**Original Research Article**

**“Nematicidal Activity of Fungal and Bacterial Metabolites Against *Meloidogyne* *incognita* Infesting Mulberry”**

**ABSTRACT**

Mulberry (*Morus alba* L.) serves as the exclusive host plant for the silkworm (*Bombyx mori* L.) and is cultivated mainly for its nutritious foliage. However, leaf yield and quality are adversely impacted by several soil-borne pathogens, with root-knot nematode (*Meloidogyne incognita*) being one of the most destructive pests. The present investigation was conducted in vitro at the Department of Plant Pathology, College of Sericulture, Chintamani, to evaluate the nematicidal efficacy of secondary metabolites extracted from selected biocontrol agents, including *Paecilomyces lilacinus*, *Lecanicillium lecanii*, and *Pseudomonas fluorescens*. A total of five treatments were tested, each replicated four times, and both egg hatching inhibition and juvenile mortality of *M. incognita* were assessed at four concentrations (25%, 50%, 75%, and 100%) over a 72-hour period under *in-vitro* conditions. The study revealed that these metabolites effectively suppressed egg hatching and increased juvenile mortality when compared to the untreated control. Notably, *Paecilomyces lilacinus* exhibited the highest suppression, recording 85.60 per cent egg hatching inhibition and 70.50 per cent juvenile mortality after 72 hours of incubation at 100 per cent metabolite concentration. These results suggest that fungal secondary metabolites, particularly from *P. lilacinus*, offer promise potential as eco-friendly alternatives for managing *M. incognita* in mulberry cultivation systems.

**Keywords:** M*ulberry,* *Meloidogyne incognita,* *Paecilomyces lilacinus,* secondary metabolites, biocontrol agents, *in-vitro*

1. **Introduction**

Mulberry (*Morus alba* L.) is a hardy, perennial, and deep-rooted plant that is widely cultivated for its leaves, which serve as the sole food source for the domesticated silkworm (*Bombyx mori* L.) (Datta, 2000). The success of sericulture depends heavily on the nutritional quality and availability of mulberry foliage, as it directly influences cocoon yield and silk quality (Krishnaswami, 1978). Owing to its adaptability, mulberry is grown across both tropical and temperate regions of the world.

India is the second-largest producer of silk globally, following China, and holds the unique distinction of being the only country that produces all four major types of silk—mulberry, eri, tasar, and muga (CSB, 2024). The sericulture industry in India is a vital agro-based livelihood sector, providing employment to approximately 9.2 million people, mainly in rural and semi-urban areas. According to recent data, India produced 36,582 metric tonnes of raw silk in 2022–23, with mulberry silk contributing 27,654 metric tonnes from about 2.53 lakh hectares of cultivated area. Karnataka alone accounted for over 32% of this production, with other key states including Andhra Pradesh, Tamil Nadu, West Bengal, Uttar Pradesh, Jammu & Kashmir, and northeastern regions (CSB, 2024).

Despite favorable climatic conditions and government support, mulberry cultivation is affected by several biotic and abiotic stresses. Among the biotic factors, plant-parasitic nematodes pose a major threat to mulberry health and productivity. The root-knot nematode *Meloidogyne incognita* (Kofoid and White) is one of the most destructive species, causing root galling that interferes with water and nutrient uptake, thereby reducing leaf yield and quality (). The infestation also negatively affects silkworm growth and cocoon formation, ultimately impacting silk quality and farmer income.

Although synthetic nematicides have been widely used for nematode management, their prolonged and indiscriminate application has raised significant concerns, including environmental contamination, human health risks, resistance development, and harm to beneficial soil microflora (Akhtar & Malik, 2000). These drawbacks have driven a shift toward sustainable and eco-friendly approaches to nematode control.

Biological control using microbial agents especially fungi and bacteria has emerged as a promising alternative. Several biocontrol organisms, such as *Trichoderma* spp. and *Pseudomonas* spp., produce secondary metabolites that exhibit nematicidal properties through mechanisms like cuticle degradation, paralysis, egg shell disruption, and inhibition of egg hatching (Muthulakshmi and Devrajan, 2015). These microbial metabolites are gaining attention for their specificity, biodegradability, and minimal ecological impact. However, despite growing interest, there remains a significant gap in understanding the specific effects of fungal and bacterial metabolites on the egg stage of *Meloidogyne incognita* infesting mulberry. Most studies to date have concentrated on juvenile mortality or root gall suppression, while the inhibitory action on egg hatching an early and vulnerable stage in the nematode life cycle has received limited attention, particularly under *in vitro* conditions. Therefore, the present study was undertaken to evaluate the *in vitro* nematicidal activity of selected fungal and bacterial secondary metabolites on the eggs and juveniles of *Meloidogyne incognita* infesting mulberry. The findings aim to support the development of biobased, environmentally sound strategies for nematode management in sericulture.

1. **Material and Methods**

**2.1 Collection of samples**

Root samples were obtained from mulberry fields across various locations in Chintamani taluk, Karnataka state selected based on the presence of visible symptoms. Plants exhibiting signs of stunted growth and leaf yellowing were carefully uprooted using a scoop or spade. From each field, samples were randomly collected from 4 to 6 different points within the root zone of actively growing mulberry plants (Fig. 1).

**Fig 1: Mulberry fields infested by *Meloidogyne incognita***

**2.2 Collection of egg masses of *Meloidogyne incognita***

Root-knot-infected mulberry roots were collected from the sick plot and gently washed under running tap water to remove adhering soil particles Egg masses were clearly visible on the root surface, positioned directly above the developed galls. These egg masses were picked with the help of forceps under a stereo microscope and were transferred to a Petri plate containing sterile water.

**2.3 Extraction of juveniles (J2) from egg masses of *Meloidogyne incognita***

Three egg masses from infected mulberry plants were carefully picked with the help of forceps under a stereo binocular microscope (Olympus SZ61) and transferred to a Petri plate containing sterile water and incubated at room temperature for 48-96 h to facilitate egg hatching and release of juveniles from the eggs. After 48-96 h, the hatched juveniles were harvested from the suspension. One milliliter of suspension was placed on a nematode counting dish, and the number of juveniles was counted under a stereo binocular microscope. The concentration was adjusted to 100 juveniles per mL.

**2.4 Extraction of secondary metabolites from potential biocontrol agents against** **eggs of** ***Meloidogyne infesting* mulberry**

Secondary metabolites were extracted from *the* biocontrol agents *Paecilomyces lilacinus, Lecanicillium lecanii, and Pseudomonas fluorescens.*

150 mL each of nutrient broth (for bacteria) and potato dextrose broth (for fungi) were prepared in 250 mL conical flasks and sterilized. A single bacterial or fungal colony was inoculated into the respective sterilized broth aseptically and incubated at 28°C in mechanical shaker for continuous agitation at 100 rpm for 24 h. After incubation, the culture broth was subjected to centrifuge at 9000 rpm for 15-20 min at 4°C and supernatant was collected in sterilized conical flask. Ethyl acetate was used in extraction process due to its moderate polarity and low toxicity for secondary metabolite extraction.In order to extract the secondary metabolites, 150 ml of ethyl acetateorganic solvent was added to the 150 ml of supernatant collected in flask (1:1). The mixture was shaken well to mix supernatant and ethyl acetate organic solvent and the mixture was transferred to the separating funnel for separation into two layers of solvent and aqueous phase (Fig. 2). The solvent phase was collected in a separate sterilized glass bottle for further experiments. The secondary metabolites were extracted and diluted to different concentrations for testing, with sterile distilled water used as the control

Solvent phase

Aqueous phase

**Fig 2. Extract of secondary metabolites from, bioagent *Paecilomyces lilacinus***

**2.5 Effect on Inhibition of Egg Hatching**

Three egg masses were collected from infected mulberry roots and was transferred to each of the Petri plates (5 cm) separately which were filled with 10 mL of extracted secondary metabolite suspension of different concentrations (25, 50, 75 and 100 per cent) of bioagents and a Petri plate with sterile water served as a control. Three replications of each treatment were maintained and were incubated at room temperature. The treated plates were observed under a stereo binocular microscope(Olympus SZ61) for egg hatching after every 24 h of incubation for 3 days (24, 48 and 72h) and number of hatched eggs was counted at each 24 h interval. The per cent egg hatching inhibition was calculated using the following (Abbott, 1987) formula:

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

**2.6 Effect on juvenile mortality**

The freshly hatched juveniles from egg masses were collected and juvenile population was made up to 100 juveniles per mL of suspension as shown in section 3.2.5.2. One mL of suspension with 100 juveniles was placed in 10 mL of secondary metabolites extracts of different concentrations (25, 50, 75 and 100 per cent) of bio-agents separately in sterilized Petri plates (5 cm) and a Petri plate with sterile water served as a control. Three replications of each treatment were maintained and were incubated at room temperature. The plates were observed for juvenile mobility after 24, 48 and 72 h at 24-hour intervals. Based on the movement of juveniles on probing with a needle, the juvenile was considered as dead and number of dead larvae was counted in each treatment. Juvenile mortality was calculated according to the (Abbott, 1987) formula:

where, JM: Juvenile mortality, T: Number of dead J2 in treatment, C: Number of J2 used in control

**Experiment details:**

“The experiment was laid out in a Completely Randomized Design (CRD) with 5 treatments, 4 replications and a total of 20 experimental units.”

TABLE 1:

|  |  |
| --- | --- |
| **Sl.No.** | **Treatments** |
| **T1** | *Paecilomyces lilacinus* @ 25, 50, 75,100 per cent dilutions |
| **T2** | *Pseudomonas fluorescens* @ 25, 50, 75,100 per cent dilutions |
| **T3** | *Lecanicillium lecanii @* 25, 50, 75,100 per cent dilutions |
| **T4** | Velume prime (Positive check) |
| **T5** | Distilled water (Negative check) |

1. **Results and Discussion**

**3.1 Egg hatching inhibition of *Meloiodgyne incognita***

The efficacy of four concentrations of secondary metabolites (25, 50, 75 and 100%) extracted from three potential biocontrol agents was evaluated for their ability to suppress egg hatching in *Meloidogyne incognita*. The observations were recorded at 24 h interval for three continuouslydays after treatment (Table 2,3 and 4).

**3.1.1 After 24 hours of treatment**

At twenty-five per cent concentration of secondary metabolites extract, there was a significant difference in egg hatching between the bio-agent-treated batches over the control (distilled water). The egg hatching was ranged from 23.75 to 28.25 (average number of eggs hatched) in the bioagents treated batches, while in the control it was 49.00. The minimum egg hatching was noticed in *P. lilacinus* (23.75) amounting to 51.53 per cent suppression, which was significantly greater than all other treatments. The maximum eggs hatched was in the treatment with *P. fluorescens* (28.25) and *L. lecanii* (26.00) which lead to 42.35 and 46.94 per cent inhibition, respectively over control.

At fifty per cent concentration of secondary metabolites extract, egg hatching was ranged from 20.75 to 26.00 (average number of eggs hatched) in the secondary metabolites extract treated treatments and were significantly distinct from the control. The lowest number of eggs hatched was recorded in *P. lilacinus* (20.75) leading to 57.65 per cent suppression. Meanwhile, *P. fluorescens* and *L. lecanii* recorded of 26.00 and 24.00 eggs hatched, respectively, with 46.94 and 51.02 per cent inhibition compared to control. A significant difference was noticed among the treatments.

Egg hatching in the secondary metabolites extract from bioagents treated groups was ranged from 17.25 to 23.00 (average number of eggs hatched) at seventy-five per cent concentration. The minimum (17.25) number of eggs hatched was recorded in *P. lilacinus* leading to 64.80 per cent suppression*,* which was significantly superior over all other treatments. The maximum (23.00) number of eggs hatched was observed in *P. fluorescens* amounting to 53.06 per cent suppression, followed by *L. lecanii* (20.25) with inhibition per cent of 58.67, they were significantly distinct from each other.

All the bioagents significantly inhibited the egg hatching compared to the control at 100 per cent concentration of secondary metabolites extract. Egg hatching in the secondary metabolites extract treated plates was varied from 15.50 to 20.75, while in the control it was 49.00. The treatment *P. lilacinus* (15.50) exhibited the minimum egg hatching with 68.37 per cent suppression. The next best treatment was *L. lecanii* with average number of eggs hatched(18.25), and 62.76 per cent suppression of egg hatching. The maximum (20.75) number of eggs hatched was observed in *P. fluorescens* amounting to 57.65 per cent suppression and all of which were significantly different from one another.

**3.1.2 After 48 hours of treatment**

At twenty-five per cent concentration of secondary metabolites extract, there was a significant difference in egg hatching was observed between the bio-agent-treated plates and the control (distilled water). In the bioagents treated batches, the egg hatching was ranged from 23.00 to 30.50 (average number of eggs hatched), while in the control it was 60.50. The minimum (23.00) number of eggs hatched was noticed in *P. lilacinus* amounting to 61.98 per cent suppression, which was significantly higher than all other treatments. However, the maximum number of eggs hatched was in *P. fluorescens* (30.50) and *L. lecanii* (26.75) amounting to 49.59 and 55.79 per cent inhibition, respectively as compared to untreated control.

Egg hatching fluctuated between 20.25 to 27.25 (average number of eggs hatched) in the bioagents treated plates were significantly distinct from the control. The lowest (20.25) number of eggs hatched was recorded in *P. lilacinus* leading to 66.53 per cent suppression. The highest number (27.25) of eggs hatched was recorded in *P*. *fluorescens,* followed by the *L. lecanii* (23.25) leading to 54.96 and 61.57 per cent inhibition, respectively as compared to control. A significant difference in egg hatching was observed.

At seventy-five per cent concentration of secondary metabolites extract, the egg hatching was varied from 17.50 to 24.00 in the bioagents treated treatments which were significantly distinct from the control. The minimum number of eggs hatched was noticed in *P. lilacinus* (17.50), followed by the *L. lecanii* (21.00) resulting to 71.07 and 65.29 per cent inhibition, respectively as compared to control. The maximum (24.00) number of eggs hatched was recorded in *P. fluorescens* resulting in 60.33 per cent suppression.

All the bioagents significantly reduced the egg hatching compared to the control at 100 per cent concentration of secondary metabolites extract. Egg hatching in the bioagnets treated batches was varied from 13.50 to 20.75, compared to the control it was 60.50. The minimum egg hatching was recorded in the *P. lilacinus* (13.50) leading to 77.69 per cent suppression followed by *L. lecanii* (16.75) with inhibition per cent of 72.31. Among the bioagents, the highest (20.75) number of eggs hatched was noticed in *P. fluorescens* amounting to 65.70 per cent suppression

**3.1.3 After 72 hours of treatment**

Egg hatching in the bioagents treated treatments was varied from 18.00 to 26.75 (average number of eggs hatched) at twenty-five per cent concentration of secondary metabolites extract, while in the control it was 64.25. The minimum (18.00) number of eggs hatched was noticed in the case of *P. lilacinus* amounting to 71.98 per cent suppression, which was significantly superior than all other treatments. However, among the bioagents, the maximum number of eggs hatched was recorded in *P. fluorescens* (26.75)and *L. lecanii* (21.25) amounting to 58.37 and 66.93 per cent inhibition, respectively as compared to untreated control.

At fifty per cent concentration of secondary metabolites extract, the average number of eggs hatched was fluctuated between 15.50 to 24.00 in the bioagents treated batches which were significantly distinct from the control. The lowest number of eggs hatched was noticed in *P. lilacinus* (15.50), followed by the *L. lecanii* (19.25) resulting in 75.88 and 70.04 per cent inhibition, respectively as compared to control. The highest number of eggs hatched was recorded with *P. fluorescens* (24.00) resulting to 62.65 per cent suppression. A significant difference was observed among the bioagent-treated plates."

All the bioagents significantly reduced the egg hatching compared to the control at 75 per cent concentration of secondary metabolites extract. The average number of eggs hatched in the bioagent treated batches ranged from 12.00 to 21.75. The fewest (12.00) eggs were hatched in the *P. lilacinus* leading to 81.32 per cent suppression. The next best treatment was *L. lecanii* (16.25) with average number of eggs hatched and 74.71 per cent suppression of egg hatching and were significantly different from each other. However, among the bioagents, the maximum (21.75) number of eggs hatched was observed in *P. fluorescens* amounting to 66.15 per cent suppression.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatments** | **Concentrations of secondary metabolites extract (%)** | | | | | | | |
| **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 = *Paecilomyces lilacinus* | 23.75 | 51.53 | 20.75 | 57.65 | 17.25 | 64.80 | 15.50 | 68.37 |
| T2 = *Pseudomonas fluorescens* | 28.25 | 42.34 | 26.00 | 46.94 | 23.00 | 53.06 | 20.75 | 57.65 |
| T3= *Lecanicillium lecanii* | 26.00 | 46.93 | 24.00 | 51.02 | 20.25 | 58.67 | 18.25 | 62.76 |
| T4 = Velume prime | 14.25 | 70.91 | 12.00 | 75.51 | 9.25 | 81.12 | 7.00 | 85.71 |
| T5 *=* Distilled water | 49.00 | 0 | 49.00 | 0 | 49.00 | 0 | 49.00 | 0 |
| SEm ± | 0.41 |  | 0.42 |  | 0.48 |  | 0.49 |  |
| CD @ 1 % | 1.25 |  | 1.28 |  | 1.48 |  | 1.49 |  |

**Table 2. Egg hatching inhibition of *Meloidogyne incognita* as influenced by secondary metabolites of bioagents after 24 hours of treatment**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatments** | **Concentrations of secondary metabolites extract (%)** | | | | | | | |
| **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*= Paecilomyces lilacinus* | 23.00 | 61.98 | 20.25 | 66.53 | 17.50 | 71.07 | 13.50 | 77.69 |
| T2 = *Pseudomonas fluorescens* | 30.50 | 49.59 | 27.25 | 54.96 | 24.00 | 60.33 | 20.75 | 65.70 |
| T3= *Lecanicillium lecanii* | 26.75 | 55.79 | 23.25 | 61.57 | 21.00 | 65.29 | 16.75 | 72.31 |
| T4 = Velume prime | 13.50 | 77.69 | 11.00 | 81.82 | 8.25 | 86.36 | 5.00 | 91.74 |
| T5 =Distilled water | 60.50 | 0 | 60.50 | 0 | 60.50 | 0 | 60.50 | 0 |
| SEm ± | 0.44 |  | 0.43 |  | 0.52 |  | 0.43 |  |
| CD @ 1 % | 1.34 |  | 1.31 |  | 1.60 |  | 1.33 |  |

**Table 3. Egg hatching inhibition of *Meloidogyne incognita* as influenced by secondary metabolites of bioagents after 48 hours of treatment**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatments** | **Concentrations of secondary metabolites extract (%)** | | | | | | | |
| **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 = *Paecilomyces lilacinus* | 18.00 | 71.98 | 15.50 | 75.88 | 12.00 | 81.32 | 9.25 | 85.60 |
| T2 = *Pseudomonas fluorescens* | 26.75 | 58.37 | 24.00 | 62.65 | 21.75 | 66.15 | 17.25 | 73.15 |
| T3= *Lecanicillium lecanii* | 21.25 | 66.93 | 19.25 | 70.04 | 16.25 | 74.71 | 13.25 | 79.38 |
| T4 =Velume prime | 12.00 | 81.32 | 10.00 | 84.44 | 5.75 | 91.05 | 3.00 | 95.33 |
| T5 =Distilled water | 64.25 | 0 | 64.25 | 0 | 64.25 | 0 | 64.25 | 0 |
| SEm ± | 0.45 |  | 0.41 |  | 0.50 |  | 0.44 |  |
| CD @ 1 % | 1.37 |  | 1.27 |  | 1.52 |  | 1.34 |  |

**Table 4. Egg hatching inhibition of *Meloidogyne incognita* as influenced by secondary metabolites of bioagents after 72 hours of treatment**

Egg hatching was ranged from 9.25 to 17.25 (average number of eggs hatched) in the bioagents treated treatments at 100 per cent concentration of secondary metabolites extract. The lowest (9.25) number of hatched eggs was recorded in *P. lilacinus* leading to 85.60 per cent suppression, followed by *L. lecanii* (13.25) leading to 79.38 per cent inhibition. Among the bioagents, the highest (17.25) number of eggs hatched was observed in *P. fluorescens* leading to 73.15 per cent inhibition. Significant difference were observed among the bioagents treatments.

From the above observations, it can be inferred that there was a positive relationship between the concentration of secondary metabolites, duration of the treatment and the percentage of egg hatching inhibition. As the concentration of secondary metabolites increased, egg hatching period extended, inhibition of egg hatching was increased compared to the untreated control.

However, when compared with all the treatments the positive control Velume prime recorded the minimum number of eggs hatched with maximum percent of egg hatching inhibition

**3.2 Juvenile mortality of *Meloidogyne incognita***

The efficacy of four concentrations of secondary metabolite extracts (25%, 50%, 75%, and 100%) from potential biocontrol agents was assessed for their ability to induce juvenile mortality in *Meloidogyne incognita*. Observations were recorded at 24, 48, and 72 hours after treatment (Table 5).

**3.2.1 After 24 hours of treatment**

At twenty-five per cent concentration of secondary metabolites extract after 24 h of treatment, significant difference was noticed between bio-agents treatment and the control on mortality of juveniles. The maximum mortality of juveniles was recorded in positive check Velume prime. However, among the bioagents tested, the *P. lilacinus* treated batch (35.25%) was recorded maximum juvenile mortality, followed by *L. lecanii* (29.25%). However, among the bioagents, the minimum juvenile mortality was noticed in the treatment with extracts of *P. fluorescens* amounting to 24.50 per cent.

Juvenile mortality ranged from between 32.25 to 41.75 per cent at fifty per cent concentration of secondary metabolites extract. The maximum mortality of juveniles was observed in the case of *P. lilacinus* resulting to 41.75 per cent which was significantly distinct from rest of the treatments, followed by the *L. lecanii* resulting to 37.25 per cent. However, among the bioagents, the minimum mortality was observed in treatment *P. fluorescens* amounting to 32.25 per cent. All the treatments were significantly different from each other.

At seventy-five per cent concentration of secondary metabolites extract, the juvenile mortality varied from 36.25 to 46.00 per cent. The treatment *P. lilacinus* (46.00%) was exhibited highest juvenile mortality. The next best treatment was *L. lecanii* with juvenile mortality of 39.50 per cent. Whereas among the bioagents, the minimum juvenile mortality of 36.25 per cent was exhibited by *P. fluorescens*.

Each bioagent substantially increased the death of juveniles at 100 per cent concentration of secondary metabolites extract. The mortality of juveniles varied from 40.00 to 45.75 per cent in the bioagents treated batches. The highest juvenile mortality was observed in *P. lilacinus* (51.75%)*,* followed by *L. lecanii* (45.75%). Among the bioagents, the lowest juvenile mortality was noticed in the *P. fluorescens* resulting to 40.00 per cent. Significant differences were observed among the treatments

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatments** | **Per cent juvenile mortality at different incubation period (hours)** | | | | | | | | | | | | |
| **24 hours** | | | | **48 hours** | | | | **72 hours** | | | | |
| **Concentrations of secondary metabolites extract (%)** | | | | | | | | | | | | |
| **25** | **50** | **75** | **100** | **25** | **50** | **75** | **100** | **25** | **50** | **75** | **100** |
| T1= *Paecilomyces lilacinus* | 35.25 | 41.75 | 46.00 | 51.75 | 49.50 | 54.00 | 59.25 | 64.75 | 53.75 | 58.25 | 64.50 | 70.50 |
| T2=*Pseudomonas fluorescens* | 24.50 | 32.25 | 36.25 | 40.00 | 38.25 | 47.25 | 49.25 | 54.00 | 43.00 | 45.50 | 51.25 | 58.00 |
| T3 =*Lecanicillium lecanii* | 29.25 | 37.25 | 39.50 | 45.75 | 45.00 | 49.75 | 54.50 | 58.00 | 49.00 | 52.00 | 58.00 | 63.75 |
| T4 = Velume prime | 46.00 | 55.00 | 61.25 | 70.75 | 58.75 | 72.00 | 79.50 | 89.50 | 65.75 | 78.50 | 87.00 | 94.50 |
| T5 = Distilled water | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SEm ± | 0.37 | 0.32 | 0.45 | 0.41 | 0.32 | 0.57 | 0.24 | 0.31 | 0.30 | 0.33 | 0.40 | 0.38 |
| CD @ 1 % | 1.14 | 0.98 | 1.38 | 1.25 | 1.00 | 1.73 | 0.73 | 0.94 | 0.92 | 1.02 | 1.22 | 1.16 |

**Table 5. Per cent juvenile mortality of *Meloidogyne incognita* as influenced by secondary metabolites of bioagents**

**3.2.2 After 48 hours of treatment**

At twenty-five per cent concentration of secondary metabolites extract after 48 h of treatment, a significant difference was noticed between bio-agents treatment and the control on mortality of juveniles. The juvenile mortality was ranged from 38.25 to 49.50 per cent in the bioagents treatment batches. Maximum juvenile mortality was exhibited by the treatment *P. lilacinus*, followed by *L. lecanii* amounting to 49.50 and 45.00 per cent, respectively. Among the bioagents, the minimum juvenile mortality was noticed in the case of *P. fluorescens* amounting to 38.25 per cent. *All treatments were significantly different from each other.*

Juvenile mortality was significantly increased by each bioagent at 50 per cent concentration of secondary metabolites extract and it ranged from 47.25 to 54.00 per cent in the bioagents treatment batches. The treatment *P. lilacinus* exhibited highest juvenile mortality of 54.00 per cent. Among the bioagents, the mortality of *L. lecanii* and *P. fluorescens* amounting to 49.75 and 47.25 per cent, respectively. However, it was observed that each treatment was significantly distinct from the others.

Juvenile mortality was ranged from 49.25 to 59.25 per cent at seventy-five per cent concentration of secondary metabolites extract. The maximum mortality of juveniles was observed in the case of *P. lilacinus* resulting to 59.25 per cent which was significantly distinct from rest of the treatments, followed by the *L. lecanii* resulting in 54.50 per cent. The minimum juvenile mortality was observed in the bioagent *P. fluorescens* amounting to 49.25 per cent.

Juvenile mortality ranged from 54.00 to 64.75 per cent at 100 per cent concentration of secondary metabolites extract. The maximum juvenile mortality was recorded in the case of *P. lilacinus* resulting to 64.75 per cent which was significantly superior than other treatments. The minimum mortality was observed in *P. fluorescens* amounting to 54.00 per cent. The treatment *L. lecanii* wasthe second highest performingbioagent, with a juvenile mortality of 58.00. per cent.

**3.2.3 After 72 hours of treatment**

After 72 hours, the juvenile mortality was ranged between 43.00 and 53.75 per cent at twenty-five per cent concentration of secondary metabolites extract. Maximum juvenile mortality was noticed in the case of *P. lilacinus* (53.75%), followed by *L. lecanii* (49.00%), they were significantly distinct from each other. However, among the bioagents, *P. fluorescens* exhibited the minimum juvenile mortality of 43.00 per cent.

Juvenile mortality was markedly influenced by all the bioagents and it varied from 45.50 to 58.25 per cent at fifty concentrations of secondary metabolites extract. The highest mortality (58.25%) was observed with *P. lilacinus,* significantly greater than all other treatments. The next best treatment was *L. lecanii* with a juvenile mortality of 52.00 per cent. The minimum juvenile mortality was observed in the bioagent *P. fluorescens* amounting to 45.50 per cent. At seventy-five per cent concentration of secondary metabolites extract, the juvenile mortality ranged from 51.25 to 64.50 per cent. The maximum juvenile mortality was recorded in the *P. lilacinus*, followed by *L. lecanii* leading to 64.50 per cent and 58.00 per cent. Among the bioagents, the minimum mortality was recorded in the *P. fluorescens* resulting to 51.25 per cent. All the treatments were significantly differed from one another.

Juvenile mortality was appreciably affected by every bioagent and it ranged from 58.00 to 70.50 per cent at hundred per cent concentration of secondary metabolites extract. The maximum juvenile mortality of 70.50 per cent was recorded in the *P. lilacinus* treatment, followed by the bioagent *L. lecanii* amounting to 63.75 per cent. However, among the bioagents the minimum juvenile mortality of 58.00 per cent was noticed in the *P. fluorescens* extract. Significant differences were observed among all treatments.

The observed effects in this study may be attributed to the production of toxic secondary metabolites and antibiotics such as leucinostatin, paecilotoxin, and acetic acid by fungal bioagents in the suspension that has nematicidal activity against Meloidogyne incognita, as reported by Pandey *et al.* (2021).

The present results are in align with the findings of Sharma *et al.* (2020), who reported that the ethyl acetate extract of fungal filtrate has the most promising effects on egg hatching inhibition and juvenile mortality of *Meloidogyne incognita* than hexane extracts, indicating that active nematicidal compounds are intermediary in polarity. This is consistent with previous reports by Siddiqui *et al*. (2000).

The protease and chitinase enzymes of *P. lilacinus* drastically altered the eggshell structures, reducing the hatching of *M. javanica* (Khan *et al*., 2004).

Gapasin *et al*. (2011) reported that the extraction of metabolites from *Paecilomyces lilacinus* with ethyl acetate resulted in 88.32 per cent mortality at a concentration of 500 mg/ml under *in vitro*. They revealed that more toxic metabolites were present in the mycelial extract compared to the culture filtrate extract, both of which were nematoxic against the rice root-knot nematode, *M. graminicola*. PGPRs involved in production of volatile compounds like benzene acetaldehyde, decanal, 2-nonanone, dimethyl disulphide and 2-undecanone that were effective against both eggs and juveniles of *Meloidogyne incognita* (Huang *et al.*, 2009). The observed depletion of egg hatching and death of juveniles in the current study might be also due to presence of antibiotic genes indicating the antimicrobial potentialfor bacterial bio-agents. There was a study supporting the present results by Xia *et al*. (2011).

1. **Conclusion**

The findings of the present study highlight the promising role of fungal secondary metabolites aseffective biocontrol agents against *Meloidogyne incognita*, a major pest limiting mulberry productivity. Under *in-vitro* conditions, the secondary metabolites, particularly those derived from *Paecilomyces lilacinus*, demonstrated significant nematicidal activity by inhibiting egg hatching. Among the tested agents, Given the superior efficacy of *P. lilacinus*, this study underscores the potential for developing sustainable*,* environmentally friendly strategies for nematode management in sericulture. Incorporating such biocontrol agents could reduce reliance on chemical nematicides and contribute to healthier mulberry ecosystems and improved silk production.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests, non-financial interests, or personal relationships that could have appeared to influence the work reported in this paper.

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.

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