**Original Research Article**

**Extraction of secondary metabolites from potential biocontrol agents against** ***Meloidogyne 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 undertaken under *in-vitro* conditions in the Department of Plant Pathology, College of Sericulture, Chintamani, to assess the nematicidal efficacy of secondary metabolites extracted from selected biocontrol agents. The study revealed that these metabolites effectively suppressed egg hatching and increased juvenile mortality when compared to the 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 promising 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 (Govindaiah & Sharma, 1994; Sivakumar & Gopi, 2006). 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 control, their long-term application has raised environmental and health concerns, including the development of resistance, soil degradation, and toxicity to non-target organisms (Akhtar & Malik, 2000). As a result, there is growing interest in the use of eco-friendly alternatives such as biocontrol agents. Fungal bioagents, particularly their secondary metabolites, have shown promise in suppressing nematode populations through various antagonistic mechanisms (Siddiqui & Shaukat, 2004). Their application offers a sustainable approach to managing nematode infestations while preserving environmental health and supporting sustainable mulberry production systems.

1. **Material and Methods**

**2.1 Collection of samples**

Root samples were obtained from mulberry fields across various locations in Chintamani taluk, 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: 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 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.

**2.3 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 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. The juvenile population in the suspension was counted under a stereo binocular microscope by transferring 1 mL of suspension on a nematode counting dish and the population was made up to 100 juveniles per mL of suspension.

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

Secondary metabolites were extracted from *Paecilomyces lilacinus, Lecanicillium lecanni* and *Pseudomonas fluorescens* bio control agents

 Nutrient broth and Potato dextrose broth of 150 ml was prepared in 250 mL conical flask and sterilized. Single bacterial colony and funal colony was inoculated into sterilized nutrient 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). Shaked well to mix the 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 separate sterilized glass bottle for further experiments. The secondary metabolites were extracted and diluted to different concentrations and compared for sterile distilled water (control).

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

**2.5 Effect on egg hatching inhibition**

Three egg mass was 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 for egg hatching after every 24 h of incubation for 3 days (24, 48 and 72h) and number of hatched eggs were counted at each 24 h interval. The per cent egg hatching inhibition was calculated using 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 with equal interval. 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:**

**Design:** Completely Randomized Design (CRD), **Number of treatments:** 5, **Number of replications:** 4, **Number of units:** 20

**TABLE 1. Treatment details:**

|  |  |
| --- | --- |
| **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 were evaluated for their ability to suppress egg hatching in *Meloidogyne incognita*. The observations were recorded at 24 h interval for three days continuouslyafter treatment (Table 1,2 and 3).

**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 varied 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* showed egg hatching of 26.00 and 24.00, 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 bioagnets treated treatments was varied 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 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. Significant difference noticed in between bioagents treated plates.

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 to 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, while in 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 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 to 75.88 and 70.04 per cent

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **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 treatmen**

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. Significant difference was noticed in between bioagents treated treatments.

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 bioagnets treated batches was varied 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.

Egg hatching was fluctuated between 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 was noticed in between bioagents treated 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 decreased, and as the exposure period extends, inhibition of egg hatching was increased compared to the untreated control.

However, when compared with all the treatments the positive control Velume prime recorded significantly 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 1).

**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 was ranged 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 was 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 bioagnets, the minimum juvenile mortality of 36.25 per cent was exhibited by *P. fluorescens*. However, it was observed that each treatment was significantly distinct from the others.

Each bioagent substantially increased the death of juveniles at 100 per cent concentration of secondary metabolites extract. The mortality of juveniles was varied between 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 noticed in between 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. Here, every treatment was significantly distinct from each other.

Mortality of juveniles was significantly diminished by each bioagent at 50 per cent concentration of secondary metabolites extract and it was 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 between to 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 to 54.50 per cent. The minimum juvenile mortality was observed in the bioagent *P. fluorescens* amounting to 49.25 per cent.

Juvenile mortality was fluctuated between 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* was exhibited the minimum juvenile mortality of 43.00 per cent.

Juvenile mortality was markedly influenced by all the bioagents and it was 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 was 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 was 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 difference was noticed in between all the treatments.

The observed results in this study might be attributed to the production of potential toxic metabolites and antibiotics viz., peptidal antibiotics like lecinostain and paecilo toxin, acetic acid by fungal bioagent 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 have 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 also falls in line with the previous reports of 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. 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 proving the antimicrobial potential for 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 as effective biocontrol tools against the root-knot nematode *Meloidogyne incognita*, a major constraint in mulberry cultivation. Under *in-vitro* conditions, the secondary metabolites, particularly those derived from *Paecilomyces lilacinus*, demonstrated significant nematicidal activity by inhibiting egg hatching and increasing juvenile mortality. With *P. lilacinus* showing the highest efficacy, this approach 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 OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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