***In-vitro* evaluation of biocontrol agents against eggs of *Meloidogyne* *incognita* infesting Mulberry**

**ABSTRACT**

The present study aimed to assess the *in-vitro* efficacy of selected biocontrol agents in inhibiting egg hatching of *Meloidogyne incognita* to evaluate the efficacy of various bioagents *viz.*, *Trichoderma harzianum, Paecilomyces lilacinus*, *Lecanicillium lecanii, Bacillus subtilis,* *Pseudomonas fluorescens,* microbial consortia1 (*P. fluorescens+ B. subtilis+ L. lecanii)* and microbial consortia2 (*T. harzianum* +*P. fluorescens*+ *L. lecanii*)*.* Experimental results showed that all the bioagents significantly reduced the hatching rate of juveniles and increased the egg hatching inhibition rate. Among the bioagents, microbial consortia 1 (*P. fluorescens* + *B. subtilis* + *L. lecanii*) was found to be the most effective treatment with a minimum juvenile hatching rate of 9.67% and maximum egg hatching inhibition rate of 84.97% at 100% concentration after 72 hours, followed by microbial consortia 2 (*T. harzianum* + *P. fluorescens* + *L. lecanii*) and *Paecilomyces lilacinus*.

Keywords: *Meloidogyne incognita*, Bioagents, Microbial consortia, Egg hatching inhibition, Mulberry.

1. **INTRODUCTION**

Mulberry is a hardy perennial deep-rooted, fast-growing tree species widely adaptable to different environmental climatic conditions and only food for silkworm (*Bombyx mori*). The quality and quantity of mulberry leaves decide the cocoon production and in turn quality of silk (Bharath *et al.,* 2024) However, mulberry leaf yield and quality gets affected by various biotic and abiotic factors. India is the second largest producer of silk in the world after China. The country achieved a raw silk production of 36,582 MT against the target of 40,800 MT during 2022-23, with 4.8 % increase over 2021-22. Mulberry sector achieved 27,654 MT raw silk production (BV-8,904 MT & CB-18,750 MT) covering 2.53 lakh ha area under mulberry plantation. The major constraints in the cultivation of mulberry and production of quality leaves are attack by pests and diseases including plant parasitic nematodes. Phytonematodes affecting the mulberry are considered to be a serious threat to sericulture industry (Ramakrishnan and Senthilkumar, 2003). More than 42 species of nematodes belonging to 24 genera are reported to cause the root knot disease in mulberry all over the world. Among them, *Meloidogyne*0*incognita* (Kofoid & White) Chitwood is the one affecting more than 80 % of mulberry plantations in various mulberry growing regions (Nandan *et al*., 2022).

It causes around 10- 12 % leaf yield loss in mulberry (Govindaiah *et al*., 1991). The management of root-knot nematodes is more challenging than that of other pests, as nematodes primarily inhabit the soil and attack the underground parts of the mulberry plant (Sukumar *et al.,* 2004). Various management practices *viz.*, cultural, physical, chemical and biological methods are there for management of root knot nematode infestation.

Among them, chemical methods through the application of nematicides used by farmers were known to have better efficacy in field conditions. However, the use of soil fumigants and nematicides for the control of nematode infestation may lead to soil fertility degradation, environmental pollution and also cause possible toxicity to silkworms. Hence, restrictions were imposed on the usage of these chemical compounds in agriculture to reduce the possible deleterious effects and chemicals have limited the availability of management strategies against plant-parasitic nematodes making to think of eco-friendly approaches for its management from the point of view of both mulberry and silkworm (Shruthi *et al*., 2024).Considering these concerns, there is a greater emphasis on biological control methods, which are more feasible, economical, and environmentally safer Because of this, the present studies were carried out on eco-friendly management of root knot nematode which helps to overcome the deleterious effect of nematicides on soil health by using various biocontrol agents under *in-vitro* conditions.

1. **MATERIAL AND METHODS**

*In-vitro* studies were undertaken in the Pathology laboratory, Department of Plant Pathology, College of Sericulture, Chintamani during the year of 2023-24.

**2.1 Collection of samples and egg masses**

Root knot infected mulberry roots collected from a known infested field (sick plot) located in Chintamani Taluk, Karnataka State. (Fig.1) and washed gently under running tap water to clean all soil particles adhering to the roots. Egg masses were clearly seen attached to the surface of roots exactly above the galls developed (Fig.2). These egg masses were picked with the help of forceps under a stereo microscope and were These egg masses were picked with the help of forceps under a stereo microscope and were transferred to a petriplate containing sterile water.

**Fig. 2:** Egg mass of mulberry root knot nematode

**Fig. 1**: Collection of root knot infected samples from infested field

**2.2 Preparation of culture filtrates of fungal and bacterial bioagents**

Five different biocontrol agents were used in this study *viz., Trichoderma harzianum, Paecilomyces lilacinus, Pseudomonas fluorescens, Bacillus subtilis, Lecanicillium lecanii* along with two microbial consortia. Velume prime as a nematicide (Fluopyram 34.48 % SC) was used as a standard check: different concentrations of culture filtrates were prepared and sterile distilled water served as a control.

The potato dextrose broth (PDB) for fungal bioagents and nutrient broth (NB) for bacterial bioagent agents were prepared, inoculated with respective bio agents in 1000 mL sterilized conical flasks and incubated at 28°C in mechanical shaker at 100 rpm ensuring continuous agitation for 48 h. After incubation, the culture broth was centrifuged at 6000 rpm for 15-20 min at 4°C and supernatant was collected sterilized 1000 mL conical flask, which served as a stock filtrate of 100 % concentration. The stock filtrate was diluted to 25, 50, 75 % concentration using sterile distilled water.

**Table 1. Treatment details**

|  |  |
| --- | --- |
| **Sr. No.** | **Treatments** |
| **T1** | Cell-free culture filtrates of *Trichoderma harzianum* at 25, 50, 75,100 % dilutions |
| **T2** | Cell-free culture filtrates of *Paecilomyces lilacinus* at 25, 50, 75,100 % dilutions |
| **T3** | Cell-free culture filtrates of *Lecanicillium lecanii* at 25, 50, 75,100 % dilutions |
| **T4** | Cell-free culture filtrates of *Bacillus subtilis* at25, 50, 75,100 % dilutions |
| **T5** | Cell-free culture filtrates of *Pseudomonas fluorescens* at 25, 50, 75, 100 % dilutions |
| **T6** | Cell-free culture filtrates of microbial consortia1 (*Pseudomonas fluorescens+ Bacillus subtilis+Lecanicillium lecanii)* at 25, 50, 75,100 % dilutions |
| **T7** | Cell-free culture filtrates of microbial consortia2 (*Trichoderma harzianum* + *Pseudomonas fluorescens* + *Lecanicillium lecanii*) at 25, 50, 75,100 % dilutions |
| **T8** | Velume prime (Positive check) |
| **T9** | Distilled water (Negative check) |

**2.3. Effect of bioagents on egg-hatching**

From the freshly collected samples, three egg masses were carefully excised and transferred into each of the Petri plates (5 cm diameter). Each plate contained approximately 200-300 eggs, depending on the size and maturity of the egg masses. The plates were filled with 10 mL of culture filtrates at concentrations of 25, 50, 75, and 100% of the respective bacterial and fungal bio-agents. A Petri plate with sterile distilled water served as a control. The egg masses were gently scratched to release the eggs and ensure uniform exposure to the treatments. Each treatment was replicated three times and incubated at room temperature. The plates were examined under a stereo binocular microscope at 24-hour intervals for three consecutive days (24, 48, and 72 hours), and the number of hatched juveniles was recorded. The percentage of egg hatching inhibition was calculated using Abbott's formula (1987).

where,

I: Inhibition of the egg hatching, T: Number of eggs hatched in suspension in treatment, C: Number of eggs hatched in the control

1. **RESULTS AND DISCUSSION**

The rate of egg hatching inhibition at four different concentrations *viz*., 25, 50, 75 and 100 % culture filtrates of isolated bacterial and fungal bioagents were given in Table 2, 3 and 4.

**3.1 After 24 hours of treatment**

Egg hatching in bioagents inoculated plates ranged from 24.67 to 42.33 (average number of eggs hatched) and 59.33 in control (distilled water) at 25 % concentration of culture filtrate. The minimum eggs (24.67) were hatched in MC1 (*P. fluroscens + B. subtilis +L. lecanii)* culture filtrate amounting to 58.43 % followed by the MC2 (*T. harzianum* + *P. fluorescens* + *L. lecanii)* (28.33), amounting to 52.25 % egg hatching inhibition. The % inhibition of egg hatching in *P. lilacinus* (32.00)*, L. lecanii* (34.67), *P.  fluorescens* (37.33) and *T. harzianum* (39.33) were 46.07, 41.57, 37.08 and 33.71 %, respectively and they were significantly different from each other. However, among the biocontrol agents the maximum number of eggs hatched was in culture filtrate of *B. subtilis* (42.33), amounting to 28.65 % inhibition of egg hatching.

At fifty % concentration, the average number of eggs hatched in bioagents treated plates ranged between 21.67 to 39.33 and in control (distilled water) it was 59.33. Minimum eggs hatched (21.67) was in MC1 (*P. fluroscens + B. subtilis + L. lecanii)* culture filtrate amounting to 63.48 % suppression followed by MC2 (*T. harzianum* + *P. fluorescens* + *L. lecanii)*  with 24.33 average number of eggs and 58.99 % egg hatching inhibition and maximum eggs hatched was in *B. subtilis* (39.33), followed by *T. harzianum* (36.00), *P. fluroscens* (34.33), *L. lecanii* (31.00) and *P. lilacinus* (28.67) with inhibition % of 33.71, 39.33, 42.13, 47.75 and 51.69 respectively, but they were significantly different from each other.

All the bioagents treated treatments significantly reduced the egg hatching compared to the control at 75 % culture filtrate of bioagents. The average number of eggs hatched ranged between 17.67 to 35.67. The minimum eggs hatched was recorded in MC1 (*P. fluroscens + B. subtilis + L. lecanii)* (17.67), amounting to 70.22 % suppression and it was significantly higher than MC2 (*T. harzianum* + *P. fluorescens* + *L. lecanii)* (21.33) and *P. lilacinus* (24.67) followed by *L. lecanii* (28.67) amounting to 64.04, 58.43 and 51.69 % suppression, respectively. Maximum (35.67) eggs hatched was in the treatment *B. subtilis*, amounting to 39.89 % suppression followed by *T. harzianum* (33.00) with inhibition % of 44.38.

At 100 % concentration, the average number of eggs hatched in the bioagent-treated treatments ranged between 14.00 to 32.33 against 59.33 in control (distilled water). Minimum eggs hatched was in the MC1 (*P. fluorescens + B. subtilis + L. lecanii)* (14.00) amounting to 76.40 % suppression and was significantly higher than all other bioagents treated treatments. The maximum eggs hatched was in the culture filtrate of *B. subtilis* (32.33) followed by *T. harzianum* (30.00), *P. fluroscens* (28.33), *L. lecanii* (25.33), *P. lilacinus* (21.67) and MC2 (*T. harzianum*+ *P. fluorescens* + *L. lecanii)* (17.33) with inhibition % of 45.51, 49.44, 52.25, 57.30, 63.48 and 70.79, respectively and were significantly different from each other.

**3.1.1 After 48 hours of treatment**

At 25 % concentration of the culture filtrate, the egg hatching in the bioagents treated treatments was ranged from 23.67 to 42.67 (average number of eggs hatched) compared to control (distilled water) (64.33). The fewest eggs were hatched in the MC1 (*P. fluorescens + B. subtilis + L. lecanii*) filtrate (23.67), resulting in 63.21 % inhibition of egg hatching, which was significantly higher than all other treatments. The next best treatment was MC2 (*T. harzianum + P. fluorescens + L. lecanii*) with average number of eggs hatched (26.33), and 59.07 % suppression of egg hatching. The highest average number of eggs hatched among the bio control agents were in the culture filtrateof *B. subtilis* (42.67), with 33.68 % inhibition, followed by *T. harzianum* (38.67), *P. fluorescens* (37.00), *L. lecanii* (33.67) and *P. lilacinus* (31.33) with inhibition % of 39.90, 42.49, 47.67 and 51.30, respectively.

Among the different biocontrol agents, egg hatching ranged from the 19.67 to 39.67 (average number of eggs hatched), in contrast to untreated control (64.33) at fifty % concentration of the culture filtrate of bioagents. The minimum number of eggs hatched were in MC1 (*P. fluorescens + B. subtilis + L. lecanii*) (19.67, with 69.43% inhibition) and it was significantly distinct from other treatments. However, maximum eggs hatched was in *B. subtilis* (39.67), followed by *T. harzianum* (36.33), *P. fluorescens* (33.33), *L. lecanii* (30.67), *P. lilacinus* (27.33) and MC2 (*T. harzianum + P. fluorescens + L. lecanii*) (23.33), with inhibition % of 38.34, 43.52, 48.19 ,52.33, 57.51 and 63.73 % respectively.

At 75 % concentration, all bioagents substantially inhibited the egg hatching. MC1 (*P. fluorescens + B. subtilis + L. lecanii*) (16.67) exhibited the minimum egg hatching with 74.09 % inhibition followed by MC2 (*T. harzianum + P. fluorescens + L. lecanii*) (19.33 no. of eggs and 69.95% inhibition). The maximum eggs hatched was in the culture filtrate of *B. subtilis* (36.00), followed by the *T. harzianum* (33.33), *P. fluroscens* (31.33), *L. lecanii* (27.67) and *P. lilacinus* (24.33) amounting to 44.04, 48.19, 51.30, 56.99 and 62.18 % inhibition, they were significantly different from one another.

At hundred % concentration of culture filtrate of bioagents, significant differences were noticed between the treatments with respect to average number of eggs hatched and percent egg hatching inhibition. Less (13.00) number of eggs were hatched in the MC1 (*P. fluorescens + B. subtilis + L. lecanii*) culture filtrate treated treatment resulting in 79.79 % suppression. The culture filtrate of MC2 (*T. harzianum + P*. *fluorescens + L. lecanii)* resulted in average 16.33 eggs hatched and 74.61 % inhibition. The maximum number of hatched eggs among biocontrol agents were noticed in *B. subtilis* (32.67) with 49.22 % inhibition, followed by *T. harzianum* (29.33), *P. fluroscens* (26.33), *L. lecanii* (24.00) and *P. lilacinus* (21.00) amounting to 54.40, 59.07, 62.69 and 67.36 % inhibition.

**3.1.2 After 72 hours of treatment**

Effect on egg hatching of *Meloidogyne incognita* by different bioagents were documented after 72 hours of treatment and observations are conferred in Table 3 and Fig . All the bioagnets significantly inhibited egg hatching compared to control treatment under *in-vitro* experiments.

At twenty-five % concentration, the average number of egg hatching in bioagents treated plates were ranged from 21.00 to 42.67 compared to the control (distilled water) (69.33). The minimum (21.00) eggs hatched was noticed in MC1 (*P. fluorescens + B. subtilis + L. lecanii)* followed by MC2 (*T. harzianum + P. fluorescens + L. lecanii*) (24.33), *P. lilacinus* (29.33) and *L. lecanii* (32.00) amounting to 69.71, 64.90, 57.69 and 53.85 % suppression, respectively, and they were significantly different from each other. Maximum (42.67) number of eggs hatched was noticed in the case of *B. subtilis* resulting in 38.46 % inhibition, followed by *T. harzianum* (37.67) amounting to 45.67 % inhibition.

Egg hatching in the bioagents inoculated treatments were varied from 17.67 to 39.00 at fifty % concentration. The lowest (17.67) number of eggs hatched was in MC1 (*P. fluorescens + B. subtilis + L. lecanii*) amounting to 74.52 % suppression followed byMC2 (*T. harzianum + P. fluorescens + L*. *lecanii)* (21.33) amounting to 69.23 % inhibition, and were significantly distinct from each other. The highest number of eggs hatched among bioagents was in *B. subtilis* (39.00) leading to 43.75 % suppression followed by *T. harzianum* with average number of 35.00 eggs and 49.52 % inhibition.

At seventy-five % concentration, the egg hatching was ranged between 14.33 to 35.67 in culture filtrates of bioagents. The minimum egg hatching of 14.33 was recorded in MC1 (*P. fluorescens + B. subtilis + L. lecanii*) resulting in 79.33 % inhibition followed by MC2 (*T*. *harzianum + P. fluorescens + L. lecanii*) (18.00) amounting to 74.04 % inhibition. However, *B. subtilis* exhibited the maximum (35.67) egg hatching among bioagents, followed by *T. harzianum* (32.00), *P. fluorescens* (28.67), *L. lecanii* (27.00) and *P. lilacinus* (24.00) amounting to 48.56, 53.85, 58.65, 61.06 and 65.38 % suppression, respectively and they were significantly distinct from one another.

.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatments** | **Concentration of culture filtrates (%)** | | | | | | | |
| **25%** | | **50%** | | **75%** | | **100%** | |
| Avg no. of eggs hatched | % inhibition over control | Avg no. of eggs hatched | %  inhibition over control | Avg no. of eggs hatched | %  inhibition over control | Avg no. of eggs hatched | %  inhibition over control |
| T1 = *Trichoderma harzianum* | 39.33 | 33.71 | 36.00 | 39.33 | 33.00 | 44.38 | 30.00 | 49.44 |
| T2 = *Paecilomyces lilacinus* | 32.00 | 46.07 | 28.67 | 51.69 | 24.67 | 58.43 | 21.67 | 63.48 |
| T3 = *Lecanicillium lecanii* | 34.67 | 41.57 | 31.00 | 47.75 | 28.67 | 51.69 | 25.33 | 57.30 |
| T4 = *Bacillus subtilis* | 42.33 | 28.65 | 39.33 | 33.71 | 35.67 | 39.89 | 32.33 | 45.51 |
| T5 = *Pseudomonas fluorescens* | 37.33 | 37.08 | 34.33 | 42.13 | 31.33 | 47.19 | 28.33 | 52.25 |
| T6 = MC1 (*P. fluroscens + B. subtilis +L. lecanii)* | 24.67 | 58.43 | 21.67 | 63.48 | 17.67 | 70.22 | 14.00 | 76.40 |
| T7= MC2 (*T. harzianum*+ *P. fluorescens* + *L. lecanii)* | 28.33 | 52.25 | 24.33 | 58.99 | 21.33 | 64.04 | 17.33 | 70.79 |
| T8 = Velume prime | 16.67 | 71.91 | 14.33 | 75.84 | 10.67 | 82.02 | 8.33 | 85.96 |
| T9 =Distilled water | 59.33 | 0 | 59.33 | 0 | 59.33 | 0 | 59.33 | 0 |
| SEm ± | 0.36 |  | 0.40 |  | 0.41 |  | 0.40 |  |
| CD @ 1 % | 1.10 |  | 1.20 |  | 1.24 |  | 1.20 |  |

**Table 2. Egg hatching inhibition of *Meloidogyne incognita* after 24 hours of treatment of culture filtrates of bioagents**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatments** | **Concentration of culture filtrates (%)** | | | | | | | |
| **25%** | | **50%** | | **75%** | | **100%** | |
| Avg no. of eggs hatched | %  inhibition over control | Avg no. of eggs hatched | %  inhibition over control | Avg no. of eggs hatched | %  inhibition over control | Avg no. of eggs hatched | %  inhibition over control |
| T1 = *Trichoderma harzianum* | 38.67 | 39.90 | 36.33 | 43.52 | 33.33 | 48.19 | 29.33 | 54.40 |
| T2 = *Paecilomyces lilacinus* | 31.33 | 51.30 | 27.33 | 57.51 | 24.33 | 62.18 | 21.00 | 67.36 |
| T3 = *Lecanicillium lecanii* | 33.67 | 47.67 | 30.67 | 52.33 | 27.67 | 56.99 | 24.00 | 62.69 |
| T4 = *Bacillus subtilis* | 42.67 | 33.68 | 39.67 | 38.34 | 36.00 | 44.04 | 32.67 | 49.22 |
| T5 = *Pseudomonas fluorescens* | 37.00 | 42.49 | 33.33 | 48.19 | 31.33 | 51.30 | 26.33 | 59.07 |
| T6 = MC1(*P. fluorescens + B. subtilis +L. lecanii)* | 23.67 | 63.21 | 19.67 | 69.43 | 16.67 | 74.09 | 13.00 | 79.79 |
| T7= MC2(*T. harzianum*+ *P. fluorescens* + *L. lecanii)* | 26.33 | 59.07 | 23.33 | 63.73 | 19.33 | 69.95 | 16.33 | 74.61 |
| T8 = Velume prime | 14.67 | 77.20 | 11.67 | 81.87 | 9.67 | 84.97 | 7.00 | 89.12 |
| T9 =Distilled water | 64.33 | 0 | 64.33 | 0 | 64.33 | 0 | 64.33 | 0 |
| SEm ± | 0.36 |  | 0.43 |  | 0.36 |  | 0.45 |  |
| CD @ 1 % | 1.10 |  | 1.28 |  | 1.10 |  | 1.37 |  |

**Table 3. Egg hatching inhibition of *Meloidogyne incognita* after 48 hours of treatment of culture filtrates of bioagents**

**Table 4. Egg hatching inhibition of *Meloidogyne incognita* after 72 hours of treatment of culture filtrates of bioagents**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatments** | **Concentration of culture filtrates (%)** | | | | | | | |
| **25%** | | **50%** | | **75%** | | **100%** | |
| Avg no. of eggs hatched | %  inhibition over control | Avg no. of eggs hatched | %  inhibition over control | Avg no. of eggs hatched | %  inhibition over control | Avg no. of eggs hatched | %  inhibition over control |
| T1 = *Trichoderma harzianum* | 37.67 | 45.67 | 35.00 | 49.52 | 32.00 | 53.85 | 27.33 | 57.51 |
| T2 = *Paecilomyces lilacinus* | 29.33 | 57.69 | 27.00 | 61.06 | 24.00 | 65.38 | 17.33 | 73.06 |
| T3 = *Lecanicillium lecanii* | 32.00 | 53.85 | 30.00 | 56.73 | 27.00 | 61.06 | 21.00 | 67.36 |
| T4 = *Bacillus subtilis* | 42.67 | 38.46 | 39.00 | 43.75 | 35.67 | 48.56 | 30.67 | 52.33 |
| T5 = *Pseudomonas fluorescens* | 35.33 | 49.04 | 33.00 | 52.40 | 28.67 | 58.65 | 24.33 | 62.18 |
| T6 = MC1 (*P. fluoroscens + B. subtilis +L. lecanii)* | 21.00 | 69.71 | 17.67 | 74.52 | 14.33 | 79.33 | 9.67 | 84.97 |
| T7 =MC2 (*T. harzianum*+ *P. fluorescens* + *L. lecanii)* | 24.33 | 64.90 | 21.33 | 69.23 | 18.00 | 74.04 | 13.00 | 79.79 |
| T8 = Velume prime | 11.33 | 83.65 | 8.00 | 88.46 | 5.33 | 92.31 | 2.67 | 95.85 |
| T9 =Distilled water | 69.33 | 0 | 69.33 | 0 | 69.33 | 0 | 64.33 | 0 |
| SEm ± | 0.44 |  | 0.60 |  | 0.53 |  | 0.52 |  |
| CD @ 1 % | 1.33 |  | 1.82 |  | 1.59 |  | 1.56 |  |

Egg hatching in the bioagents treated plates was varied from 9.67 to 30.67 at hundred % concentration culture filtrates of bioagents. Egg hatching exhibited was minimum (9.67) in the MC1 (*P. fluorescens + B. subtilis + L. lecanii*) treated batches achieving 84.97 % inhibition, followed by MC2 (*T. harzianum* + *P. fluorescens* + *L. lecanii*) (13.00) amounting to 79.79 % inhibition and were significantly different. The maximum egg hatching among bioagents were observed in *B. subtilis* (30.67), followed by *T. harzianum* (27.33) and *P. fluorescens* (24.33) resulting to 52.33, 57.51 and 62.18 % inhibition over untreated control respectively.

Based on the above observations, it can be inferred that there was positive correlation between the concentrations of culture filtrate, duration of incubation and egg hatching inhibition percent and juvenile mortality. The culture filtrates at 100 % concentration were found to be most effective in suppressing the hatching of *M. incognita* eggs. Any how when compared with all the treatments the positive control Velume prime recorded significantly lower number of eggs hatched with maximum percent of egg hatching inhibition.

The present findings are in accordance with that of Koulagi *et al*. (2024) who reported that among the different bio consortia treatments, combination of the *T. harzianum + B. subtilis + P. lilacinum + B. bassiana + M. anisophile + P. fluroscens* recorded the lowest egg hatching % of 10.35, 29.35, 39.85 and 55.65, respectively at different time intervals with inhibition percent of 44.35.

The credible reason for the suppression of egg hatching might be release of unknown compounds, plantazolicin, which is structurally similar to telomerase inhibitor telomestatin, which might be responsible for nematicidal activity of bioagent *Bacillus* as reported by Liu *et al*. (2013).

Turatto *et al*. (2018) reported the inhibitory action of Plant Growth Promoting Rhizobacteria (PGPR) which might be associated with the production of chitinase and some other cell wall degrading substances that degraded the wall of eggs of *Meloidogyne javanica* and eventually, diminished the development of juveniles of Ditylenchus spp. The inhibitory effect of *Pseudomonas* and *Bacillus* on egg hatching was reported by El-Sherif *et al*. (1994). The bioagent *B. subtilis* produces various antibiotic compounds, nematicidal volatiles and lipopolypeptides which are antagonistic towards the egg hatching of *Meloidogyne* spp. The antibiotic compound, fengycin produced by *B. subtilis* showed a strong lethal activity against the nematodes as reported by Huang *et al*. (2010) and Kavitha *et al*. (2012). The suppression of egg hatching by *Bacillus* spp. might be also due to high production of chitinase, chitosanase and protease, using colloidal chitin and soluble chitosan as carbon sources effectively inhibited egg hatching by altering the eggshell structures. *Bacillus subtilis* exhibited the greatest inhibitory effect, achieving 77.97 % inhibition of egg hatching in *Meloidogyne incognita* asreported by Soliman *et al*. (2019).

Present results are in conformity with the findings of Soliman *et al*. (2019), who reported higher mortality of juveniles (J2) (97.00%) of *Meloidogyne incognita* upon inoculation with culture filtrates of *Bacillus subtlis*. They showed that production of chitinase, chitosanase, and protease activities effectively inhibited egg hatching, and altered the eggshell structures. Moreover, eggs treated with the produced chitinase displayed large and more vacuoles in the chitin layer and increasing the mortality percentage of *M. incognita* J2 in *in-vitro* tests. Hajji-Hedfi*et al*. **(**2023) reported that the culture filtrate (100%) of *Lecanicillium* spp. was highly effective against root-knot nematode, with 91 % rate of second-stage juvenile (J2) mortality.

1. **CONCLUSION**

From the results it can be concluded that all the tested fungal and bacterial bioagents were able to control root knot nematode by suppression of egg hatching and increasing the mortality of second stage juveniles under *in vitro* conditions. Among the treatments, the maximum egg hatching inhibition was recorded in the Microbial consortia1 (*P. fluorescens+ B. subtilis+ L. lecanii*) (84.97%) and (64.33%) over untreated control. 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.

**Disclaimer (Artificial intelligence)**

Option 1:

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 the writing or editing of this manuscript.

**COMPETING INTERESTS**: Authors have declared that no competing interests exist.

**REFERENCES**

Bharath, K. B., Vinoda, K. S., Kavitha, T. R., & Banuprakash, K. G. (2024). Nematode diversity and root-knot nematode management approaches for sustainable mulberry production. *Annual Research & Review in Biology*, 39(9), 79–86. <https://doi.org/10.9734/arrb/2024/v39i92121>

El‑Sherif, M. A., Ali, A. H., & Barakat, M. I. (1994). Suppressive bacteria associated with plant parasitic nematodes in Egyptian agriculture. *Japanese Journal of Nematology, 24*(2), 55–59. https://doi.org/10.3725/jjn1993.24.2\_55.

Govindaiah, D., Dandin, S. B., & Sharma, D. D. (1991). Pathogenicity and avoidable leaf yield loss due to *Meloidogyne* in mulberry (*Morus alba* L.). *Indian Journal of Nematology, 21*(1), 96–99.

Hajji‑Hedfi, L., Hlaoua, W., Rhouma, A., Al‑Judaibi, A. A., Arcos, S. C., Robertson, L., Ciordia, S., et al. (2023). Biological and proteomic analysis of a new isolate of the nematophagous fungus *Lecanicillium* spp. *BMC Microbiology, 23*(1), 108. https://doi.org/10.1186/s12866-023-02855-4

Huang, X., Niu, Q., Zhang, L., Xu, J., Yang, D., Wei, K., et al. (2010). A Trojan horse mechanism of bacterial pathogenesis against nematodes. *Proceedings of the National Academy of Sciences, 107*(38), 16631–16636. https://doi.org/10.1073/pnas.1007276107.

Kavitha, P. G., Jonathan, E. I., & Nakkeeran, S. (2012). Effects of crude antibiotic of *Bacillus subtilis* on hatching of eggs and mortality of juveniles of *Meloidogyne incognita*. *Nematologia Mediterranea, 40*, 203–206.

Koulagi, S., Rathod, V. K., & Hejjegar, I. (2024). *In‑vitro* efficacy of microbial consortia against tomato root‑knot nematode (*Meloidogyne incognita* [Kofoid & White]). *Biological Forum – An International Journal, 15*(12), 1–4. DOI not located.

Liu, Z., Budiharjo, A., Wang, P., Shi, H., Fang, J., & Borriss, R., et al. (2013). The highly modified microcin peptide plantazolicin is associated with nematicidal activity of *Bacillus amyloliquefaciens* FZB42. *Applied Microbiology and Biotechnology, 97*(23), 10081–10090.

Nandan, M., Venkataravana, P., Devaraja, Mahesh, M., & Ramakrishna Naika. (2022). Eco‑friendly management of root‑knot nematode in mulberry – An overview. *Mysore Journal of Agricultural Sciences, 56*(4), 1–9.

Ramakrishnan, S. & Senthilkumar, T. (2003). Plant parasitic nematodes, a serious threat to mulberry-A review*. Indian J. Seric*., 42(2), 82-92.

Shruthi, G. H., Karthik, V. C., Bharathi, B. K. M., Dodmani, B. A., Samreen, K., Ramya Harika, K., & Kishan Kumar, R. (2024). Role of bio-control agents in mulberry pest management: Effective strategies and key challenges. *International Journal of Environment and Climate Change*, 14(9), 454–460. <https://doi.org/10.9734/ijecc/2024/v14i94429>

Soliman, G. M., Ameen, H. H., & Abdel‑Aziz, S. M. (2019). In‑vitro evaluation of some isolated bacteria against the plant parasite nematode *Meloidogyne incognita*. *Bulletin of the National Research Centre, 43*(1), 171–177.

Sukumar, J., Padma, S. D., & Bongale, U. D**.** (2004). Biological control of mulberry root-knot nematode *Meloidogyne incognita* by *Trichoderma harzianum*. *Indian Journal of Sericulture*, 43(2), 177–180.

Turatto, M. F., Dourado, F. D. S., Zilli, J. E., & Botelho, G. R. (2018). Control potential of *Meloidogyne javanica* and *Ditylenchus* spp. using fluorescent *Pseudomonas* and *Bacillus* spp. *Brazilian Journal of Microbiology, 49*(1), 54–58.