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In vitro Evaluation of Entomopathogenic Fungi for the Management of Broad Mite, *Polyphagotarsonemus latus* Occurrence on Mulberry

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Authors' contributions

This work was carried out in collaboration between both authors. Author BSR designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author BS managed the analyses of the study and prepared the manuscript. Both authors read and approved the final manuscript.

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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.

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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% with. 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. Yield and quality of mulberry leaf has direct impact on silkworm rearing influencing the cocoon quality and productivity. To achieve the production of good quality silkworm cocoon crop, factors such as mulberry leaf, climate, rearing technique, silkworm race and silkworm egg play an important role [1]. Throughout the year, mulberry cultivation and silkworm rearing which are the two important farmbased 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 mulberry crop is a significant factor in determining productivity and profitability in sericulture [2]. Across the world, more than 300 species of both insect and non-insect pests have been identified to infest mulberry and affecting the various stages of growth during different seasons [3]. Among the major insect orders that attack the mulberry crop, the most numerous species are orders Lepidoptera, Hemiptera, Coleoptera, Thysanoptera, Orthoptera and Isoptera. Additionally, acarids and mollusks have also been observed to cause damage [4]. Some species the mites have gained attention as crops pests and mulberry have not been spared from their impact [5]. Recently, in Karnataka non-insect pests have become threat and there has been a concerning rises of Broad mite occurence in mulberry cultivation. Additionally mite species have been found on both wild and cultivated species of mulberry [6].

Broad mite, *P. latus* and two spotted spider mites, *Tetranychus* spp. found in mulberry and these mites cause severe damage top leaves by sucking the sap from ventral surface leading to detrimental effects [7]. The Broad mite damage has reached alarming levels and is causing significant loss to the 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 elimination of natural enemies due to increase in usage of modern pesticides [8].

At present, the primary approach for managing mites in mulberry cultivation relies heavily on the use of acaricides. Broad mite infestation occur during sprouting and leaf harvesting phases of the mulberry cultivation and the use of pesticides especially during harvesting leads to residue problems in the mulberry leaves required for the silkworm rearing. Therefore there is an urgent requirement to develop management strategies using biological control agents, specifically tailored to Broad mite management in mulberry ecosystem. The current study aims to promote practical application of entomopathogenc fungi in managing the Broad mite occurring on mulberry. This approach holds great promise for effectively managing Broad mite, 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 great impact on plant growth, leaf yield as well as the improvement in biochemical constituents which are the most important factors contributing to quality mulberry leaf, cocoon and silk production.

2. MATERIALS AND METHODS

The current investigation has been undertaken at 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] and methodologies followed during the period of investigations are presented hereunder.

2.1 The Materials Used in the Study

2.1.1 Mite pest

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

2.1.2 Entomopathogenic fungi

Entomopathogenic fungi are microorganisms that infect and often kill susceptible insect population through conidia. Use of fungal entomo-pathogens serves as an alternative to insecticides. In the current study, the entomopathogenic fungi, *Leconicillium lecani* and *Hirsutella thomsonii* were used to know the pathogenesis against Broad mite, *P. latus* damaging mulberry. Stock culture of *L. lecanii* was obtained from 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. Culture were prepared by inoculating 100 ml fresh sterilized potato dextrose broth by 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, matured mulberry leaves grown in separate pots without any insecticidal sprays were collected, washed and cleaned with tissue paper. The leaf disc of 60 mm diameter were prepared and kept in paper lined 100 mm glass petridishes. The leaf discs were kept in the abaxial side with upwards direction. Released the 25 adult mites over the leaf disc and the spore suspension of *L. lecanii* and *H. thompsonii* were sprayed on the Broad mite each at three concentrations [1.50, 2.5 and 3.0 ml/l] separately using hand atomizer. Observations were recorded on the mortality of mites on one, three, five seven days after spray (DAS). Dead mites were collected and placed over moist sterile filter paper for incubation. Growth of fungal mycelium over the body of mites was observed under the stereo zoom microscope to confirm the 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 on managing the Broad mite. ONE-WAY Analysis of Variance (ANOVA) was employed using SPSS statistical package (Ver. 21.0) following the methods outlined by Gomez and Gomez [9].

3. RESULTS

Two entomopathogenic fungi namely *L. lecanii* and *H. thompsonii*were evaluated under laboratory conditions (*in-vitro*) using Broad mite and their efficacy levels were recorded at different days after their usage. Significant variations were observed with respect to 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 Fig. 1].

3.1 One Day After Spray

Statistical [F-Value=195.1**] variation observed in mortality of *P. latus* at 1 DAS. 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. Highest rate of mortality [82.00 ± 1.05%] was observed at 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

Mortality rate was recorded significant [F-Value=554.4**] variation of *P. latus* at five DAS. Highest rate of mortality [95.60 ± 1.31%]was observed at 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 ± 0.78in treatment T_2:*L. lacanii*[CFU-2x10^8/ml] @ 2.5ml/l,81.33 ± 1.56in treatment T_5:*H. thompsonii* [CFU-2x10^8/ml] @ 2.5ml/l, 81.07 ± 1.89 in treatment T_4: *H. thompsonii* [CFU-2x10^8/ml] @ 1.5 ml/l and in T1: *L. lacanii* [CFU-2x10^8/ml] @ 1.5 ml/l, lowest mortality rate [69.20 ± 1.97].

3.4 Seven Days after Spray

Significant [F-Value=1805.1**] variation noticed in the rate of mortality of *P. latus* at seven DAS. 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 finding indicated that using entomopathogenic fungi as a biological agent show high potential for effectively managing theBroad mite, *P. latus*infesting mulberry.

Table 1. Effect of *In-vitro* evaluation of entomopathogenic fungi, *Lecanicilliumlecanii*and *Hirsutellathompsonii*on mortality rate of Broad mite, *P. latus*

Treatment	Cumulative mortality [%]			
	1 DAS	3 DAS	5 DAS	7 DAS
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≤0.01*

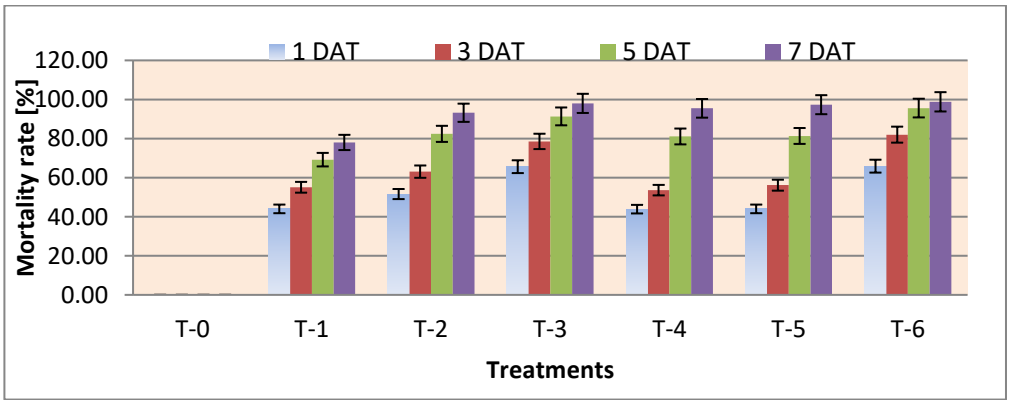

Fig. 1. In-vitro evaluation of entomopathogenic fungi, *L. lecanii* and *H. thompsonii* on mortality of Broad mite, *P. latus*

Theaverage rate of mortality was observed [80.11 ± 0.20%], [71.56 ± 0.62%], [55.49 ± 0.86%] and [44.90 ± 0.68%] in 7,5,3,1 DAS respectively [Table 1 and Fig. 1].

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 was 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 with Srinivasa et al. [6] who conducted a study on the efficacy of entomopathogenic fungi against Red Spider mite in mulberry. *Lecanicillium lecanii* [*Verticillium lecanii*] @ CFU-1.2x10^8/ml registered higher per cent mycosis but, it was statistically on par with remaining two fungi, *Beauveria bassiana* and *Metarhizium anisopliae*. According to Monchn et al. [10], 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 highest virulence in controlling both larval and adult stages of Broad mite when used at $2x10^8$ conidia/ml and it was not having an ovicidal effect when treated against Broad mite eggs. The application of *M. anisopliae* for managing Broad mite on tree type of mulberry resulted in a significant reduction in the population.

Satpathy et al. [11] evaluated the infectivity of talc based formulations at different concentrations of three entomopathogenic fungi. Among these *Paecilomyces fumosoroseus* and *Beauveria bassiana* recorded significantly higher mortality of Yellow mite than *Lecanicillium leccanii* at 3 days of post treatment, significantly highest mortality was observed in *Paecilomyces fumosoroseus* [30.35%] followed by *Beauveria bassiana* [21.59%] and *Lecanicilliumleccanii* [4.87%]. The infectivity of *Lecanicilliumleccanii* at both the concentrations was significantly less than *Paeciliomycesfumosoreus* and *Beauveria bassiana*. Due to higher infectivity of *Paecilomycesfumosoroseus,* it can be ideally used for the control of yellow mite.

According to Sreeramkumar and Vershney [8], mycelial-conidial liquid formulation of *Hirsutella thompsoni* on all sampling dates significantly reduced the number of Broad mites on both bottom and top leaves in comparison with the untreated check. It has been inferred that *Hirsutella thompsonii* was consistently the best in reducing the Broad mite density and there was no significant difference between the two concentrations of the fungal formulations used [12,13].

Use of microbial biological control agent of genus, *Metarhizium* was separately applied in a single dose of 1 ml of conidial suspension at 1×10^8 conidia/ ml on adult spider mite [*Tetranychus truncates* Ehara] on mulberry, *Metarhizium* sp. BCC 4849 resulted 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 than the upper part [14].

5. CONCLUSION

The results indicate that both *Lecanicillium lecanii* and *Hirsutella thompsonii* are effective biological control agents against Broad mite, *Polyphagotarsonemus latus,* with *H. thompsonii* showing higher efficacy, at the concentration of 3.0 ml/l. The mortality rates significantly increased over time, with the highest efficacy observed at 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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

We are hereby declaring that no generative AI technologies used during preparation of the manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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