



# Nematicidal Activity of Fungal and Bacterial Metabolites against *Meloidogyne incognita* Eggs Infesting Mulberry

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

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

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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 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 when compared to untreated control plates. Notably, *Paecilomyces lilacinus* exhibited the highest suppression, recording 85.60 per cent egg hatching inhibition 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:** Mulberry; *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 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 (Siddiqui & Shaukat, 2004; Kumar et al. 2022). 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 of *Meloidogyne incognita* infesting mulberry. The findings aim to support the development of biobased, environmentally sound strategies for nematode management in sericulture.

## 2. MATERIALS 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).

### 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 Secondary Metabolites from Potential Biocontrol agents against Eggs of *Meloidogyne incognita* 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 acetate organic 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.

### 2.4 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 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:

$$I(\%) = \frac{(C-T)}{C} \times 100$$

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

## 3. RESULTS AND DISCUSSION

### 3.1 Egg Hatching Inhibition of *Meloidogyne incognita*

The efficacy of four concentrations of secondary metabolites (25, 50, 75 and 100%) extracted from three potential biocontrol agents was



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



→ Solvent phase  
→ Aqueous phase

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

evaluated for their ability to suppress egg hatching in *Meloidogyne incognita*. The observations were recorded at 24 h interval for three continuously days after treatment (Tables 2,3 and 4).

**Table 1. List of the treatments**

Sl.No.	Treatments
T <sub>1</sub>	<i>Paecilomyces lilacinus</i> @ 25, 50, 75,100 per cent dilutions
T <sub>2</sub>	<i>Pseudomonas fluorescens</i> @ 25, 50, 75,100 per cent dilutions
T <sub>3</sub>	<i>Lecanicillium lecanii</i> @ 25, 50, 75,100 per cent dilutions
T <sub>4</sub>	Velume prime (Positive check)
T <sub>5</sub>	Distilled water (Negative check)

### 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.

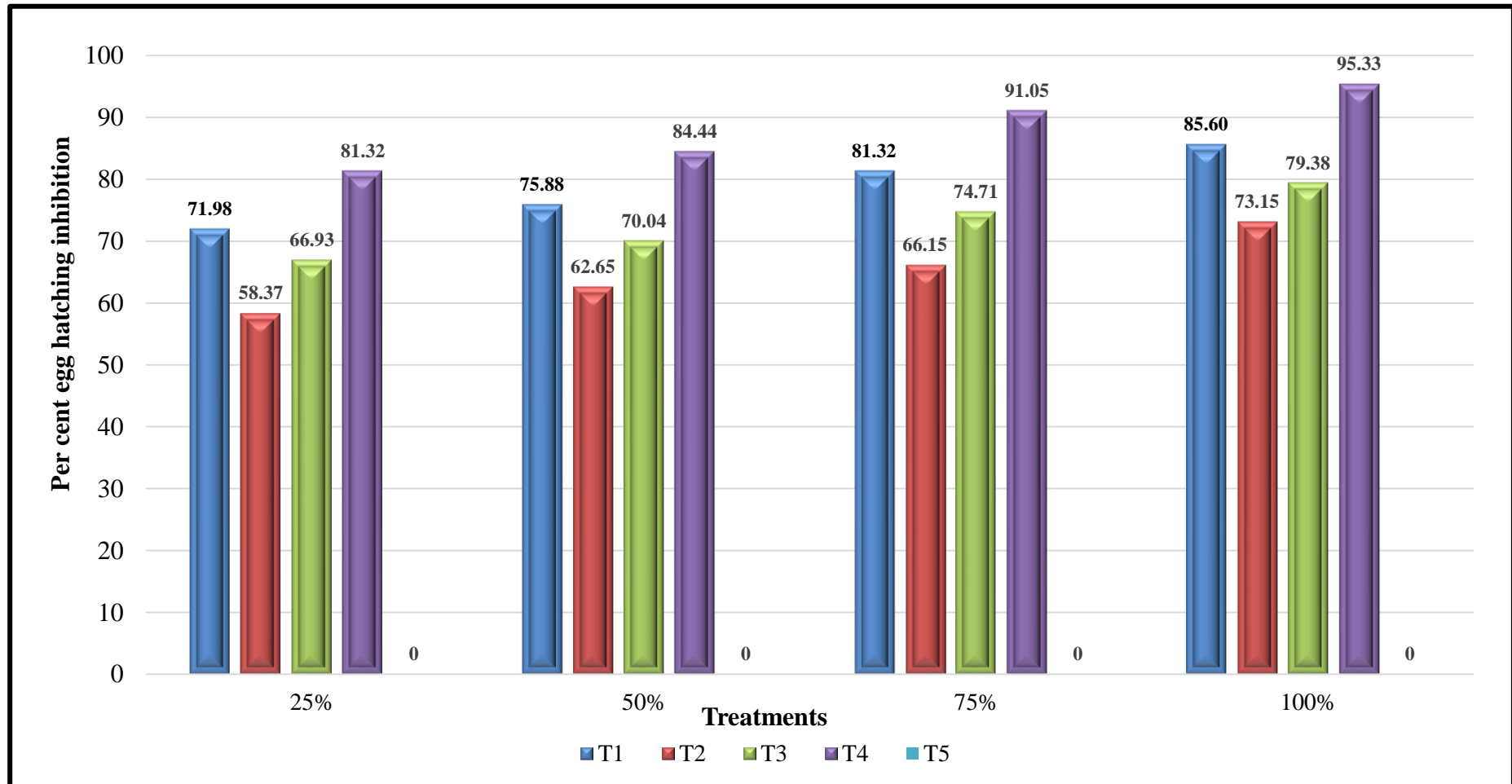


Fig. 3. Per cent egg hatching inhibition of *Meloidogyne incognita* in as influenced by secondary metabolites of bioagents after 72 hours

**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
T <sub>1</sub> = <i>Paecilomyces lilacinus</i>	23.75	51.53	20.75	57.65	17.25	64.80	15.50	68.37
T <sub>2</sub> = <i>Pseudomonas fluorescens</i>	28.25	42.34	26.00	46.94	23.00	53.06	20.75	57.65
T <sub>3</sub> = <i>Lecanicillium lecanii</i>	26.00	46.93	24.00	51.02	20.25	58.67	18.25	62.76
T <sub>4</sub> = Velume prime	14.25	70.91	12.00	75.51	9.25	81.12	7.00	85.71
T <sub>5</sub> = 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 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
T <sub>1</sub> = <i>Paecilomyces lilacinus</i>	23.00	61.98	20.25	66.53	17.50	71.07	13.50	77.69
T <sub>2</sub> = <i>Pseudomonas fluorescens</i>	30.50	49.59	27.25	54.96	24.00	60.33	20.75	65.70
T <sub>3</sub> = <i>Lecanicillium lecanii</i>	26.75	55.79	23.25	61.57	21.00	65.29	16.75	72.31
T <sub>4</sub> = Velume prime	13.50	77.69	11.00	81.82	8.25	86.36	5.00	91.74
T <sub>5</sub> = 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 4. Egg hatching inhibition of *Meloidogyne incognita* as influenced by secondary metabolites of bioagents after 72 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
T <sub>1</sub> = <i>Paecilomyces lilacinus</i>	18.00	71.98	15.50	75.88	12.00	81.32	9.25	85.60
T <sub>2</sub> = <i>Pseudomonas fluorescens</i>	26.75	58.37	24.00	62.65	21.75	66.15	17.25	73.15
T <sub>3</sub> = <i>Lecanicillium lecanii</i>	21.25	66.93	19.25	70.04	16.25	74.71	13.25	79.38
T <sub>4</sub> = Volume prime	12.00	81.32	10.00	84.44	5.75	91.05	3.00	95.33
T <sub>5</sub> = 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	

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 bioagents 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.

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 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 Volume prime recorded the minimum number of eggs hatched with maximum percent of egg hatching inhibition.

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 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 inhibition 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, Shao et al. 2020).

They revealed that more toxic metabolites were present in the mycelial extract compared to the culture filtrate extract, both of which were nematotoxic 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 of *Meloidogyne incognita* (Huang et al. 2009). The observed depletion of egg hatching in the current study might also be due to the presence of antibiotic genes indicating the antimicrobial potential bio-agents. There was a study supporting the present results by Xia et al. (2011).

#### 4. CONCLUSION

The findings of the present study highlight the promising role of fungal secondary metabolites as effective 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.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

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 writing or editing of this manuscript.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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