Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

J E Z S Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2016; 4(6): 335-338

Received: 16-09-2016
Accepted: 17-10-2016

R Raja Rishi
Scientist, Forest and Wood Protection Division, Institute of Wood Science and Technology, Bengaluru, Karnataka, India

Dr. Shailesh Pandey
Scientist, Forest Protection Division, Rain Forest Research Institute, Jorhat, Assam, India

Rajesh Kumar
Scientist, Forest Protection Division, Rain Forest Research Institute, Jorhat, Assam, India

Correspondence
R Raja Rishi
Scientist, Forest and Wood Protection Division, Institute of Wood Science and Technology, Bengaluru, Karnataka, India

Management of *Heortia vitessoides* Moore. A major insect pest of *Aquilaria malaccensis* Lamk. in North East India

R Raja Rishi, Dr. Shailesh Pandey and Rajesh Kumar

Abstract
Aquilaria malaccensis Lamk. (Agar tree) is one of the most valuable economically important commercial tree species of North East India. Pest survey conducted during 2012-2015 in different Agar plantations revealed that *Heortia vitessoides*, a lepidopteran insect is responsible for severe defoliation of seedlings and saplings. An attempt was made to manage this pest using different fungal isolates, *viz.*, *Fusarium* sp., *Beauveria bassiana*, *Metarhizium anisopliae, Aspergillus flavus, Paecilomyces* sp., *Trichoderma hamatum*, *Mucor* sp., *Verticillium lecanii*, *Aspergillus ochraceus*, *Fusarium oxysporum*, *Trichophyton* sp., and *Penicillium chrysogenum,* as biocontrol agents. Spore concentrations, *viz.* 2.4 x 10^{10}, 2.4 x 10^{8}, 2.4 x 10^{6} and 2.4 x 10^{4} spores/ ml of the biocontrol fungi were tested against *H. vitessoides* in laboratory condition. The highest spore concentration of *B. bassiana*, *M. anisopliae, V. lecanii* and *Paecilomyces* sp., were found to cause percent mortality of 100, 76, 58 and 51, respectively. Further, these four potential isolates were found to cause mortality percent of 90, 52, 38 and 36.6, respectively in field conditions. The present study suggests that field application of *B. bassiana* and *M. anisopliae* is an effective management approach against *H. vitessoides.*

Keywords: *Beauveria bassiana*, *Metarhizium anisopliae, Verticillium lecanii, Heortia vitessoides, Aquilaria malaccensis*

Introduction
Aquilaria malaccensis Lamk., is an indigenous tree species of northern tropical wet-evergreen, evergreen and semi-evergreen forests of North eastern states of India [1]. It is renowned for its oleoresin oil (commonly called as Agar oil), which is highly prized for its fragrance in perfumery industry. Agarwood is fetching high price in international market, especially in Egypt and Arabian countries, owing to its highly priced resinous wood and Agar oil (4 to 6 lakh/litre). Unfortunately, this species has come to the risk of extinction due to large scale exploitation from natural forests. As a result, it has been included in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and considered 'critically endangered' in India [2]. Besides human exploitation, this species is highly prone to *Heortia vitessoides,* a major defoliator of Agar [3]. This pest is active for about 7 months from February to September depending upon the weather condition. Life cycle of the pest is completed in about 32-35 days and there are 4-5 overlapping generations in a year. The effective utilization of entomopathogenic fungi in control of number of insect pests of agricultural and horticultural importance were attempted by many researchers [4-5]. Keeping in mind the serious nature of pest incidence and the high demand of Agar oil in international market, an attempt was made to evaluate the native entomopathogenic fungi against *H. vitessoides* in laboratory and field conditions.

2. Material and Methods
2.1. Study area and period
Soil samples were collected from different land use systems as well as from the natural forest in the state of Assam during the period from 2012-15 and the soil samples were subjected to insect bait by using *Galleria mellonella* (Wax moth) to trap the entomopathogenic fungi. The lab experiments were conducted at the research laboratories in the Rain Forest Research Institute, and the field experiments were conducted in the *A. malaccensis* field station located at Sotai, Jorhat, Assam State, India (N 26º 46' 59.5"; E 094º 17'35.3").

2.2 Isolation and identification of fungal pathogens

Twelve fungal isolates were successfully recovered from the infected cadavers of *Galleria mellonella* using insect bait method. The fungal isolates were identified as *F. oxysporum*, *B. bassiana*, *M. anisopliae, A. flavus, Paecilomyces* sp., *T. hamatum*, *Mucor* sp., *V. lecanii, A. ochraceus, Fusarium* sp., *Trichophyton* sp., and *P. chrysogenum* on the basis of microscopic and colony characteristics [6-8]. Pure cultured fungi isolated from the infested cadavers of *Galleria mellonella* through insect bait were used for the preparation of aqueous solutions [9]. The spore suspensions were then filtered through several layers of muslin cloth to remove mycelial mats. The concentration of spores in the final suspension was determined by haemocytometry [10].

2.3 Rearing of Test insect

The egg patches of *Heortia vitessoides,* major pest of *Aquilaria malaccensis* were collected from the field were brought to laboratory for mass rearing at a room temperature 25 – 28 °C. The targeted pest larvae were fed with fresh leaf materials at regular intervals.

2.4 Conidial preparations

For conidial preparations, the spores of the fungal isolates grown on the SDA media were harvested and the aqueous solutions were prepared. Then the aqueous solution prepared were filtered using muslin cloth to get the pure spore suspension and to remove mycelial growths and the concentration of the final suspension was determined. The purified stock fungus suspension was made up to 10 ml with water containing 0.1% wetting agent (Tween-80). This solution ensured thorough mixing and uniform distribution of fungal spores. There were two dilution factors: 1 ml made up to 10 ml = 10 times; 1 ml made up to 100 ml = 100 times. With the haemocytometer, the fungal suspension was drawn to the '1' (One) mark and made up to 11 mark with distilled water containing 0.1% wetting agent. One drop was introduced into the groove of the haemocytometer after placing the standard coverslip over the slide. The spores were allowed to settle down and then counted in about 25 of the 1/400 sq. mm squares with the help of a research microscope under 10 x 40 magnification. Spore suspensions of known concentration were prepared from the stock solution by suitable dilution with distilled water.

2.5 Estimation of spore concentration

If the number of spores counted from 25 of 1/400 sq.mm squares is X and the original dilution is 100 times, then the spores/ml is calculated as:

$$\text{The spores in 1 ml} = \frac{X \times 400 \times 10 \times 1000 \times 100 \times 10}{25}$$

Then the concentration will be $X \times 10^{10}$ Spores/ml

2.6 Bioefficacy of EPF isolates

Third instar larvae of *H. vitessoides* were used for conducting bioassay studies. Twelve fungal isolates, *viz. Fusarium* sp., *Beauveria bassiana*, *Metarhizium anisopliae, Aspergillus flavus, Paecilomyces* sp., *Trichoderma hamatum*, *Mucor* sp., *Verticillium lecanii*, *Aspergillus ochraceus*, *Fusarium oxysporum*, *Trichophyton* sp., and *Penicillium chrysogenum* with four spore concentrations, *viz.* 2.4 x 10^{10}, 2.4 x 10^8, 2.4 x 10^6 and 2.4 x 10^4 spores/ ml were tested against *H. vitessoides* in laboratory condition. Healthy third instar larvae of *H. vitessoides* reared in the laboratory were surface sterilized with 1-5% sodium hypochlorite and sprayed with the fungal inoculums of known concentrations. The larvae were released to feed on the leaves of *A. malaccensis* sprayed with different concentration of fungal inoculums. Twenty larvae were used per replication and five replications were maintained for each concentration. Larvae sprayed with only distilled water were used as control. Larval mortality was recorded at every 48 h interval and recording of data was concluded on the seventh day of the experiment.

Likewise, in the field testing, foliar spray of four potential isolates, *viz. B. bassiana*, *M. anisopliae, V. lecanii* and *Paecilomyces* sp., with all the four concentrations, *viz.* 2.4 x 10^{10}, 2.4 x 10^8, 2.4 x 10^6 and 2.4 x 10^4 spores/ ml was done and the healthy III instar larvae of *H. vitessoides* were released on treated *A. malaccensis* seedlings. The mortality was observed up to 7 days after spraying of spores suspensions.

2.7 Statistical analysis

The data on percent mortality were subjected to Analysis of Variance (ANOVA) and different treatments were compared using Duncan's Multiple Range Test (DMRT) using SPSS version 16.

3. Results and Discussion

Third instar larvae of *H. vitessoides* were used for conducting bioassay studies. Twelve isolates, *viz. F. oxysporum*., *B. bassiana*, *M. anisopliae, A. flavus, Paecilomyces* sp., *T. hamatum*, *Mucor* sp., *V. lecanii, A. ochraceus, F. oxysporum, Trichophyton sp.,* and *P. chrysogenum* with four spore concentrations, *viz.* 2.4 x 10^{10}, 2.4 x 10^8, 2.4 x 10^6 and 2.4 x 10^4 Spores/ ml were tested against *H. vitessoides* in laboratory condition**.** Significant differences among the entomopathogenic fungi were observed in causing the larval mortality at different concentrations (P<0.05). The highest spore concentration, i.e. 2.4 x 10^{10} spores/ ml of *B. bassiana*, *M. anisopliae*, *V. lecanii* and *Paecilomyces* sp., were found to cause percent mortality of 100, 76, 58 and 51 respectively after 7 days, whereas the spore concentration 2.4 x 10^8 spores/ ml resulted in mortality percent of 100, 70, 56.2 and 47.2, respectively. The fungi, *viz. A. flavus*, *T. hamatum*, and *Mucor* sp., were similar in their effect and found less effective than *B. bassiana*, *M. anisopliae, V. lecanii* and *Paecilomyces* sp., were found to cause 5.6, 5.4 and 5.4 percent larval mortality in the higher concentration sprayed. The concentration of 2.4 x 10^{10} spores/ ml of *F. oxysporum* had moderate effect and resulted in 41 percent larval mortality (Table. 1). All other fungal pathogens, viz. *A. ochraceus*, *F. oxysporum*, *Trichophyton sp*., and *P. chrysogenum* were found to have no effect in causing the larval mortality.

Table 1: Effect of different concentrations of entomopathogenic fungi on the larval mortality percentage of *H. vitessoides* in laboratory condition.

Entomopathogens	2.4 x 10^{10} Spores/ ml	2.4 x 10^8 Spores/ ml	2.4 x 10^6 Spores/ ml	2.4 x 10^4 Spores/ ml
B. bassiana	100^a	100^a	100^a	98±1.00^a
M. anisopliae	76±1.41^b	70±0.70^b	62±0.70^b	50±1.41^b
V. lecanii	58±1.41^c	56.2±1.48^c	52±2.23^c	48±1.22^c

Paecilomyces sp.	51±0.70[d]	47.2±1.30[d]	46±1.22[d]	40±1.58[d]
Fusarium oxysporum	41±1.22[e]	39.6±1.14[e]	35.8±1.48[e]	31.4±1.51[e]
A. flavus	5.6±0.54[f]	4.8±0.83[f]	2.4±0.54[f]	2.2±0.44[f]
Trichoderma hamatum	5.4±0.54[f]	4.6±0.54[f]	2.2±0.44[f]	2.0±0.70[f]
Mucor sp.	5.4±0.54[f]	4.2±0.83[f]	2.2±0.44[f]	2.0±0.70[f]
Control	0.0[g]	0.0[g]	0.0[g]	0.0[g]

In the column, values having the same letter are not statistically different (P<0.05) according to DMRT.

In the field testing, foliar spray of four potential isolates, *viz. B. bassiana*, *M. anisopliae, V. lecanii* and *Paecilomyces* sp., with all the four concentrations, *viz.* 2.4 x 10^{10}, 2.4 x 10^{8}, 2.4 x 10^{6} and 2.4 x 10^{4} spores/ ml was done and the healthy third instar larvae of *H. vitessoides* were released on treated Agar seedlings. The mortality was observed up to 7 days after spraying of spore suspensions. All the isolates were found significantly different from each other at the highest concentration. The four potential isolates were found to cause mortality percent of 90, 52, 38 and 36.6 respectively in the field conditions in the higher concentration 2.4 x 10^{10} spores/ ml. The concentration 2.4 x 10^{8} spores/ ml resulted in larval mortality percent of 84, 40, 33.6 and 30.2, respectively. (Table. 2). The data shows linear relationship between dose concentration and mortality, i.e., an increase in the third instar larval mortality with an increase of the spore concentration was observed.

Table 2: Effect of different concentrations of entomopathogenic on the larval mortality percentage of *H. vitessoides* in field condition**.**

Entomopathogens	2.4 x 10^{10} Spores/ ml	2.4 x 10^{8} Spores/ ml	2.4 x 10^{6} Spores/ ml	2.4 x 10^{4} Spores/ ml
B. bassiana	90±0.70[a]	84±1.22[a]	62±0.70[a]	48±1.73[a]
M. anisopliae	52±1.87[b]	40±1.00[b]	32±0.70[b]	20±1.00[b]
V. lecanii	38±1.00[c]	33.6±1.51[c]	20.4±1.51[c]	15.8±1.64[c]
Paecilomyces sp.	36.6±0.89[c]	30.2±2.28[c]	19.6±1.14[c]	15.8±1.09[c]
Control	0.0[d]	0.0[d]	0.0[d]	0.0[d]

In the column, values having the same letter are not statistically different (P<0.05) according to DMRT.

B. bassiana plays an important role in the regulation of pest population [11]. Bio-efficacy of fungal bio-pesticides has been studied by many workers against many economically important insect pests. For instance, *B. bassiana* at the concentration 1.25 x10^{8} spores/ml was reported to cause 86.7 percent larval mortality on *Helicoverpa armigera* [12]. *B. bassiana* was also reported as a promising biocontrol agent on the bark feeding borer, *Indarbela quadrinotata* [13]. The efficacy and persistence of *B. bassiana* was reported on the diamond back moth larvae, *Plutella xylostella* [14]. The pathogenicity of *B. bassiana* on *Hyblaea puera* and *Eligma narcissusss*, pests of *Tectona grandis* and *Ailanthus triphysa*, respectively, has been confirmed and the methods of collection of infected cavader samples, isolation of fungal colonies, their culture and bioassay have also been described [15]. An increase in percent mortality of third instar larvae of *H. armigera* with an increase in spore concentration, i.e. from 0.1x10^{8} spores/ml to 0.25x10^{8} spores /ml, as compared to 23.3 percent in control was also reported [16]. Similarly, *B. bassiana* isolate Bb10 at a concentration of 10^{8} spore/ml, showed maximum larvicidal activity, minimum pupal weight and low number of spore emergence against *Spodoptera litura* [17]. *B. bassiana* was non-pathogenic to natural enemies like *Coccinella septempunctata*, *Chrysoperla carnea* and *Dicyphus tamaninii* as well as beneficial soil insect *Heteromurus nitidus* [18]. The pathogenicity of *B. bassiana* and *M. flavoviride* was tested against the grasshopper *Schistocerca americana* (Orthoptera: Acrididae). Both the fungi were effective in controlling the pest [19]. The pathogenicity of the isolates of *B. bassiana* and *M. anisopliae* were assessed in laboratory against adults of *Leptoglossus zonatus* and *Pachycoris klugii*, the two most frequent pest species in physic nut (*Jatropha curcas*) plantations in Nicaragua, where using dipping bio-assay method, the medium lethal concentration (LC_{50}) of the most efficient strain, *M. anisopliae* NB, was determined [20]. The efficacy of *B. bassiana* and *M. anisopliae* with the concentrations 2.4 × 10^{10} and 2.4 × 10^{8} spores/ml was also found effective on *Crespedonta leayana* in lab and field conditions [21].

B. bassiana and *M. anisopliae* have demonstrated considerable potential in microbial control of many hazardous insect pests with limited harm to non-target organisms especially within Integrated Pest Management programs. Their restricted host ranges allow for control of insect pests with limited harm to non-target organisms including predators, parasites and other pathogens. From this study it was clearly understood that *B. bassiana* and *M. anisopliae* are two important entomopathogens having the potential to control the major pest *H. vitessoides* of Agar.

4. Conclusion

The present study demonstrated the efficacy of the potential fungal isolates against *H. vitessoides,* one of the major pests of *A. malaccensis,* in both lab and field condition. *B. bassiana* spore concentrations of 2.4 x 10^{8} and 2.4 × 10^{10} spores/ml were found effective against *H. vitessoides*, causing 84-90 percent larval mortality. *M. anisopliae* was also effective in controlling the pest by causing 40-52 percent larval mortality. The present study suggests the possibility of *B. bassiana* and *M. anisopliae* for the management of *H. vitessoides.*

5. Acknowledgements

The authors are thankful to the Director, Rain Forest Research Institute, Jorhat for providing the laboratory facilities. Financial support for this work by the Indian Council of Forestry Research and Education, Dehradun, India is gratefully acknowledged.

6. References

1. Beniwal BS. Silvicultural characteristics of *Aquilaria agallocha* Roxb. Indian Forester. 1989; 115(1):17-21.
2. CITES, Resolution, in Proceedings of the 9th Meeting of the Conference of the Parties (COP '94), Fort Lauderdale, Fla, USA, 1994.
3. Sudhir S, Barthakur ND, Gurung D. Bioecology of *Heortia vitessoides* Moore (Lepidoptera: Pyralidae: Odentiinae), A major defoliator of *Aquilaria malaccensis* Lam. (Indian Eagle- wood), 2000.

4. Gurr GM, Wratten SD, Luna JM. Multifunction agricultural biodiversity: pest management and other benefits. Basic Applied Ecology. 2003; 4:107-116.
5. Sergio R, Sanchez P, Jorge SJL, Raul FM. Occurrence of Entomopathogenic fungi from agricultural and natural ecosystems in Sattillo, Mexico and their virulence towards Thrips and White flies. J Insect Sci. 2011; 11(1):1-10.
6. Samson RA, Evans HC, Latge JP. Atlas of entomopathogenic fungi, New York; Springer Verlag, 1988, 187.
7. Richard A, Humber. Entomopathogenic fungal identification (updated Nov.2005), Workshop report prepared jointly by the American Phytopathological Society and Entomological Society of America, Las Vegas, Nevada- 1998, 2005, 33.
8. Gilman JG. A manual of Soil Fungi, Biotech Books, Delhi, India. 1998, 392.
9. Zimmermann G. The *Galleria* bait method for detection of entomopathogenic fungi in soil. J Appl. Entomol. 1986; 102:213-215.
10. Tamuli AK, Gurusubramanium G. Entomopathogenicity of white muscardine fungus *Beauveria bassiana* (Balls.) Vuill. (Deuteromyotina: Hyphomycetes) (BBFF-135) against *Odontotermes* (Rambur) (Isoptera: Termitidae). Assam University Journal of Science and Technology. 2011; 7(1):118-125.
11. Ferron P. Pest control by the fungi *Beauveria* and *Metarhizium.* In: Microbial Control of Insects and Mites. Burges HD, ed. Academic Press, New York, USA. 1981, 465-482.
12. Arti P, Nilofer S. Evaluating prospects of fungal biopesticide *Beauveria bassiana* (Balsamo) against *Helicoverpa armigera* (Hubner): An ecosafe strategy for pesticidal pollution. Society of Applied Sciences. 2010; 1(3):596-601.
13. Balu A, Sasidharan KR, Rishi RR. A new record of the promising biocontrol agent *Beauveria bassiana* on the bark feeder/stem borer*, Indarbela quadrinotata*. Sylva plus. 1998; 6(1):6.
14. Vanderberg JD, Shelton AM, Wilsey WT, Ramos M. Assessment of *Beauveria bassiana* sprays for control of diamond back moth on Crucifers. J Econ. Ent. 1998; 91(3):624-630.
15. Ali MI, Varma RV, Sudheendrakumar. Microbial pathogens associated with forest insect pests in Kerala. Emerging Trends in Biological Control of Phytophagous insects Pub. Mohan Primlani for Oxford and IBH Pub. Co. New Delhi. 1992; 255.
16. Prasad A, Syed N. Evaluating prospects of fungal biopesticide *Beauveria Bassiana* (Balsamo) against *Helicoverpa armigera* (Hubner): An ecosafe strategy for pesticidal pollution. Asian J Exp. Biol. Sci. 2010; 1(3):596-601.
17. Baskar K, Antony RG, Murali, MP, Lingathurai S, Ambrose T, Muthu C. Larvicidal and growth inhibitory activities of entomopathogenic fungus *Beauveria bassiana* against Asian army worm, *Spodoptera litura* Fab. (Lepidoptera: Noctuidae). Journal of Entomology. 2012; 9(3):155-162.
18. Thungrabeab M, Tongma S. Effect of entomopathogenic fungi, *Beauveria bassiana* (Balsam) and *Metarhizium anisopliae* (Metsch) on non target insects. Kmitl Sci. Tech. J 2007; 7(1):8-12.
19. Sieglaff DH, Pereira RM, Capinera JL. Pathogenicity of *Beauveria bassiana* and *Metarhizium flavoviride* to *Schistocerca americana* (Orthoptera: Acrididae). J Econ. Ent.1997; 90(6):1539-1545.
20. Grimm C, Guharay F. Control of leaf footed bug *Leptoglossus zonatus* and shield backed bug *Pachycoris klugii* with Entomopathogenic fungi. Bio-con. Sci. and Tech. 1998; 8(3):365-376, 29.
21. Rishi RR, Pandey S, Borah RK, Kumar R, Borthakur ND. Efficacy of entomopathogenic fungi on *Craspedonta leayana*, a serious insect pest of *Gmelina arborea*. Current Life Sciences. 2016; 2(1):15-19.