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# Effect on Hatching and Mortality of Root-knot Nematode (*Meloidogyne javanica*) by Bio-agents

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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

Investigations were carried out in *vitro* condition to evaluate the antagonistic effect of fungal and bacterial bio-agents. For effective management of root-knot nematode experiment was conducted in laboratory condition using the culture filtrate (5, 10 and 20 percent concentrate) of bio-agents (*viz., Trichoderma viride, Trichoderma harzianum, Purpureocillium lilacinum, Pochonia chlamydosporia* and *Pseudomonas fluorescens*). Experimental results showed that all the tested bio-agents significantly reduced the percent hatched juveniles and increased the percent mortality

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of juveniles. Among the tested bio-agents *T. viride* was found most effective treatment with minimum percent hatched juveniles and maximum percent mortality on eggs and juveniles of *Meloidogyne javanica.*

*Keywords: Root-knot nematode; meloidogyne spp.; bio-agent; hatching; mortality.*

## 1. INTRODUCTION

Nematodes are hidden enemy of plant. Among the nematodes, root-knot nematode, *Meloidogyne* spp. is an important pest of vegetables all around the world and causes severe damage to brinjal crop in India [1], the root-knot nematode was first reported by [2] on tea (*Thea sinensis*) roots from Devala territory of Tamil Nadu, South India. Arya 1957 [3], reported it "first time in Rajasthan from Jodhpur, while in 1966 Yadav and Naik [4], found *Meloidogyne* spp. and its wide distribution in the soils of Rajasthan". "The nematode infected plant shows reduced root system with less feeder roots" [5]. "Overall, plant-parasitic nematodes cause 21.3% crop losses amounting to Rs. 102040 million (1.58 billion USD) annually; the losses in 19 horticultural crops were assessed at Rs. 50,225 million, while for 11 field crops it was estimated at Rs. 51,815 million. Among the vegetable crops comparatively more losses recorded in tomato (Rs. 6035.2 million), brinjal (Rs. 3499.12 million) and okra (2480.86 million)" [6]. "Management of nematode is much difficult as compare to other pathogen because nematodes mainly attack underground parts of plants" [7]. "The nematode control mainly depended on synthetic nematicides" [8]. "Although, nematicides are efficient and fast acting, yet they are currently being reappraised as there are relatively unaffordable to many small-scale farmers. The potential negative effect on environment and ineffectiveness after prolonged use have led to a total ban or restricted use of most chemical nematicides and an urgent need for safe and more effective alternatives" [9]. "Biological control appears the alternative strategies for management of plant parasitic nematodes in the soil" [10,11]. "Application of microorganisms antagonistic to root-knot nematodes or compounds produced by these microbes could provide an additional option for managing the damage caused by root-knot nematodes. Fungi and bacteria are among the most dominant soil-borne groups in natural soil ecosystem and some of them have shown great potential as biological control agents for root-knot nematodes" [12]. So, the present investigation was carried out on test the effect of different bio- agents' cultural filtrate on root-knot nematode.

## 2. MATERIALS AND METHODS

The efficacy of bio-agents tested against hatching and mortality of juveniles of root-knot nematode in laboratory condition.

### 2.1 Preparation and Maintenance of Pure Culture of *M. javanica*

*M. javanica* infected brinjal plant were uprooted from the pure culture plots of Division of Nematology, RARI, Durgapura, Jaipur and brought to the laboratory. The roots were first rinsed carefully in water to remove adhering soil particles. Egg masses were carefully detached from roots, then egg masses were kept in distilled water in watch glasses at room temperature for hatching.

### 2.2 Preparation of Culture Filtrates of Fungal and Bacterial Bio-agents

"The potato dextrose agar (PDA) for fungal agents and nutrient agar (NA) 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 hrs. 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" [13].

One ml of sterilized double distilled water was added on fully grown fresh mother culture of bio-agents *Trichoderma viride, Trichoderma harzianum, Purpureocillium lilacinum, Pochonia chlamydosporia* and *Pseudomonas fluorescens* 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.

## 2.3 Effect of Bio-agents on Root-knot Nematode in Laboratory Condition

### 2.3.1 Effect of bio-agent on hatching of *M. javanica*

To test the effect of bio-agents on nematode hatching the experiment was conducted in completely randomized design (CRD) with four replications. One healthy average sized egg mass of *M. javanica* were collected from infected brinjal roots and kept in glass cavity block (1 egg mass/ cavity block) containing 3 ml of bio-agents formulation 5, 10 and 20 percent (*Trichoderma viride, Trichoderma harzianum, Purpureocillium lilacinum, Pochonia chlamydosporia* and *Pseudomonas fluorescens*) concentrations respectively. A distilled water control was also maintained simultaneously. Number of juveniles hatched were recorded after 24, 48 and 72hrs under binocular microscope.

The percent of egg hatching was calculated by using formula:

Hatching percent = (C/T) × 100 %

Where,

C = number of parasitized nematodes after 24, 48 and 72hrs exposure.
T = total number of nematodes in a cavity block.

### 2.3.2 Effect of bio-agent on mortality of *M. javanica*

The experiment on test the effect of bio-agents on nematode mortality was laid out in completely randomized design (CRD) with four replications. Freshly hatched second stage juveniles of *M. javanica* were transferred to different cavity blocks (10 juveniles/ cavity block) containing 3 ml of bio-agents formulation *i.e.,* 5, 10 and 20 percent *(Trichoderma viride, Trichoderma harzianum, Purpureocillium lilacinum, Pochonia chlamydosporia* and *Pseudomonas fluorescens)* concentrations respectively. A distilled water control was maintained simultaneously. Percent juvenile mortality rate was counted at intervals of 24, 48 and 72 hrs. The dead juveniles attained the shape of straight line and the mortality was ensured by touching the juvenile with a fine needle.

The percent mortality was calculated by using formula:

Percent 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.

## 2.4 Statistical Analysis

After completion of experiment, data were statically analyzed for interpretation of finding. The critical deference was calculated 5 % level of significance. Summary table along with SEm± and CD were worked out.

## 3. RESULTS

Effect of bio-agents were tested on the hatching and mortality of *M. javanica* under laboratory conditions. 5, 10 and 20 percent concentration of bio-agents culture filtrate were used for hatching and mortality of *M. javanica.* Number of hatched larvae out from egg masses and dead larvae were observed after 24, 48 and 72 hrs. Data presented in Tables 1 and 2 showed that all the bio-agents significantly decreased number of hatched juveniles and increased mortality compared to untreated check. The minimum number of hatched juveniles and maximum number of dead juveniles were observed with the *T. viride* at 20 percent concentration followed *P. lilacinum* and *P. fluorescens* respectively at 20 percent concentration. While, maximum number of hatched juveniles and no mortality were observed in untreated check.

## 3.1 Effect of Bio-agents on Percent Hatching

### 3.1.1 Hatching at 5 percent

Data presented in Table 1 showed that all the bio-agents significantly decreased number of hatched juveniles compared to untreated check. The minimum number of hatched juveniles (24.38percent) were observed with the *T. viride* after 72 hrs followed by *P. lilacinum* (25.39percent) and *P. fluorescens* respectively (26.88percent) after 72 hrs. While, maximum number of hatched juveniles (75.78percent) were observed in untreated check.

### 3.1.2 Hatching at 10 percent

Data revealed that the minimum number of hatched juveniles (20.70percent) were observed with the *T. viride* after 72 hrs followed *P. lilacinum* (21.17percent) and *P. fluorescens*

**Table 1. Effect of bio-agent on percent hatching of root-knot nematode juveniles**

| Concentration | 5% | | | 10% | | | 20% | | |
|---|---|---|---|---|---|---|---|---|---|
| Treatment | 24 h | 48 h | 72 h | 24 h | 48 h | 72 h | 24 h | 48 h | 72 h |
| *T. viride* | 19.45 | 21.48 | 24.38 | 16.72 | 18.28 | 20.70 | 12.03 | 13.59 | 14.53 |
| *T. harzianum* | 25.00 | 26.41 | 28.44 | 22.97 | 24.84 | 26.25 | 20.31 | 22.03 | 23.36 |
| *P. lilacinum* | 21.56 | 23.59 | 25.39 | 18.20 | 19.77 | 21.17 | 13.75 | 15.00 | 16.41 |
| *P. chlamydosporia* | 23.91 | 25.39 | 27.50 | 21.72 | 23.05 | 24.30 | 18.52 | 20.08 | 21.56 |
| *P. fluorescens* | 21.95 | 24.06 | 26.88 | 20.16 | 21.72 | 23.44 | 16.72 | 18.44 | 20.00 |
| Control | 31.95 | 55.16 | 75.78 | 31.95 | 55.16 | 75.78 | 31.95 | 55.16 | 75.78 |
| SEm± | 1.16 | 1.479 | 1.965 | 1.34 | 1.630 | 2.048 | 1.324 | 1.680 | 2.044 |
| CD 5% | 3.45 | 4.39 | 5.837 | 3.97 | 4.844 | 6.084 | 3.93 | 4.993 | 6.072 |
| CV % | 9.69 | 10.08 | 11.31 | 12.18 | 12.02 | 12.82 | 14.02 | 13.97 | 14.29 |

** Average of four replications*

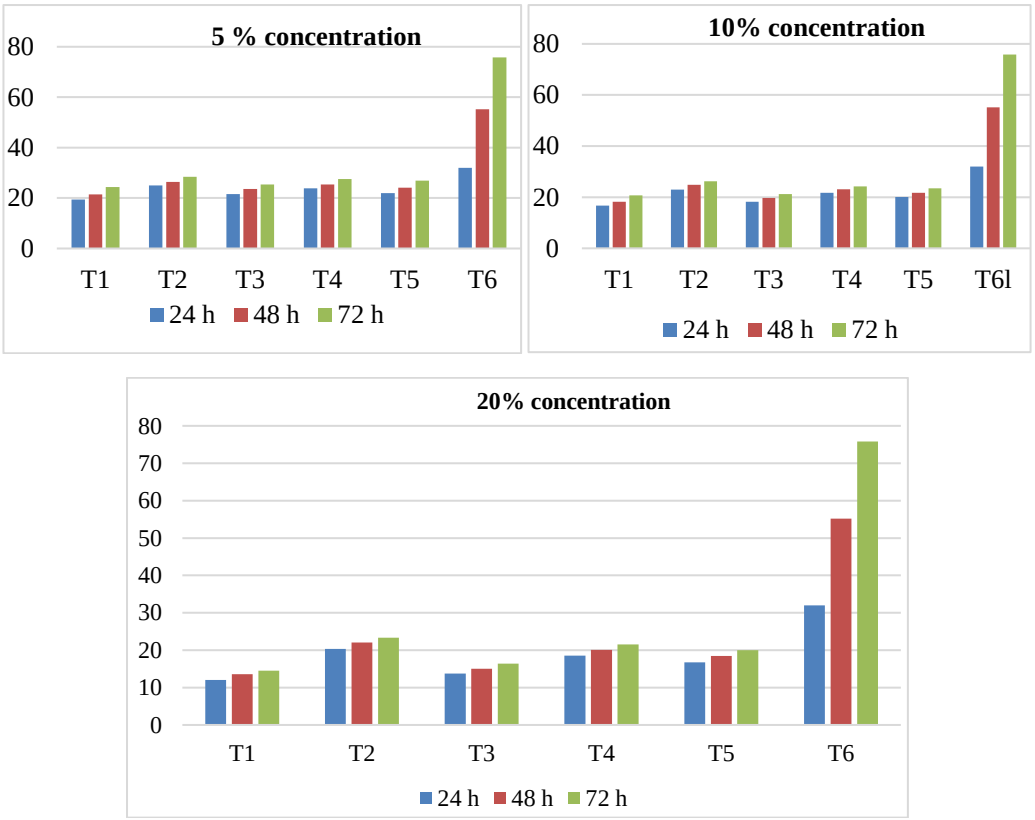


**Fig.1. Effect of bio-agents on percent hatching of root-knot nematode juveniles**

respectively (23.44percent) after 72 hrs. Whereas, maximum number of hatched juveniles (75.78percent) were observed in untreated check. All the bio-agents significantly decreased number of hatched juveniles over untreated check.

### 3.1.3 Hatching at 20 percent

Data showed that *T. viride* after 72 hrs was observed most effective treatment with minimum number of hatched juveniles (14.53) followed *P. lilacinum* (16.41percent) and *P. fluorescens* respectively (20.00 percent) after 72 hrs. However, untreated check was observed least effective treatment with maximum number of hatched juveniles (75.78 percent).

## 3.2 Effect of Bio-agents on Percent Mortality

### 3.2.1 Mortality at 5 percent

All the bio-agents significantly increased percent mortality of juveniles over untreated check. Data

**Table 2. Effect of bio-agent on root-knot nematode juveniles percent mortality**

| Concentration | 5.00% | | | 10.00% | | | 20.00% | | |
|---|---|---|---|---|---|---|---|---|---|
| Treatment | 24 h | 48 h | 72 h | 24 h | 48 h | 72 h | 24 h | 48 h | 72 h |
| *T. viride* | 35.00 | 45.00 | 52.50 | 45.00 | 50.00 | 60.00 | 55.00 | 65.00 | 80.00 |
| *T. harzianum* | 12.50 | 17.50 | 22.50 | 15.00 | 25.00 | 27.50 | 25.00 | 32.50 | 40.00 |
| *P. lilacinum* | 25.00 | 32.50 | 40.00 | 35.00 | 40.00 | 47.50 | 45.00 | 50.00 | 65.00 |
| *P. chlamydosporia* | 17.50 | 22.50 | 27.50 | 25.00 | 32.50 | 37.50 | 32.50 | 42.50 | 55.00 |
| *P. fluorescens* | 22.50 | 30.00 | 35.00 | 32.50 | 40.00 | 42.50 | 42.50 | 45.00 | 60.00 |
| Control | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SEm± | 2.43 | 2.700 | 3.173 | 2.57 | 3.281 | 3.118 | 2.500 | 2.764 | 4.082 |
| CD 5% | 7.22 | 8.02 | 9.428 | 7.63 | 9.748 | 9.264 | 7.43 | 8.212 | 12.130 |
| CV % | 25.92 | 21.97 | 21.45 | 20.21 | 21.00 | 17.40 | 15.00 | 14.11 | 16.33 |

** Average of four replications*


**Fig. 2. Effect of bio-agents on percent mortality of root-knot nematode juveniles**

presented in Table 2 showed that the maximum juvenile's mortality percent (52.50 percent) were observed with the *T. viride* after 72 hrs followed by *P. lilacinum* (40.00 percent) and *P. fluorescens* (35.00 percent). While, juvenile's mortality was not observed in untreated check.

### 3.2.2 Mortality at 10 percent

The maximum percent mortality of juveniles (60.00 percent) were observed with the *T. viride* after 72 hrs followed by *P. lilacinum* (47.00 percent) and *P. fluorescens* (42.00 percent).

Whereas, juvenile's mortality was not observed in untreated check. All the bio-agents significantly increased mortality of juveniles over untreated check.

### 3.2.3 Mortality at 20 percent

Data presented in Table 2 showed that all the bio-agents significantly increased percent mortality of juveniles over untreated check. Among all the tested bio-agents *T. viride* was observed best treatment with maximum percent mortality of juveniles (80.00 percent) after 72hrs followed by *P. lilacinum* (65.00 percent) and *P. fluorescens* (60.00 percent). However, untreated check was observed least effective with no mortality of juveniles.

## 4. DISCUSSION

The obtained results were similar with the findings of [14] they found "culture filtrates of *A. strictum* was very effective against the nematode in regards to egg parasitism, egg hatching inhibition and mortality compared to controls. *A. strictum* was caused greater mortality of the second stage juveniles ($J_2$). *A. terreus* did not show egg parasitism but was found to be highly toxic against second stage juveniles ($J_2$) causing high mortality". Sharma et al. [15] showed that "*Paecilomyces lilacinus* culture filtrate from Karanj cake medium killed 100% *Meloidogyne incognita* larvae while only 78.28% mortality was recorded by Czapeck-Dox filtrate within 12 h of exposure". Annapurna *et al* 2018, showed *T. harzianum* was caused highest egg hatch inhibition and juvenile mortality of *M. incognita*. The culture filtrate of *T. harzianum* showed the highest activity with a LC50 value at 96 hrs of exposure. Popal et al. [16], also studied the efficacy of different bio-agents on egg-hatching and larval mortality of *M. incognita* after 24, 48 and 72 hours of inoculation. *T. viride* was superior over *P. penetrans* as against untreated check in regards to egg-hatching. While, regarding larval mortality of the nematode *P. lilacinus* and *P. penetrans* were at par with each other and were superior over *T.* viride. Kumari et al. [17], tested bio-control agents on hatching and juvenile mortality of *M. incognita* after 24, 48, 72 and 96 hrs exposure period and found that *T. viride* and *T. asperellum* were at par and significantly effective on hatching inhibition and larval mortality of *M. incognita*. "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" [18,19,20,21]. "All bio-agent 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" [22,23]. "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" [24].

## 5. CONCLUSION

All the bio-agents significantly decreased the number of hatched juveniles and increased the mortality compared to untreated check. The minimum number of hatched juveniles and maximum number of dead juveniles were observed with the *T. viride* at 20 percent concentration followed *P. lilacinum* and *P. fluorescens* respectively at 20 percent concentration. While, maximum number of hatched juveniles and no mortality were observed in untreated check.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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