- *M. anisopliae* 2×10^7 spore/ml	2.66	3.33	5.00	13.00	19.00
T_9- *B. bassiana* 2×10^6 spore/ml	4.66	5.66	7.66	21.00	27.00
T_{10}- *B. bassiana* 2×10^7 spore/ml	4.00	4.66	6.66	18.00	24.66
T_{11}– Control	2.33	3.00	5.33	7.66	12.00

Note: 50 juveniles per cavity block*. Data are average value of three replications.

The experiment was conducted to study the bioefficacy of different fungal bioagents on juvenile mortality of root-knot nematode under *in vitro* condition. The fungus T. *harzianum, T. viride, P. lilacinum, M. anisopliae* and *B. bassiana* first enters in the nematode body through natural openings of nematodes and sucking out the nutrients from nematode body after depleting all nutrients from juvenile the mycelium penetrates and ruptures the cuticle of

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the infected juvenile and then emerges out to infect other juveniles in the vicinity. The nematodes that showed any mobility or appeared as winding shapes were considered alive while the others that did not show any movement and their body shape remained straight were considered dead. Average number of parasitized juveniles of three replications were recorded and presented in Table 1 at all dilutions after 24, 48, 72, 96 and 120 hrs exposure period. Percentage mortality of juveniles were recorded and presented in Table 2.

All the fungal bio-agents showed significant increase in mortality of *M. incognita* J_2 irrespective of concentrations of the culture filtrates as compared to the controls. No mortality of *M. incognita* J_2 was recorded in controls. Results (Table 1-2, Fig 1) shows that all the treatments, causes J_2 mortality of *M. incognita* in different concentrations at different exposure time interval. The concentration of culture filtrate of treatments is increased there is corresponding increasing in mortality of J_2 of *M. incognita*. Among the bio-agents, culture filtrate of T_3 - *T. viride* @ 2×10^6 spore/ml (86.00 per cent) was found to cause maximum mortality of J_2 in all the concentrations and at different time of exposure as compared to other bio-agents. After *T. viride* @ 2×10^6 spore/ml the best bio-agent was found to be T_1 - *T. harzianum* @ 2×10^6 spore/ml (82.00 per cent) which also caused considerably increased mortality of J_2 followed by T_5 – *P. lilacinum* @ 2×10^6 spore/ml (76.00 per cent), T_4 - *T. viride* @ 2×10^6 spore/ml (72.00 per cent) than the other bioagents. Whereas T_8 – *M. anisopliae* @ 2×10^6 spore/ml dilution (38.00 per cent) was found least effective after 120 hrs (Table 1, Fig 1). However, other bio-agents were also effective in causing mortality of J_2 with varying degrees at different dilution of culture filtrates and at different exposure time.

The reason behind causing mortality of *M. incognita* J_2 might be due to release of lytic enzymes by *Trichoderma* spp. *viz*., chitinases (13), lipases (14, 15, 16, 17, 18) and acetic acid (19) in the filtrates that cause break down of nematode cuticle proteins (13). The variable effect of tested fugal filtrate on *M. incognita* was observed in the present investigation can be attributed to have ability to the production of such toxic metabolites as well as protease enzymes in the filtrates.

These findings are in agreement with the results of (20) who reported 89.12 per cent juvenile mortality of root-knot nematode by *T. viride* after 120 hrs. The above findings are also in accordance with (21) who reported 60.00 per cent toxicity in culture filtrates of *T. viride* as compared to *P. lilacinus* (25.00 per cent) against juveniles of *M. incognita* and 65.00 per cent inhibition of hatching of *M. incognita* eggs by culture filtrates of *T. viride* was

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compared to *P. lilacinus* (40.00 per cent). Results of present study showed similarity with (22) who found 45.00 per cent and 30.00 per cent juvenile mortality after 48 hrs of exposure through treatment with *Pseudomonas fluorescens* and *P. lilacinus* as compared to water control. (23) who reported that the highest inhibition of egg hatching and juvenile mortality in *T. harzianum* followed by *T. viride.* (24) who reported that *T. viride* was observed best treatment with maximum per cent mortality of juveniles (80.00) after 72 hrs followed by *P. lilacinum* (65.00 per cent) over the untreated control. *T. viride* and *T. asperellum* were found effective on larval mortality of *M. incognita*. Among different dilutions, *T. viride* at 10^6 dilutions (66.66 per cent) gave maximum percent mortality of juveniles after 96 hrs followed by *T. asperellum* at 10^6 dilutions (65.33 per cent) (25). (26) who reported that at 20 per cent concentration, maximum 95.00 per cent larval mortality of root-knot nematode was recorded in culture filtrate of *T. harzianum* followed by *Bacillus subtilis* (92.50 per cent).

These studies clearly indicated that fungal biocontrol agents were effectively on juvenile mortality of root-knot nematode.

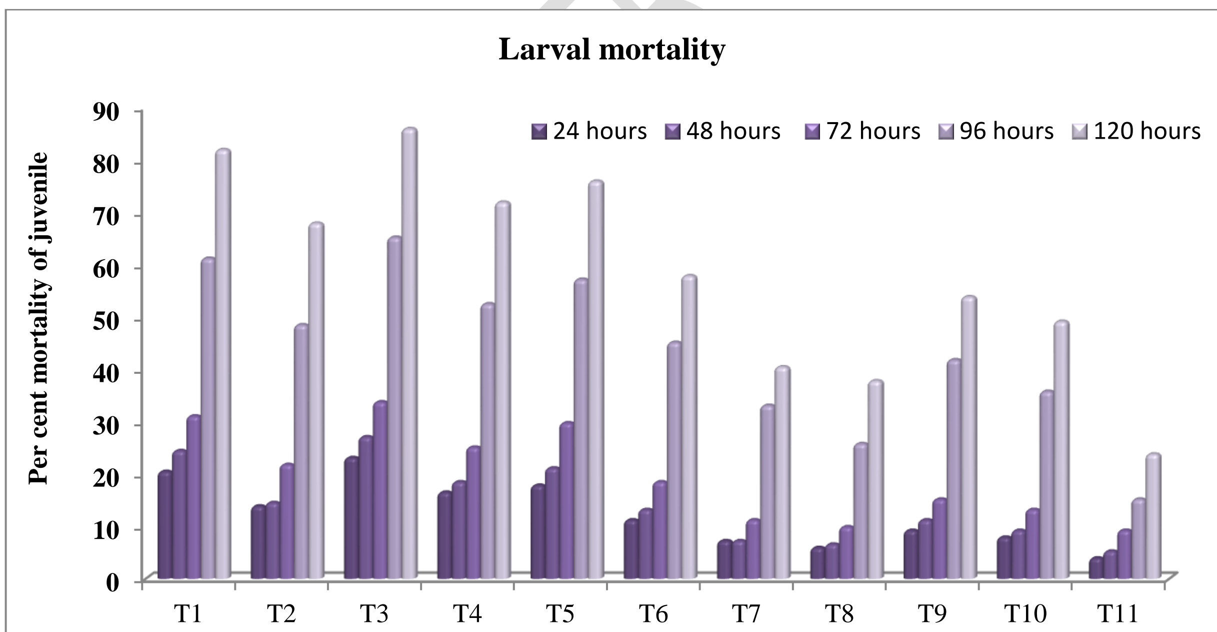

Fig 1: Effect of fungal bio-agents on mortality of root-knot nematode, *Meloidogyne incognita* at different exposure periods.

Table 2: Effect of fungal bio-control agents on mortality of root-knot nematode, *Meloidogyne incognita* under *in vitro* conditions.

Spore suspension dilution	**Per cent mortality of juveniles after an exposure period**				
	24 hours	**48 hours**	**72 hours**	**96 hours**	**120 hours**
T_1 – *Trichoderma harzianum* 2×10^6 spore/ml	20.66 (27.03)	24.66 (29.76)	31.32 (34.02)	61.32 (51.55)	82.00 (65.06)
T_2 – *Trichoderma harzianum* 2×10^7 spore/ml	14.00 (21.96)	14.66 (22.50)	22.00 (27.96)	48.66 (44.23)	68.00 (55.57)
T_3- *Trichoderma viride* 2×10^6 spore/ml	23.32 (28.86)	27.32 (31.51)	34.00 (35.67)	65.32 (53.92)	86.00 (68.23)
T_4 - *Trichoderma viride* 2×10^7 spore/ml	16.66 (24.08)	18.66 (25.59)	25.32 (30.20)	52.66 (46.52)	72.00 (58.06)
T_5 - *Purpureocillium lilacinum* 2×10^6 spore/ml	18.00 (25.10)	21.32 (27.49)	30.00 (33.20)	57.32 (49.21)	76.00 (60.70)
T_6 - *Purpureocillium lilacinum* 2×10^7 spore/ml	11.32 (19.65)	13.32 (21.39)	18.66 (25.58)	45.32 (42.31)	58.00 (49.61)
T_7 - *Metarhizium anisopliae* 2×10^6 spore/ml	7.32 (15.69)	7.32 (15.69)	11.32 (19.65)	33.32 (35.25)	40.66 (39.61)
T_8- *Metarhizium anisopliae* 2×10^7 spore/ml	5.32 (13.33)	6.66 (14.95)	10.00 (18.43)	26.00 (30.65)	38.00 (38.05)
T_9- *Beauveria bassiana* 2×10^6 spore/ml	9.32 (17.77)	11.32 (19.65)	15.32 (23.04)	42.00 (40.39)	54.00 (47.29)
T_{10}- *Beauveria bassiana* 2×10^7 spore/ml	8.00 (16.41)	9.32 (17.77)	13.32 (21.38)	36.00 (36.86)	49.32 (44.61)
T_{11}– Control	4.66 (12.46)	6.00 (14.29)	10.66 (19.05)	15.32 (23.23)	24.00 (29.32)
SEm ±	**0.35**	**0.37**	**0.55**	**1.11**	**1.79**
CD at 5%	**1.01**	**1.09**	**1.60**	**3.22**	**5.18**
CV(%)	**4.85**	**4.50**	**4.77**	**4.40**	**5.28**

Note: Data are per cent of average value of three replications over control.

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