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In-vitro Study of Different Fungal Bio-agents against Root-knot Nematode, *Meloidogyne incognita*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

To assess the antagonistic effect of fungal bio-control agents, such as *Trichoderma harzianum, T. viride, Purpureocillium lilacinum, Metarhizium anisopliae,* and *Beauveria bassiana*, on the hatching of egg masses of the root-knot nematode, *Meloidogyne incognita*, investigations were conducted *in vitro*. In comparison to the control, bio-control agents were tested at 10^6 and 10^7 dilutions on *M.*

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incognita egg hatching after 24, 48, 72, 96, and 120 hours of exposure. *P. lilacinum* 10^7 concentration and *T. harzianum* 10^6 concentration (83.90 % and 83.04 %) were found at par and significantly effective on egg hatching inhibition of *M. incognita*. Among different concentration, *P. lilacinum* at 10^6 concentration (85.46 %) gave maximum egg hatching inhibition followed by *P. lilacinum* at 10^7 concentration (83.90 %), *T. harzianum* at 10^6 concentration (83.04 %) and *T. viride*at 10^6 concentration (82.00 %) after 120 hrs. *M. anisopliae* at 10^7 concentration (69.46 %) 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*.

Keywords: Fungal bio agents; root knot nematode; antagonistic; In vitro; egg hatching.

1. INTRODUCTION

"Root-knot nematode (*Meloidogyne* spp.) is among the most significant polyphagous pest in agriculture. Root-knot nematode (RKN) is one of the most damaging plant infections to crops and is ranked among the top five plant pathogens harming global food production. Overall, plant parasitic nematodes (PPNs) cause an annual loss of 21.3% in crop yield, or Rs. 102,039.79 million (or $1.58 billion USD)" [1]. The most significant RKN in terms of economic impact was *Meloidogyne incognita*, which cost Rs. 6035.2 million in tomato yield losses [1]. "It is likely that *M. incognita* will further contribute to tomato yield decline, as the trend towards intensification of production will support increased nematode population densities. To prevent further tomato yield losses due to the nematodes and improve productivity, a sound nematode management scheme is essential" [1].

The utilization of different fungal bio-agents in the management of nematode parasites is gaining importance. Among the various bio-control agents, *Trichoderma harzianum*, *T. viride*, *Purpureocillium lilacinum*, *Metarhizium anisopliae* and *Beauveria bassiana* have been found to be promising against root-knot nematodes [2]. "Chemical nematicides will no longer be allowed because they not only pose environmental hazards but also increase resistance in the target disease. The use of bioagents has been found to improve attention in reducing such conditions, and these bioagents provide an efficient, secure, long-lasting, and natural defense against *M. incognita"* [3]. On the other hand, a variety of naturally occurring enemies of *Meloidogyne* spp. in the soil can be employed as bio-agents to effectively manage *Meloidogyne* spp. [4]. Fungal bio-agents are a special kind of natural enemy that help control nematodes in soil. These bioagents demonstrated their antagonistic properties toward *M. incognita,* including parasitism, antibiosis, and predation. Nonetheless, the nematode vitality was induced by these fungal bio-agents' capacity to release antibiotics, metabolites, protease enzymes, etc. [5] into the environment. The effectiveness of bioagents in decreasing nematode viability, however, differed throughout species. To make use of efficient bio-control agents, the possible advantages must be investigated.

Keeping this in view, the present investigations were undertaken to study the efficacy of culture filtrate of different fungal bio-agents in the managing of *M. incognita* infecting tomato under *in- vitro* condition.

2. MATERIALS AND METHODS

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. 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" [2].

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" [2].

Surface sterilization of egg masses: The collected egg masses were surface sterilized in 0.4 per cent sodium hypochlorite (NaOCl) for two minutes [6]. 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 eggs from egg masses": Surface sterilized egg masses were taken in a petridish and subjected to 0.5 % sodium hypochlorite solution for two minutes, with frequent stirring followed by a 30 seconds settleling to dissolve the gelatinous matrix. The eggs released through gelatinous matrix and further disinfested in 0.4 % NaOCl followed by three washing with sterile water. Eggs were then collected on a 500mesh sieve and washed thoroughly with sterilized distilled water to remove the traces of NaOCl. A measured quantity of suspension was prepared with eggs in the distilled water in a measuring cylinder. The egg suspension was prepared in such a way that 1 ml of it contained 100 eggs. The counting of eggs in the suspension was made by using Hawkshley counting dish. Five aliquots of 1 ml suspension were counted and their average number was multiplied with total volume of suspension prepared" [2].

2.1 Preparation of Media

Potato Dextrose Agar (PDA): "The following components were used to prepare PDA: 200 g of peeled potatoes, 20 g of dextrose, 20 g of agar, 1000 ml of water, and a pH of 6.0 to 6.5. After peeling, the potatoes were sliced into slices and cooked in 500 milliliters of distilled water until a glass rod could easily pass through them. After passing the extract through a double layer of muslin cloth, a precise quantity of dextrose was added. The remaining 500 ml of distilled water were transferred to another flask, and the agar-agar was allowed to melt by boiling. After being squeezed through two layers of muslin cloth, the melted agar-agar was combined with the potato extract solution. A 1000ml volume was achieved by adding distilled water. The media's pH was brought 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" [7].

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: As previously noted, "100 ml of potato dextrose broth was made in 250 ml Erlenmeyer flasks and seeded with verified fungal bio-agents to prepare the fungal culture filtrates. The inoculated flasks were incubated in a BOD incubator for 15 days at 25± 2°C. After that, Whatman filter paper no. 1 was used to filter the fungal culture filtrates. After that, the filtrates were centrifuged again at 2000 rpm to get rid of any remaining mycelia and spores. After that, supernatants were gathered and utilized for the *in vitro* research" [2].

Process of spore counting: "For estimation of spores, pure culture of isolated bio-control 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" [2].

Treatments and experimental layout: "Experiment was conducted to investigate the antagonistic effect of fungal bio-control agents on hatching of root-knot nematode, *M. incognita* under *in-vitro* conditions. 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" [2].

Preliminary preparation of experimentation: "Cavity blocks filled with five ml of sterilized distilled water were kept and then uniform sized single sterilized egg mass of *M. incognita* was transferred into them with fungi spore suspension diluted to 10^6 and 10^7 separately. Two drops of 0.1 per cent streptomycin were added to cavity blocks having fungus for avoiding bacterial contamination. One cavity block with sterilized distilled water was maintained for control. After 24, 48, 72, 96 and 120 hrs of exposure observation on hatching and mortality of larvae were recorded under compound microscope" [2].

Hatching test: Five ml of spore suspension of bio-control agents in each sterile cavity block was taken. Surface sterilization of *M. incognita* egg masses were done with 0.4 per cent sodium hypochlorite 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 120 hrs and the numbers of hatched juveniles were recorded out for every 24 hrs interval. The percent inhibition in egg hatching was calculated by using formula:

Per cent 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.

3. RESULTS AND DISCUSSION

Results of studies showed that the maximum suppression in egg hatching was observed in T5 – *P. lilacinum* @ 2×10^6 spore/ml (85.46 per cent), followed by T6 – *P. lilacinum* @ 2×10^7 spore/ml (83.90 per cent), T1- *T. harzianum* @ 2×10^6 spore/ml (83.04 per cent) and T3 - *T. viride* @ 2×10^6 spore/ml (82.00 per cent) whereas T8- *M. anisopliae* @ 2×10^7 spore/ml (69.46 per cent) was found least effective after 120 hrs. The culture filtrate studies revealed that all the tested fungal bio-control agents were effective in suppressing the egg hatching of *M. incognita* (Table 2).

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

Spore suspension concentration	No. of hatched juveniles after an exposure period				
	24 hours	48 hours	72 hours	96 hours	120 hours
T_1 – *T. harzianum* 2×10^6 spore/ml	24.00	34.33	48.00	56.00	65.33
T_2 – *T. harzianum* 2×10^7 spore/ml	29.66	41.00	54.66	65.66	75.33
T_3- *T. viride* 2×10^6 spore/ml	26.33	38.33	51.00	59.33	69.33
T_4 – *T. viride* 2×10^7 spore/ml	31.00	42.66	59.00	71.00	80.33
T_5 – *P. lilacinum* 2×10^6 spore/ml	19.33	29.00	40.00	48.33	56.00
T_6 – *P. lilacinum* 2×10^7 spore/ml	20.66	30.66	43.00	51.66	62.00
T_7 – *M. anisopliae* 2×10^6 spore/ml	40.33	51.00	72.00	85.33	105.33
T_8- *M. anisopliae* 2×10^7 spore/ml	44.00	55.66	77.66	93.33	117.66
T_9- *B. bassiana* 2×10^6 spore/ml	35.00	44.66	62.00	75.33	91.33
T_{10}- *B. bassiana* 2×10^7 spore/ml	38.00	47.33	66.00	79.33	96.33
T_{11}– Control	83.00	155.00	226.66	292.00	385.33

One egg mass of per cavity block. Data are average value of three replications

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

Spore suspension concentration	Per cent inhibition of hatching after an exposure period				
	24 hours	48 hours	72 hours	96 hours	120 hours
T_1 – *Trichoderma harzianum* 2×10^6 spore/ml	71.08 (57.47)	77.85 (62.02)	78.82 (62.71)	80.82 (64.16)	83.04 (65.86)
T_2 – *Trichoderma harzianum* 2×10^7 spore/ml	64.26 (53.30)	73.54 (59.08)	75.88 (60.64)	77.51 (61.75)	80.45 (63.85)
T_3- *Trichoderma viride* 2×10^6 spore/ml	68.27 (55.71)	75.27 (60.17)	77.49 (61.74)	79.68 (63.20)	82.00 (64.89)
T_4 - *Trichoderma viride* 2×10^7 spore/ml	62.65 (52.36)	72.47 (58.35)	73.96 (59.33)	75.68 (60.62)	79.15 (62.83)
T_5 - *Purpureocillium lilacinum* 2×10^6 spore/ml	76.71 (61.18)	81.29 (64.44)	82.35 (65.24)	83.44 (66.08)	85.46 (67.60)
T_6 - *Purpureocillium lilacinum* 2×10^7 spore/ml	75.10 (60.18)	80.21 (63.80)	81.02 (64.34)	82.30 (65.40)	83.90 (66.70)
T_7 - *Metarhizium anisopliae* 2×10^6 spore/ml	51.40 (45.80)	67.09 (54.99)	68.23 (55.69)	70.77 (57.27)	72.66 (58.48)
T_8- *Metarhizium anisopliae* 2×10^7 spore/ml	46.98 (43.26)	64.09 (53.19)	65.73 (54.18)	68.03 (55.58)	69.46 (56.47)
T_9- *Beauveria bassiana* 2×10^6 spore/ml	57.83 (49.50)	71.18 (57.54)	72.64 (58.46)	74.20 (59.48)	76.29 (60.87)

Spore suspension concentration	Per cent inhibition of hatching after an exposure period				
	24 hours	48 hours	72 hours	96 hours	120 hours
T_{10}- *Beauveria bassiana* 2×10^7 spore/ml	54.21 (47.41)	69.46 (56.48)	70.88 (57.48)	72.83 (58.63)	75.00 (60.06)
T_{11}– Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
SEm ±	**1.65**	**1.76**	**2.10**	**2.13**	**1.78**
CD at 5%	**4.70**	**5.08**	**6.07**	**6.15**	**5.15**

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

"Nematode-destroying fungi must be tested *in vitro* to determine their antagonistic activity against *M. incognita*. These fungi produce protease enzymes and/or metabolites that impact nematode viability" [8]. "The chitinous and proteinaceous egg shells of nematodes serve as barriers against fungi that parasitize eggs. These fungi bio-agents generate lytic enzymes, such as lipases, chitinases, and proteases, to break down egg shells and enable egg penetration for effective establishment in order to get past these obstacles" [9,10,11,12,13]. "The same mechanism might be possessed by tested bio-agents that may have ability to produce such type of enzymes which caused extensive network of hyphae inside the *M. incognita* eggs. Similar result were also observed that egg parasitism by fungal bio-agents and observed conidia of *T. harzianum* to stick on the gelatinous matrix around the *M. javanica* eggs masses with prolific fungal growth inside the eggs as the germinating hyphae penetrated the egg masses for parasitization" [14]. "The present investigation demonstrated early age of *M. incognita* eggs to be more susceptible *to P. lilacinum* infection than the eggs with ready to hatch" [15]. Further, they observed extensive network of hyphae of *P. lilacinum* that ramified several eggs as recorded in the present investigation as well.

These findings are in agreement with the results of [16] who reported 92.72 per cent inhibition in hatching of root-knot nematode, *M. incognita* by *T. viride* after 120 hrs. [17] showed *T. harzianum* BI most effective for its capacity to reduce the incidence and pathogenicity of the root-knot nematode *M. javanica* on tomato *In vitro* conditions. Parasitism of *M. javanica* eggs by *T. harzianum* BI ranged from 21.00 per cent in control to 84.00 per cent in antagonistic fungi. *T. harzianum* BI reduced nematode damage to tomato. Another similar reports on inhibition of egg hatching P*. lilacinum, T. viride, P. fluorescens* and *P. penetrans* have been reportred [18,19,20,21,22]. Under *in vitro* conditions, the fungal bio-agents *T. viride, T. harzianum, P. chlamydosporia,* and *P. lilacinum* were evaluated for their ability to effectively inhibit *M. incognita* [2]. The culture filtrates of these fungal bio-agents were examined with respect to their ability to prevent egg hatch and juvenile mortality of *M. incognita*, and the results showed that these bio-agents were effective at 25, 50, 75, and 100% concentrations. *T. harzianum* demonstrated the highest egg hatch inhibition and juvenile mortality of *M. incognita*among the bio-agents, which is consistent with the current findings. As a result, these bio-agents can be used even more for field evaluation.

4. CONCLUSION

Based on the results of the present investigation, it can be concluded that *Purpureocillium lilacinum, Trichoderma harzianum* and *T. viride* tested in this study produce good amount of secondary metabolites that is having lethal effect on egg hatching of *Meloidogyne incognita*. It indicated that the presence of these isolates of *P. lilacinum, T. harzianum* and *T. viride* in the soil may be helpful as a bio agent in the management of this root knot nematode under field conditions.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kumar V, Khan RM, Walia RK. Crop loss estimations due to plant parasitic nematode in major crops in India. National Academy Science Letters; 2020.
2. Annapurna M, Bhagawati B, Kurulkar Uday. *In-vitro*efficacy of native fungal bioagents against *Meloidogyne incognita*. International Journal of Current Microbiology and Applied Sciences. 2018;7(11):396-410.
3. Anita B, Samiyappan R. Induction of systemic resistance in rice by *Pseudomonas fluorescens* against rice root knot nematode *Meloidogyne graminicola.* Journal of Biopesticides. 2012;5:53-59.
4. Karssen G, Moens M, Perry R. Plant nematology. Ed 1. CABI, Oxfordshire. 2006;59–90.
5. Sharon E, Bar EM, Chet I, Herrera-Estrella A, Kleifeld O, Spiegel Y. Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. Phytopathology. 2001;91:687-693.
6. Singh S, Mathur N. *In vitro* studies of antagonistic fungi against the root-knot nematode, *Meloidogyne incognita*. Biocontrol Science and Technology. 2010;20(3):275-282.
7. Aneja KR. Experiments in Microbiology, Plant Pathology and Biochemistry, 4 th edition, New Age International Publishers. New Delhi, India;2003.
8. Nitao JK, Meyer SLF, Chitwood DJ. *In-vitro* assays of *Meloidogyne incognita* and *Heterodera glycines* for detection of nematode-antagonistic fungal compounds. Journal of Nematology. 1991;31:172–183.
9. Elad Y, Chet I, Henis Y. Degradation of plant pathogenic fungi by *Trichoderma harzianum*. Canadian Journal of Microbiology. 1982;28:719-725.
10. Lorito M, Harman GE, Hayes CK, Broadway RM, Tronsmo A, Woo SL. Chitnolytic enzymes produced by *Trichoderma harzianum*: Antifungal activity purified endochitinase and chitobiosidase. Phytopathology.1993;83:302- 307.
11. Kerry BR. Rhizosphere interactions and exploitation of microbial agents for the biological control of plant parasitic nematodes. Annual Review of Phytopathology. 2000;38:423-441.
12. Li B, Xie GL, Soad A, Coosemans J. Suppression of *Meloidogyne javanica* by antagonistic and plant growth promoting rhizobacteria. Journal of Zhejiang University. Science. 2005;496-501.
13. Kalele DN, Affokpon A, Coosemans J, Kimenju JW. Suppression of root knot nematodes in tomato and cucumber using biological control agents. African Journal of Horticultural Sciences. 2010;3:72-80.
14. Golzari H, Panjehkeh N, Ahmadzadeh M, Salari M, Sedaghati-Khoravi E. Elucidating the parasitic capabilities of *Trichoderma* against *Meloidogyne javanica* on tomato. Insight Plant Disease. 2011;1(1): 12-19.
15. Pau CG, Leong CTS, Wong SK, Eng L, Jiwan M, Kundat FR. Isolation of indigenous strains of *Paecilomyces lilacinus* with antagonistic activity against *Meloidogyne incognita*. International Journal of Agriculture and Biology. 2012; 14:197–203.
16. Rompalli R, Mehendrakar SR, Venkata PK. Evaluation of potential bio control agents on root-knot nematode, *Meloidogyne incognita* and wilt causing fungus *Fusarium oxysporum* f.sp. *conglutinansin vitro*. African Journal of Biotechnology. 2016; 15(19):798-805.
17. Naserinasab F, Sahebani N, Etebarian HR. Biological control of *Meloidogyne javanica* by *Trichoderma harzianum* BI and salicylic acid on tomato. African Journal of Food Science. 2012;5(3):276 – 280.
18. Devi G, Bora LC. Effect of some biocontrol agents against root-knot nematode (*Meloidogyne incognita* race2). International Journal of Environment, Agriculture and Biotechnology. 2018;3: 1748-1755.
19. Guru PGR, Ravichandra, NG. Evaluation of indigenous antagonists on inhibition of egg hatching and larval mortality of *Meloidogyne incognita*infecting carrot under *In vitro*. International Journal of Current Microbiology and Applied Science. 2018;7(5):917-924.
20. Anjum SS, Reddy BMR. *In vitro* evaluation of bio-agents against the root-knot nematode *Meloidogyne incognita*. Mysore Agricultural Science. 2013;47(2): 432-434.
21. Narasimhamurthy T, Reddy B, Prahalada G, Shashikumara H. *In vitro* evaluation of indigenous bio agents on egg hatching and larval mortality of root-knot nematode (*Meloidogyne incognita*) infecting mulberry. Uttar Pradesh Journal of Zoology. 2011;31(2):169-176.

22. Narasimhamurthy TN. Evaluation of indigenous and commercial bio agents for management of root knot nematode, *Meloidogyne incognita* (kofoid and white) chitwood1949 in mulberry (*Morus alba* L.). University of Agricultural Sciences. Bengaluru; 2010.

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