Original Research Article

IN VITRO EVALUATION OF ENTOMOPATHOGENIC FUNGI FOR THE MANAGEMENT OF BROAD MITE, *POLYPHAGOTARSONEMUS LATUS* OCCURRENCE ON MULBERRY

ABSTRACT

The *in vitro* evaluation of two entomopathogenic fungi, *Lecanicillium lecanii* and *Hirsutella thompsonii*, was conducted to assess their efficacy against the Broad mite, *Polyphagotarsonemus latus*, damaging mulberry. The study measured the cumulative mortality rates of Broad mites at 1, 3, 5, and 7 days after spraying (DAS) of the fungi at different concentrations (1.5, 2.5, and 3.0 ml/l). Significant variations in mite mortality were observed across different treatments and time points. The highest mortality rate was 65.87±1.61% with *H. thompsonii* at 3.0 ml/l, while the lowest was at 1.5 ml/l with a 43.86±0.90% mortality rate at one DAS. Three days after spray, *H. thompsonii* at 3.0 ml/l showed the highest mortality rate (82.00±1.05%), with the least effective being at 1.5 ml/l (53.60±3.20%). At five days after treatment, the highest mortality was 95.60±1.31% observed in *H. thompsonii* at 3.0 ml/l, whereas *L. lecanii* recorded 91.34±0.76%. By the seventh day, *H. thompsonii* at 3.0 ml/l achieved the highest mortality rate of 98.80±0.65%, while *L. lecanii* showed 98.00±0.42%.

Keywords: Broad mite; Entomopathogenic; Hirsutella thompsonii; Lecanicillium lecanii; Mulberry; Polyphagotarsonemus latus.

1. INTRODUCTION

Mulberry (*Morus* spp.) leaf is the sole food plant for the silkworm, *Bombyx mori* L. It is a deep-rooted, perennial, and fast-growing plant. The yield and quality of mulberry leaf have a direct impact on silkworm rearing, influencing cocoon quality and productivity. To achieve the production of a good-quality silkworm cocoon crop, factors such as mulberry leaf, climate, rearing technique, silkworm race, and silkworm egg play an important role (Miyashita, 1986). Throughout the year, mulberry cultivation and silkworm rearing, which are two important farm-based activities, face challenges from various pests and pathogens. These issues adversely impact cocoon quality and productivity, leading to economic losses for both farmers and the sericulture industry. The yield of the mulberry crop is a significant factor in determining productivity and profitability in sericulture (Sarkar *et al*., 1996). Across the world, more than 300 species of both insect and non-insect pests have been identified to infest mulberries, affecting the various stages of growth during different seasons (Kotikal and Devaiah, 1985). Among the major insect orders that attack the mulberry crop, the most numerous species are the orders Lepidoptera, Hemiptera, Coleoptera, Thysanoptera, Orthoptera, and Isoptera. Additionally, acarids and mollusks have also been observed to cause damage (Sengupta *et al*., 1990). Some species of mites have gained attention as crop pests, and mulberry has not been spared from their impact (Narayanaswamy *et al*., 1996). Recently, in Karnataka, non-insect pests have become a threat, and there has been a concerning rises in broad mite incidence in mulberry cultivation. Additionally, mite species have been found on both wild and cultivated species of mulberry (Srinivasa *et al*., 2012).

The Broad mite, *P. latus,* and Two Spotted Spider mites, *Tetranychus* spp., are found in mulberry, and these mites cause severe damage to top leaves by sucking the sap from the ventral surface, leading to detrimental effects (Dar *et al*., 2012). The Broad mite damage has reached alarming levels and is causing significant losses to mulberry leaf production. As a result, crop losses ranging from 20–70% have been recorded. Several factors contribute to the severity of the mite menace. These include the availability of mulberry as a preferred host for the mites, changes in climate conditions, and the elimination of natural enemies due to the increased usage of modern pesticides (Prakya Sreeramakumar and Richa Varshney, 2020).

At present, the primary approach for managing mites in mulberry cultivation relies heavily on the use of acaricides. Broad mite infestations occur during the sprouting and leaf harvesting stage of mulberry cultivation, and the use of pesticides, especially during harvesting, leads to residue problems in the mulberry leaves required for silkworm rearing. Therefore, there is an urgent requirement to develop management strategies using biological control agents, specifically tailored to Broad mite management in mulberry ecosystems. The current study aims to promote practical application of entomopathogenic fungi in managing the Broad mite occurring on mulberry. This approach holds great promise for effectively managing Broad mites while considering the socio-economic and environmental well-being of sericulture farmers. Management practices of Broad mite (*P. latus)* using biological control agents in mulberry cultivation have a great impact on plant growth, leaf yield, and the improvement of biochemical constituents, which are the most important factors contributing to quality mulberry leaf, cocoon, and silk production.

2. MATERIAL AND METHODS

The current investigation has been undertaken at the Insect Pathology and Pest Management [IPPM] Section, Karnataka State Sericulture Research and Development Institute [KSSRDI], Thalaghattapura, Bengaluru. KSSRDI is located at a latitude of 12.8547^{o} N, longitude of 77.5261^{o} E, and altitude of 874 meters above the mean sea level [MSL]. The methodologies followed during the period of investigations are presented hereunder.

2.1 The materials used in the study

2.1.1 Mite pest:

The Broad [Yellow] mite, *Polyphagotarsonemus latus* [Banks], is a polyphagous non-insect pest widely distributed in tropical and sub-tropical regions. For the current investigation, Broad mite was collected from infested mulberry gardens at KSSRDI.

2.1.2 Entomopathogenic fungi

Entomopathogenic fungi are microorganisms that infect and often kill susceptible insect populations through conidia. The use of fungal entomopathogens serves as an alternative to insecticides. In the current study, the entomopathogenic fungi, *Leconicillium lecani* and *Hirsutella thomsonii* were used to determine the pathogenesis of the Broad mite damaging mulberry. Stock cultures of *L. lecanii* were obtained from the Mulberry Pathology and Microbiology Section of KSSRDI, Bengaluru, and *H. thompsonii* was obtained from NBAIR, Bengaluru, and also procured from IPL [International Panaacea Limited] Biologicals Limited, Bengaluru.

2.1.3 Mass culture of entomopathogenic fungi

The culture of entomopathogenic fungi, *Lecanicillium lecanii* [Zimm.] obtained from Pathology Laboratory, KSSRDI, and *Hirsutella thompsonii* Fisher obtained from NBAIR, Bangalore. The fungi were sub-cultured on fresh PDA plates and incubated at 28±1^{o}C for 7 days before use. Cultures were prepared by inoculating 100 ml of fresh sterilized potato dextrose broth with a 5 mm disc cut from 7-day-old growth in plates. Sub-culturing of fungi prepared regularly at an interval of 15-20 days.

2.2 *In vitro* evaluation of entomopathogenic fungi on Broad mite

Healthy, mature mulberry leaves grown in separate pots without any insecticidal sprays were collected, washed, and cleaned with tissue paper. The leaf discs of 60 mm diameter were prepared and kept in paper-lined 100 mm glass petri dishes. The leaf discs were kept on the abaxial side in an upward direction. Release the 25 adult mites over the leaf disc, and the spore suspension *of L. lecanii* and *H. thompsonii* was sprayed on the Broad mite at three concentrations [1.50, 2.5, and 3.0 ml/l] separately using a hand atomizer. Observations were recorded on the mortality of mites one, three, five, and seven days after spray (DAS). Dead mites were collected and placed over moist, sterile filter paper for incubation. The growth of fungal mycelium over the bodies of mites was observed under a stereo zoom microscope to confirm mortality due to mycoparasitism.

2.3 Statistical analysis of the data

The data from the study has been analyzed using statistical methods to determine the efficacy of biological control agents in managing the broad mite. A one-way analysis of variance (ANOVA) was employed using the SPSS statistical package (ver. 21.0), following the methods outlined by Gomez and Gomez (1984).

3. RESULTS

Two entomopathogenic fungi, namely *L. lecanii*, and *H. thompsonii,* were evaluated under laboratory conditions (*in vitro*) using Broad mites, and their efficacy levels were recorded on different days after their usage. Significant variations were observed with respect to the cumulative mortality of Broad mite at 1, 3, 5, and 7 days after spraying at different concentrations [1.5, 2.5, and 3.0 ml/l] [Table: 1and Figure: 1].

3.1 One day after spray

Statistical [F-value=195.1**] variation observed in mortality of *P. latus* at 1 DAS. The highest rate of mortality [65.87 ± 1.67%] was observed at 1 day after spraying with treatment T_6: *H. thompsonii* [CFU-2x10^8/ml] @ 3.0 ml/l, followed by 65.60 ± 2.05% in treatment T_3: *L. lacanii* [CFU-2x10^8/ml] @ 3.0 ml/l, 51.60 ± 2.30% in treatment T_2:*L. lacanii* [CFU-2x10^8/ml] @ 2.5ml/l,44.00 ± 1.46% in treatment T_5:*H. thompsonii* [CFU-2x10^8/ml] @ 2.5ml/l, 44.00 ± 1.30% in T_1: *L. lacanii* [CFU-2x10^8/ml] @ 1.5 ml/l and least mortality percentage [43.86 ±0.90%] was observed in treatment T_4: *H. thompsonii* [CFU-2x10^8/ml] @ 1.5 ml/l.

3.2 Three days after spray

Statistically significant [F-value=248.8**] variation observed in mortality of *P. latus* at three DAS. The highest rate of mortality [82.00 ±1.05%] was observed three days after spraying with treatment T_6: *H. thompsonii* [CFU-2x10^8/ml] @ 3.0 ml/l, followed by 78.53 ±1.06% in treatment T_3: *L. lacanii* [CFU-2x10^8/ml] @ 3.0 ml/l,63.06 ±2.05% in treatment T_2:*L. lacanii* [CFU-2x10^8/ml] @ 2.5ml/l,56.13 ±1.10%in treatment T_5:*H. thompsonii* [CFU-2x10^8/ml] @ 2.5mL/L, 55.07 ±1.20% in T_1: *L. lacanii* [CFU-2x10^8/ml] @ 1.5 ml/l, and lowest mortality rate in treatment T_4: *H. thompsonii* [CFU-2x10^8/ml] @ 1.5 ml/l [53.60 ±3.20%].

3.3 Five days after spray

The mortality rate was recorded as a significant [F-value=554.4**] variation of *P. latus* at five DAS. The highest rate of mortality [95.60 ±1.31%] was observed five days after spraying with treatment T_6: *H. thompsonii* [CFU-2x10^8/ml] @ 3.0 ml/l, followed by 91.34 ±0.76% in treatment T_3: *L. lacanii* [CFU-2x10^8/ml] @ 3.0 ml/l, 82.40 ±0.78 in treatment T_2:*L. lacanii* [CFU-2x10^8/ml] @ 2.5 ml/l, 81.33 ±1.56 in treatment T5:*H. thompsonii* [CFU-2x10^8/ml] @ 2.5 ml/l, 81.07 ±1.89 in treatment T4: *H. thompsonii* [CFU-2x10^8/ml] @ 1.5 ml/l and in T_1: *L. lacanii* [CFU-2x10^8/ml] @ 1.5 ml/l, lowest mortality rate [69.20 ±1.97].

Table 1: Effect of *in vitro* evaluation of entomopathogenic fungi, *Lecanicillium lecanii* and *Hirsutella thompsonii,* on the mortality rate of the Broad mite, *P. latus*.

Treatment	**Cumulative mortality [%]**			
	1DAS	**3DAS**	**5DAS**	**7DAS**
T_0:Control	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00

T_1:*L. lecanii* [CFU = 2x10^8/ml]1.5 ml/l	44.00 ±1.30	55.07 ±1.20	69.20 ±1.97	78.00 ±1.46
T_2:*L. lecanii* [CFU = 2x10^8/ml]2.5 ml/l	51.60 ±2.30	63.06 ±2.05	82.40 ±0.78	93.20 ±1.22
T_3:*L. lecanii* [CFU = 2x10^8/ml]3.0ml/l	65.60±2.05	78.53 ±1.06	91.34 ±0.76	98.00 ±0.42
T_4:*H. thompsonii* [CFU =2x10^8/ml]1.5 ml/l	43.86 ±0.90	53.60 ±3.20	81.07 ±1.89	95.46 ±0.65
T_5:*H. thompsonii* [CFU = 2x10^8/ml]2.5 ml/l	44.00 ±1.46	56.13 ±1.10	81.33 ±1.56	97.33 ±0.52
T_6:*H. thompsonii* [CFU = 2x10^8/ml]3.0ml/l	65.87 ±1.61	82.00 ±1.05	95.60 ±1.31	98.80±0.65
Mean	44.90 ±0.68	55.49 ±0.86	71.56 ±0.62	80.11 ±0.20
F-value	195.1**	248.8**	554.4**	1805.1**

CFU: Colony forming unit DAS: Days after spray **$p \leq 0.01$

3.4 Seven days after spray

Significant [F-value=1805.1**] variation was noticed in the rate of mortality of *P. latus* at seven DAS. The highest rate of mortality [98.80±0.65%] was observed at seven days after spraying with treatment T_6: *H. thompsonii* [CFU-2x10^8/ml] @ 3.0 ml/l, followed by 98.00 ±0.42% in treatment T_3: *L. lacanii* [CFU-2x10^8/ml] @ 3.0 ml/l,97.33 ±0.52% in treatment T_5:*H. thompsonii*[CFU-2x10^8/ml] @ 2.5ml/l, 95.46 ±0.65%, in treatment T_4: *H. thompsonii* [CFU-2x10^8/ml] @ 1.5 ml/l, 93.20 ±1.22% in treatment T_2:*L. lacanii* [CFU-2x10^8/ml] @ 2.5ml/l, and lowest rate of mortality [78.00 ±1.46%] was observed in T_1: *L. lacanii* [CFU-2x10^8/ml] @ 1.5 ml/l when compared to control [0.00 ±0.00%].

4. DISCUSSION

The research findings indicated that using entomopathogenic fungi as a biological agent shows high potential for effectively managing the Broad mite *P. latus* infesting mulberry. The average rate of mortality was observed at [80.11 ±0.20%], [71.56 ±0.62%], [55.49 ±0.86%], and [44.90 ±0.68%] in 7, 5, 3, and 1 DAS, respectively [Table:1 and Figure:1]

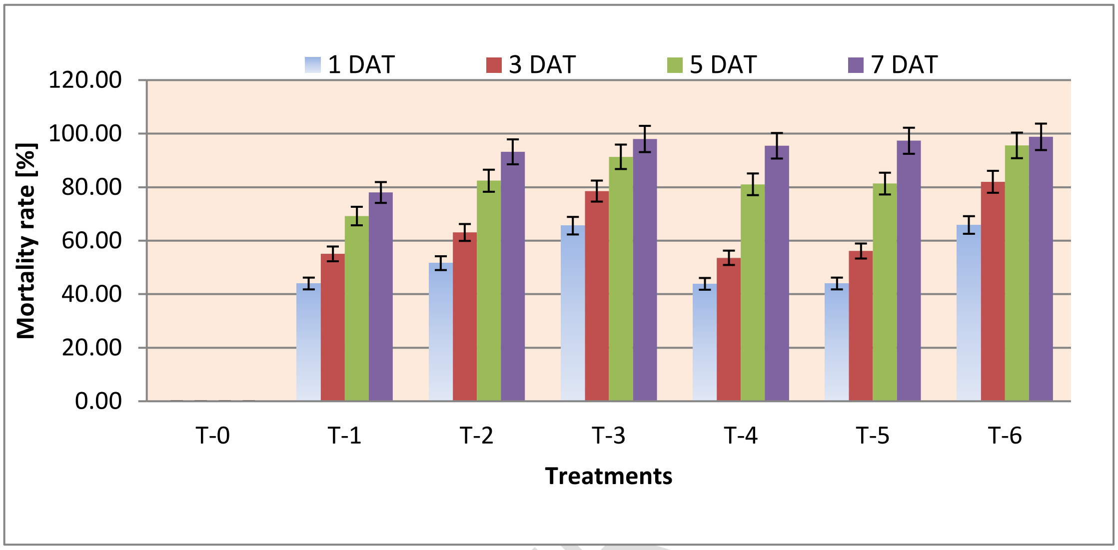

Figure: 1 *In vitro* evaluation of entomopathogenic fungi, *L. lecanii* and *H. thompsonii* on mortality of the Broad mite, *P. latus*.

The current findings revealed that entomopathogenic fungi can be used as a biological agent for effectively managing the Broad mite, *P. latus,* damaging mulberry. The rate of mortality was higher in *L. lecanii* [CFU-2x10^8/ml] at 3.0ml/l : 98.00 ± 0.42%, 91.34 ±0.76%, 78.53 ± 1.06%, and 65.60 ± 2.05% and for *H. thompsonii* [CFU-2x10^8/ml] at 3.0ml/l: was 98.80 ± 0.65%, 95.60 ± 1.31%, 82.00 ± 1.05%, and 65.87 ± 1.61% at 7, 5, 3, and 1 DAS, respectively.

The results were in close proximity to those of Srinivasa *et al*. (2005) who conducted a study on the efficacy of entomopathogenic fungi against Red Spider mites in mulberry. *Lecanicillium lecanii* [*Verticillium lecanii*] @ CFU-1.2x10^8/ml registered higher percent, mycosis but it was statistically on par with the remaining two fungi, *Beauveria bassiana* and *Metarhizium anisopliae*. According to Monchn *et al*. (2008), among the 12 entomopathogenic fungi used for the management of Broad mite, *P. latus* on tree type of mulberry, the strain *Metarhizium anisopliae* CKM-048 exhibited the highest virulence in controlling both larval and adult stages of Broad mite when used at $2x10^8$ conidia/ml, and it did not having an ovicidal effect when treated against Broad mite eggs. The application of *M. anisopliae* for managing Broad mites on tree type mulberries resulted in a significant reduction in the population.

Satpathy *et al*. (2019) evaluated the infectivity of talc based formulations at different concentrations of three entomopathogenic fungi. Among these, *Paecilomyces fumosoroseus* and *Beauveria bassiana* recorded significantly the higher mortality of Yellow mite than *Lecanicillium leccanii* at 3 days of post-treatment, the significantly highest mortality was observed in *Paecilomyces fumosoroseus* (30.35%) followed by *Beauveria bassiana* (21.59%) and *Lecanicillium leccanii* (4.87%). The infectivity of *Lecanicillium leccanii* at both concentrations was significantly less than that of *Paeciliomyces fumosoreus* and *Beauveria bassiana*. Due to the higher infectivity of *Paecilomyces fumosoroseus,* it can be ideally used for the control of Yellow mites.

According to Prakya Sreeramkumar and Richa Vershney (2020), the mycelial-conidial liquid formulation of *Hirsutella thompsoni* on all sampling dates significantly reduced the number of Broad mites on both the bottom and top leaves in comparison with the untreated check. It has been inferred that *H. thompsonii* was consistently the best at reducing Broad mite density, and there was no significant difference between the two concentrations of the fungal formulations used.

The use of a microbial biological control agent of the genus, *Metarhizium* was separately applied in a single dose of 1 ml of conidial suspension at 1 × 10^8 conidia/ml on adult Spider mites (*Tetranychus truncates* Ehara) on mulberry, *Metarhizium* sp. BCC 4849, resulting in the highest mortality (82%) on the 5th day post-inoculation (DPI). Further, it has been observed that the infected mites stopped moving and started dying by 48–72 h of PI. Elongated hyphal bodies and oval blastospores were detected in the legs. At 96–120 h of PI or longer, dense mycelia and conidial mass had colonized the interior and exterior of dead mites, primarily at the bottom rather than the upper part (Rudsamee Wasuwan *et al*., 2021).

5. CONCLUSION

The results indicate that both *Lecanicillium lecanii* and *Hirsutella thompsonii* are effective biological control agents against the Broad mite *Polyphagotarsonemus latus,* with *H. thompsonii* showing higher efficacy at a concentration of 3.0 ml/l. The mortality rates significantly increased over time, with the highest efficacy observed 7 days after spray. The study concludes that these entomopathogenic fungi can be effectively utilized in management strategies to control the Broad mite in mulberry cultivation.

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