**Evaluation of Biocontrol Agents under *in vitro* Conditions Against Eggs of *Meloidogyne* *incognita* infesting Mulberry**

**ABSTRACT**

*In-vitro* studies were undertaken in the Pathology laboratory, Department of Plant Pathology, College of Sericulture, Chintamani, to evaluate the efficacy of various bioagents *viz.*, *Trichoderma harzianum, Paecilomyces lilacinus*, *Lecanicillium lecanii, Bacillus subtilis,* *Pseudomonas fluorescens,* Microbial consortia 1 (*P. fluorescens + B. subtilis + L. lecanii)* and Microbial Consortia 2 (*T. harzianum* + *P. fluorescens* + *L. lecanii*)*.* Experimental results showed that all the bioagents significantly reduced the per cent hatched juveniles and increased the per cent egg hatching inhibition. Among the tested bio-agents, Microbial consortia 1 (*P. fluorescens* + *B. subtilis* + *L. lecanii*) was found most effective treatment with minimum per cent hatched juveniles (9.67) and increased per cent egg hatching inhibition (84.97%) @ 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. However, mulberry leaf yield and quality are 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 a 4.8 per cent 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. Phyto nematodes affecting the mulberry are considered to be a serious threat to the 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 incognita* (Kofoid & White) Chitwood is the one affecting more than 80 per cent of mulberry plantations in various mulberry growing regions (Nandan *et al*., 2022).

It causes around 10- 12 per cent leaf yield loss in mulberry (Govindaiah *et al*., 1991). The management of root knot nematodes is more challenging than that of other pests because nematodes primarily inhabit in soil and tend to attack the underground parts of mulberry. 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 (Nandan *et al*., 2022). 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 egg masses**

Root knot infected mulberry roots were collected from the sick plot and washed gently under running tap water to clear away all soil particles adhering to the roots. Egg masses were clearly seen attached to the surface of roots exactly above the galls developed. These egg masses were picked with the help of forceps under a stereo microscope and were transferred to a Petri plate containing sterile water.

**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 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 agents and nutrient broth (NB) for bacterial agents were prepared, inoculated with respective bioagents in 1000 mL sterilized conical flasks and incubated at 28°C in a 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**

|  |  |
| --- | --- |
| **Sl. No.** | **Treatments** |
| **T1** | Cell-free culture filtrates of *Trichoderma harzianum* @ 25, 50, 75, 100% dilutions |
| **T2** | Cell-free culture filtrates of *Paecilomyces lilacinus* @ 25, 50, 75, 100 % dilutions |
| **T3** | Cell-free culture filtrates of *Lecanicillium lecanii* @ 25, 50, 75, 100% dilutions |
| **T4** | Cell-free culture filtrates of *Bacillus subtilis @* 25, 50, 75, 100% dilutions |
| **T5** | Cell-free culture filtrates of *Pseudomonas fluorescens* @ 25, 50, 75, 100% dilutions |
| **T6** | Cell-free culture filtrates of Microbial Consortia 1 (*Pseudomonas fluorescens + Bacillus subtilis + Lecanicillium lecanii)* @ 25, 50, 75, 100% dilutions |
| **T7** | Cell-free culture filtrates of Microbial Consortia 2 (*Trichoderma harzianum* + *Pseudomonas fluorescens* + *Lecanicillium lecanii*) @ 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 excised and transferred to each of the Petri plates (5 cm), which were filled with 10 mL of culture filtrates of different concentrations (25, 50, 75 and 100%) of all bacterial and fungal bioagents and a Petri plate with sterile water served as a control. Egg masses were gently scratched so that eggs were exposed to different treatments. Three replications of each treatment were maintained and incubated at room temperature. The treated plates were observed under a stereo binocular microscope for egg hatching every 24 h of incubation for 3 days (24, 48, and 72h) and the number of hatched eggs was counted at every 24 h interval. The per cent egg hatching inhibition was calculated using the following Abbott's (1987) formula:

$$I (\%) =\frac{ (C-T)}{C}X100$$

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**

Per cent egg hatching inhibition at four different concentrations, viz., 25, 50, 75 and 100 per cent culture filtrates of isolated bacterial and fungal bioagents, were given in Tables 1, 2 and 3 and Fig. 1.

**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 per cent 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 per cent followed by MC2 (*T. harzianum* + *P. fluorescens* + *L. lecanii)* (28.33), amounting to 52.25 per cent egg hatching inhibition. The per cent 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 per cent, 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 per cent inhibition of egg hatching.

At fifty per cent 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 per cent suppression followed by MC2 (*T. harzianum* + *P. fluorescens* + *L. lecanii)*  with 24.33 average number of eggs and 58.99 per cent 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 per cent of 33.71, 39.33, 42.13, 47.75 and 51.69 respectively, but they were significantly different from each other.

All the bioagents treatments significantly reduced egg hatching compared to the control at 75 per cent 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 per cent 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 per cent suppression, respectively. Maximum (35.67) eggs hatched was in treatment *B. subtilis*, amounting to 39.89 per cent suppression, followed by *T. harzianum* (33.00) with inhibition per cent of 44.38.

At 100 per cent 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 per cent suppression and was significantly higher than all other bioagent 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 per cent 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 per cent concentration of the culture filtrate, egg hatching in the bioagents treatments 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 per cent 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 an average number of eggs hatched (26.33), and 59.07 per cent suppression of egg hatching. The highest average number of eggs hatched among the biocontrol agents was in the culture filtrateof *B. subtilis* (42.67), with 33.68 per cent inhibition, followed by *T. harzianum* (38.67), *P. fluorescens* (37.00), *L. lecanii* (33.67) and *P. lilacinus* (31.33) with inhibition per cent of 39.90, 42.49, 47.67 and 51.30, respectively.

Among the different biocontrol agents, egg hatching ranged from 19.67 to 39.67 (average number of eggs hatched), in contrast to untreated control (64.33) at fifty per cent concentration of the culture filtrate of bioagents. The minimum number of eggs hatched was 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 were 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 per cent of 38.34, 43.52, 48.19,52.33, 57.51 and 63.73 per cent, respectively.

At 75 per cent 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 per cent 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 *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 per cent inhibition, they were significantly different from one another.

At hundred per cent 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. Fewer (13.00) number of eggs were hatched in the MC1 (*P. fluorescens + B. subtilis + L. lecanii*) culture filtrate-treated treatment, resulting in 79.79 per cent suppression. The culture filtrate of MC2 (*T. harzianum + P*. *fluorescens + L. lecanii)* resulted in an average of 16.33 eggs hatched and 74.61 per cent inhibition. The maximum number of hatched eggs among biocontrol agents was noticed in *B. subtilis* (32.67) with 49.22 per cent 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 per cent inhibition.

**3.1.2 After 72 hours of treatment**

Effect on egg hatching of *Meloidogyne incognita* by different bioagents was documented after 72 hours of treatment and observations are conferred in Table 3 and Fig. 2. All the bioagents significantly inhibited egg hatching compared to control treatment under *in vitro* experiments.

At 25% concentration, the average number of egg hatching in bioagents-treated plates 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 per cent 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 per cent inhibition, followed by *T. harzianum* (37.67), amounting to 45.67 per cent inhibition.

Egg hatching in the bioagents inoculated treatments was varied from 17.67 to 39.00 at fifty per cent concentration. The lowest (17.67) number of eggs hatched was in MC1 (*P. fluorescens + B. subtilis + L. lecanii*), amounting to 74.52 per cent suppression, followed byMC2 (*T. harzianum + P. fluorescens + L*. *lecanii)* (21.33), amounting to 69.23 per cent 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 per cent suppression, followed by *T. harzianum* with an average number of 35.00 eggs and 49.52 per cent inhibition.

At 75% concentration, the egg hatching 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 per cent inhibition, followed by MC2 (*T*. *harzianum + P. fluorescens + L. lecanii*) (18.00), amounting to 74.04 per cent 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 per cent suppression, respectively and they were significantly distinct from one another.

Egg hatching in the bioagents-treated plates was varied from 9.67 to 30.67 at hundred per cent 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 per cent inhibition, followed by MC2 (*T. harzianum* + *P. fluorescens* + *L. lecanii*) (13.00), amounting to 79.79 per cent inhibition and were significantly different. The maximum egg hatching among bioagents was observed in *B. subtilis* (30.67), followed by *T. harzianum* (27.33) and *P. fluorescens* (24.33), resulting in 52.33, 57.51 and 62.18 per cent inhibition over untreated control, respectively.

Based on the above observations, it can be inferred that there was a positive correlation between the concentrations of culture filtrate, duration of incubation and egg hatching inhibition percent and juvenile mortality. The culture filtrates at 100 per cent 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 numbers of eggs hatched, with maximum percent of egg hatching inhibition.

The present findings are in accordance with those 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 per cent 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 also be 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 per cent inhibition of egg hatching in *Meloidogyne incognita* asreported by Soliman *et al*. (2019).

Present results conform 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* bioagent. 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 a 91 per cent rate of second-stage juvenile (J2) mortality.

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| --- | --- |
| **Treatments** | **Concentration of culture filtrates (%)** |
| **25%** | **50%** | **75%** | **100%** |
| Avg no. of eggs hatched | Per cent inhibition over control | Avg no. of eggs hatched | Per cent inhibition over control | Avg no. of eggs hatched | Per cent inhibition over control | Avg no. of eggs hatched | Per cent 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 | Per cent inhibition over control | Avg no. of eggs hatched | Per cent inhibition over control | Avg no. of eggs hatched | Per cent inhibition over control | Avg no. of eggs hatched | Per cent 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. fluroscens + 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 | Per cent inhibition over control | Avg no. of eggs hatched | Per cent inhibition over control | Avg no. of eggs hatched | Per cent inhibition over control | Avg no. of eggs hatched | Per cent 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. fluroscens + 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 |  |

**Fig. 1: Per cent egg hatching inhibition of *Meloidogyne incognita* in different concentrations of culture filtrates of bioagents after 72 hours**

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 *invitro* conditions. Among the treatments, the maximum egg hatching inhibition was recorded in the Microbial Consortia 1 (*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.

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