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International Journal of Pest Management
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ttpm20

Attempts at controlling Teak Defoliator (Hyblaea puera Cramer, Lepidoptera, Hyblaeidae) with the entomopathogenic fungus, Metarhizium anisopliae (Metsch.): laboratory, nursery and field trials

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Published online: 05 Sep 2013.

To cite this article: O. K. Remadevi , N. Sapna Bai , T. O. Sasidharan , M. Balachander & Priyadarsanan Dharmarajan (2013) Attempts at controlling Teak Defoliator (Hyblaea puera Cramer, Lepidoptera, Hyblaeidae) with the entomopathogenic fungus, Metarhizium anisopliae (Metsch.): laboratory, nursery and field trials, International Journal of Pest Management, 59:3, 236-242, DOI: 10.1080/09670874.2013.832438

To link to this article: http://dx.doi.org/10.1080/09670874.2013.832438

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International Journal of Pest Management, 2013
Vol. 59, No. 3, 236–242, http://dx.doi.org/10.1080/09670874.2013.832438

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Attempts at controlling Teak Defoliator (*Hyblaea puera* Cramer, Lepidoptera, Hyblaeidae) with the entomopathogenic fungus, *Metarhizium anisopliae* (Metsch.): laboratory, nursery and field trials

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(*Received 16 July 2012; final version received 02 August 2013*)

Defoliator pests often cause severe damage to commercially valuable timber trees, such as teak, grown in plantations, affecting the quality and quantity of the wood produced. Twenty-five isolates of an entomopathogenic fungus, *Metarhizium anisopliae* (Metsch.), derived from different forest habitats were tested for their efficacy against the Teak Defoliator, *Hyblaea puera* Cram. The median lethal concentration (LC_{50}) for the isolates ranged from 0.65 to 947.41 $\times$ 10^5 conidia mL^{-1}. Four isolates, viz. MIS2, MIS19, MIS7 and MIS1, were promising, having low LC_{50} values. A median lethal time (LT_{50}) of 3.8 d was recorded for the isolates MIS2 and MIS7 when the moth larvae were exposed to a spore load of 10^7 conidia mL^{-1}. Formulations composed of "MIS2 + MIS7 + 0.5% *Pongamia pinnata* seed oil" and "MIS2 + MIS7" proved to be superior against the Teak Defoliator, causing 94.73% and 93.93% mortality, respectively. Application of the formulation "MIS2 + MIS7 + 0.5% *P. pinnata* oil" resulted in 63.6% and 56.2% reduction in infestation in nurseries and plantations, respectively, while the formulation "MIS2 + MIS7" recorded 56.18% and 51.60% reduction. Our results indicate that *M. anisopliae* formulations could be an effective means of managing Teak Defoliator in plantations, particularly when combined with *P. pinnata* oil.

Keywords: biocontrol; entomopathogens; *Hyblaea puera*; *Metarhizium anisopliae*; teak

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1. Introduction

Teak (*Tectona grandis* L.), known as the "king of timber", is a valuable timber tree species in India (Roychoudhury and Dadwal 2010). It has been recognized for centuries as the finest hardwood in the world. It is a primary high-value wood used in furniture manufacture, and the ever-increasing need for teak timber has resulted in the establishment of large scale plantations both within and outside its range of natural distribution (Javaregowda and Naik 2007a). The most important lepidopteran defoliators, which cause defoliation in teak plantations throughout the tropics, are Teak Defoliator, *Hyblaea puera* Cramer (Hyblaeidae) and Teak Skeletonizer, *Paliga machoeralis* Walker (Pyralidae) (Tewari 1992). *Hyblaea puera* is the most widespread and serious pest in both plantations and natural forests, with as many as 14 generations per year (Beeson 1941). Defoliation does not kill the trees, but does lead to substantial timber loss (Nair 1988). Defoliation by *H. puera* in plantations four to nine years old was reported to cause about 44% volume increment loss of the trunk (Nair and Mohandas 1996). The larvae directly retard girth increment, reduce timber quality by forking, cause death of the leading shoot and result in formation of epicormic branches (Vongxomphou 2001).

With the current interest in addressing environmental pollution and developing integrated control strategies for the management of Teak Defoliator, *H. puera*, attention is now mainly focused on the use of microbial insect pathogens (Javaregowda and Naik 2008a). The entomopathogenic fungus *Metarhizium anisopliae* is one among the most exploited hyphomycetes fungi employed in insect pest management. It is a promising agent for controlling insect pests because of its wide geographical range, and its vast spectrum of pathogenicity and infectivity (Zimmermann 2007).

This investigation was focused on determining the potential of *M. anisopliae* for controlling *H. puera*. A laboratory bioassay of 25 isolates of *M. anisopliae* was initially conducted. Two promising isolates, MIS2 and MIS7 and *Pongamia pinnata* oil, individually and in different combinations were evaluated. Two promising combinations were further tested in nurseries and plantations to identify a better formulation for management of *H puera* infestation in teak plantations.

2. Materials and methods

2.1. Insect culture

Healthy larvae of *H. puera* (Figure 1) collected from the field were reared in the laboratory and allowed to pupate and develop into adults. Male and female moths were released into glass bottles covered with muslin cloth which the moths could use for oviposition. Diluted sucrose solution (10%) was provided on cotton balls as food. Any muslin cloth that eventually had eggs laid on it was surface-sterilized with 1% sodium hypochlorite for

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Figure 1. (Color online) Healthy *H. puera* larva.

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15 min and washed in sterile distilled water for 10 min and placed over blotting paper for drying. It was then covered with tender teak leaves and transferred to a glass bottle for hatching. Larvae that became established on the tender leaves were transferred with fine camel-hair brush to 5 mL of artificial diet (Mathew et al. 1990) in plastic vials covered with muslin cloth. One larva per vial was maintained. Fresh diet was provided after every 2 d. Pupae were removed from the rearing containers within 24 hours of pupation and transferred to rearing cages for adult emergence.

2.2. Fungal culture

Among the 25 fungal isolates (MIS1 to MIS25) used in this study, 16 were isolated either from soil or from infected insects and 9 procured from different institutions. Soil samples were collected from a depth of 30 cm from different study areas. The *Galleria* bait method was used to isolate the fungi from soil samples. After removing roots and gravel, soil samples were sifted through a 5 mm sieve. Thereafter, plastic boxes (10 cm height, 8 cm diameter) were filled with 100 g of soil and 10 late instar larvae of Wax Moth (*Galleria mellonella*) were introduced. Holes were punched in the lids to allow air to pass through. The larvae were incubated at 20°C in continuously dark conditions. During the first 5 days, the boxes were turned once daily to encourage the bait insects to penetrate as much soil as possible. After 7 to 10 days, boxes were examined every day and dead larvae collected. Cadavers thus obtained, as well as those collected from field, were surface-sterilized by dipping consecutively in 70% ethyl alcohol, 1% sodium hypochlorite, and sterile distilled water (each for 3 min.). The larvae were dissected and placed on Veen's medium and incubated at 28 ± 1°C and 90% relative humidity (RH) to facilitate growth and sporulation of the fungus. Slant cultures were prepared from a single colony and stored at −20°C until used.

2.3. Pathogenicity studies

2.3.1. Inoculum preparation

Culture plates of each isolate were prepared by spreading 200 μl of conidial suspension (10^7 conidia mL^{-1}) onto potato dextrose agar (Hi Media, Mumbai) medium fortified with 1% yeast extract. Plates were incubated in dark at 28 ± 1°C for 14 d to maximize spore production. Spores were harvested by flooding each plate with 10 mL of 0.05% Tween 80 in sterile distilled water and dislodging the conidia into suspension with a glass rod. The suspension was filtered through a double layer sterile cheese cloth and centrifuged at 1700 rpm for 15 min. The supernatant was discarded and the conidia re-suspended in 5 mL of sterile distilled water. This stock spore suspension was stored at 4°C for 24 h until spore viability was determined. Only cultures with $> 90\%$ viability were used. Counts of conidia were made from the stock suspension using an improved Neubauer haemocytometer. Spore suspensions containing 10^3, 10^4, 10^5, 10^6, 10^7 and 10^8 conidia mL^{-1} sterile distilled water with 0.05% Tween 80 were prepared from the stock for bioassay.

2.3.2. Bioassay

Bioassay of all the 25 isolates was carried out against *H. puera* using inoculum concentrations ranging from

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10^3 to 10^8 conidia mL^{-1} to determine the multiple dose-mortality (LC_{50}) and time-dose-mortality (LT_{50}) responses. Thirty second instar larvae of *H. puera* were placed separately in sterile 20-mL vials and 10 mL of fungal suspension was added. The vial was capped and inverted five times for a period of 5 s to ensure that the insects were completely drenched with the spore suspension. The suspension with insects was filtered through a tea strainer (6 cm diameter). For the controls, insects were treated with 0.05% Tween 80. Treated and untreated (control) *H. puera* larvae were transferred individually with a fine camel hair brush to sterile vials containing 5 mL artificial diet. Each vial was closed with sterile muslin cloth. The vials were incubated at 26 ± 1°C, 90% RH, 12 : 12 h (light : dark). Fresh diet was provided every 2 d. Four replications were maintained for each concentration of a single isolate. The mortality of larvae was recorded every 24 hours for 8 days after exposure. Dead larvae were counted and removed each day to prevent horizontal contamination. The dead larvae from each treatment were incubated in moist conditions to determine whether death resulted from mycosis (Figure 2).

The toxicity of two promising isolates, MIS2 (10^7 conidia mL^{-1}) and MIS7 (10^7 conidia mL^{-1}) and 0.5% *P. pinnata* oil were further evaluated individually and in different combinations as per the above method to determine the synergistic effect of combinations on the mortality of *H. puera.*

2.3.3. Nursery trial

The two combinations which proved more effective in the laboratory were evaluated under net-house conditions. The different treatments, viz. T1 (water formulation of MIS2 and MIS7 at 10^{14} conidia mL^{-1} + 0.08% Tween 80), T2 (oil formulation of MIS2 and MIS7 at 10^{14} conidia mL^{-1} + 0.08% Tween 80 + 0.5% *P. pinnata* oil) and T3 (control of 0.08% Tween 80) were evaluated on *H. puera* released on teak plants raised in polybags placed in a wire mesh enclosure. Germination testing of the formulations was done one day prior to application and was found to be over 80%. Twenty larvae were released on each plant one day prior to starting the experiment. The treatments were applied using a hand sprayer. Each treatment, including the control, was replicated three times with 10 plants per replication. The number of surviving larvae was recorded 3 and 7 days after the spraying. For each treatment, the average percentage reduction of larvae was calculated using Henderson and Tilton equation (Henderson and Tilton 1955).

2.3.4. Field trial

Two formulations of the isolates, MIS2 and MIS7, which proved promising in the laboratory were field-evaluated at two locations in the Kannavam forest range in Kannur district of Kerala where peak pest activity was observed. The different treatments, viz. T1 (water formulation of MIS2 and MIS7 at 10^{14} conidia mL^{-1} + 0.08% Tween 80), T2 (oil formulation of MIS2 and MIS7 at 10^{14} conidia ml^{-1} + 0.08% Tween 80 + 0.5% *P. pinnata* oil) and T3 (control of 0.08% Tween 80) were field evaluated in 3-year-old teak plantations infested by *H. puera*. A germination test of the formulations was carried out one day prior to application and the level was found to be above 80%. The experimental layout was a Randomized Block Design. Each treatment including the control was replicated four times. The subplots in each replicate had eight rows (five

Figure 2. (Color online) Mycosed cadavers of *H. puera*.

plants each) 2 m apart (six main rows and two skip rows, one on either side of main rows). Each subplot was separated from the other by two skip rows, 2 m apart (one row from each subplot). The population counts of *H. puera* were recorded one day before the imposition of treatments. The number of larvae on all the leaves of six randomly selected tagged plants in each plot was recorded. The treatments were imposed using a power sprayer. The number of surviving larvae was recorded after 3 and 7 days of treatment. For each treatment, the average of all the observations from two locations was used to determine the average percentage reduction of pest population (Henderson and Tilton 1955).

2.4. *Statistical analysis*

Median lethal concentration (LC_{50}) and median lethal time (LT_{50}) values were calculated using Probit analysis (Finney 1971). One-way and two-way analysis of variance (ANOVA) were used to analyse the field trial data.

3. Results and discussion

3.1. *Bioassay*

The bioassay of the 25 isolates against *H. puera* at different concentrations showed MIS2, MIS19, MIS7 and MIS1 to be more pathogenic, with lower LC_{50} values. The LC_{50} values of the 25 isolates ranged from 0.65 × 10^5 to 947.41 × 10^5 conidia mL^{-1}. MIS2 was the most effective isolate with the lowest LC_{50} value (0.65 × 10^5 conidia mL^{-1}) followed by MIS19 (0.88 × 10^5 conidia mL^{-1}). MIS7 showed an LC_{50} value of 1.67 × 10^5 conidia mL^{-1} and MIS1 recorded an LC_{50} value of 1.98 × 10^5 conidia mL^{-1}. MIS14 was the least effective isolate with the highest LC_{50} value, viz. 947.41 × 10^5 conidia mL^{-1} (Figure 3). The lowest LT_{50} of 3.8 d was recorded for both MIS2 and MIS7 followed by MIS1 (4.2 d) and MIS19 (4.8 d) at a spore load of 10^7 conidia mL^{-1}. MIS2 required 4.7, 5.8 and 6.6 d to kill 50% population at 10^6, 10^5 and 10^4 conidia mL^{-1} respectively, while MIS7 killed 50% of the population after 4.7, 6.2 and 6.7 d. The LT_{50} values varied from 4.2 to 8.1 d for MIS1 and from 4.8 to 6.9 d for MIS19. The highest LT_{50} value of 11.5 d was recorded for MIS12 at a spore load of 10^4 conidia mL^{-1} (Figure 4).

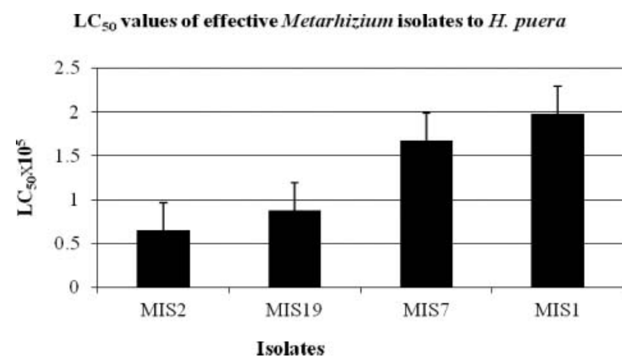

Figure 3. Dose-mortality response (LC_{50}) of four most effective *Metarhizium* isolates to *H. puera*.

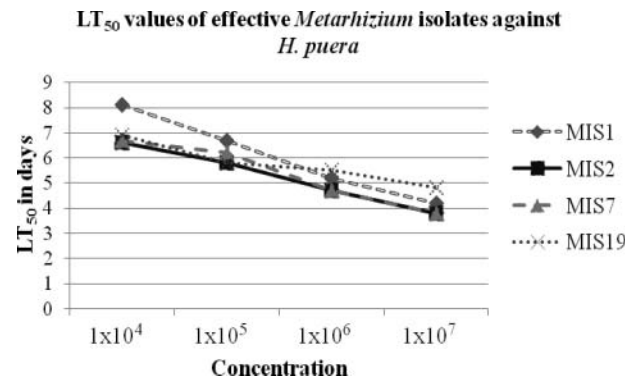

Figure 4. Time-dose-mortality response (LT_{50}) of effective *Metarhizium* isolates to *H. puera*.

The findings of Sandhu et al. (1993) on laboratory evaluation of pathogenicity of a few species of plant and entomopathogenic fungi revealed the ability of *Beauveria bassiana, M. anisopliae* and *Nomuraea rileyi* to infect *H. puera* larvae which supported our observations with regard to *M. anisopliae* isolates. Studies on the susceptibility of *H. puera* to *B. bassiana* showed optimum infection and host death at 20–30°C and the mortality decreased with the increase in larval age (Rajak et al. 1993). Apart from these studies, there has been no significant work on the use of fungal pathogens, especially *Metarhizium*, against *H. puera. Bacillus thuringiensis* was identified as one of the broad-spectrum and ecologically safe insect control agents against *H. puera* by various authors (Burgess and Hussay 1971; Kalia and Lal 2000; Javaregowda and Naik 2007b). Mortality levels up to 99.8% against early instar larvae and 81.2% against later instars were obtained using a commercial formulation of *B. thuringiensis* (Loganathan and David 2000). Efficacy of certain plant products have been tested against *H. puera* and reported to be effective (Deepa and Remadevi 2007). Sudheendrakumar et al. (1996) evaluated a nuclear polyhedrosis virus (HpNPV) for its efficacy against *H. puera* under both laboratory and field conditions and reported a fairly good control of infestation in the field. Nair et al. (1996) evaluated the effectiveness of a crude preparation of 10^5 poly occlusion body (POB) mL^{-1} of HpNPV and attained 70–76% protection of foliage. LD_{50} values of 1.38 × 10^3 POB mL^{-1} and 17.34 × 10^3 POB mL^{-1} were obtained for second instars and third instars respectively after 8 days of treatment with HpNPV (Javaregowda and Naik 2008b).

A significant difference in mortality was observed among the seven combinations tested. Increased mortality was recorded with the combination treatments compared to individual treatments. The formulation "MIS2 + MIS7 + 0.5% *P. pinnata* oil" proved to be superior to other formulations and recorded 94.73% mortality, followed by "MIS2 + MIS7" with 93.93% mortality. The "MIS2 + 0.5% *P. pinnata* oil" formulation recorded mortality of 91.30% and MIS7 + 0.5% *P. pinnata* oil formulation resulted in 90.13% mortality. MIS2, MIS7 and 0.5% *P. pinnata* oil, when tested independently, resulted in 90.48, 89.28 and 60.95% mortality, respectively (Table 1).

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Table 1. Evaluation of different combinations of *Metarhizium anisopliae* isolates and *Pongamia pinnata* seed oil against *Hyblaea puera.*

Treatment	Mean % mortality of *H. puera*
MIS2	90.48
MIS7	89.28
MIS2 + MIS7	93.93
0.5% *P. pinnata* oil	60.95
MIS2+0.5% *P. pinnata* oil	91.30
MIS7+0.5% *P. pinnata* oil	90.13
MIS2+MIS7+0.5% *P. pinnata* oil	94.73
Control	0.00

ANOVA

Treatment	Sum of Squares	df	Mean Square	*F*	Significance
Between groups	0.684	3	0.228	0.000	1.000
Within groups	29987.363	28	1070.977		
Total	29988.048	31			

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The effect of interaction among different species of entomopathogenic fungi was tested by Mahmoud (2009). He analysed the synergistic and antagonistic interactions based on a comparison of mortality of adults by the fungi when used alone and in combination. The combination of *B. bassiana* + *M. anisopilae* gave a synergistic response while the combination of *B. bassiana* + *L. lecanii* and *M. anisopliae* + *L. lecanii* gave an antagonistic response. In our study we observed a synergistic effect with respect to mortality when *Metarhizium* isolates were used in combinations. The possibility of using mixtures of different species of entomopathogenic fungi for the control of Western Flower Thrips, *Frankliniella occidentalis*, was reported by Gouli et al. (2008). Oil-based formulations have shown better tolerance to temperature and desiccation, enhanced speed of germination of conidia, improved environmental stability and overall performance as fungal biopesticides (Jackson et al. 2010). In the present study, usage of *P. pinnata* seed oil would have provided these advantages in addition to its insecticidal activity.

3.2. Nursery trial

After 3 days of treatment in the nursery trial, treatment T2 recorded a density of 7.12 larvae per plant while T1 recorded 8.54 larvae per plant, which was significantly different from the control in which a density of 18.16 larvae per plant was recorded. After 7 days of treatment, densities of 5.80, 7.00 and 17.34 larvae were recorded per plant in treatments T2, T1 and T3, respectively. Treatments T1, T2 and T3 showed significant difference in reduction of infestation between them. Treatment T2 recorded a larval density of 6.46 larvae per plant amounting to 63.61% reduction of infestation, and 56.18% reduction was observed in T1 with a density of 7.77 larvae per plant (Table 2).

3.3. Field trial

The pre-treatment larval density in the field ranged from 7.10 to 7.30 per plant in location I and 6.97 to 7.02 in location II. After 3 days of treatment, T2 recorded a larval density of 3.58 and 3.87 larvae per plant in locations I and

Table 2. Reduction of *Hyblaea puera* infestation on teak plants (net-house study).

Treatment	Mean number of larvae per plant				Reduction of infestation (%)
		After treatment			
	Before treatment	3rd day	7th day	Mean	
T1	20	8.54	7.00	7.77	56.18
T2	20	7.12	5.80	6.46	63.61
T3	20	18.16	17.34	17.75	

T1: MIS2 + MIS7, T2: MIS2 + MIS7 + *Pongamia pinnata* oil (0.5%); T3 : 0.08% Tween 80.

ANOVA

Treatment	Sum of squares	df	Mean square	*F*	Significance
Between groups	0.376	3	0.125	0.003	1.000
Within groups	305.421	8	38.178		
Total	305.797	11			

Table 3. Reduction of *Hyblaea puera* infestation on juvenile teak trees (field study).

	Average number of larvae per plant										
	Location I				Location II				Location mean		
Treatment	1 DBT	3 DAT	7 DAT	Mean	1 DBT	3 DAT	7 DAT	Mean	DBT	DAT	R I (%)
T1	7.30	4.80	3.34	4.07	7.02	4.01	3.25	3.63	7.16	3.85	51.60
T2	7.19	3.58	3.82	3.70	6.97	3.87	2.57	3.22	7.08	3.46	56.26
T3	7.10	7.81	8.27	8.04	7.00	7.90	7.46	7.68	7.05	7.86	

T1: MIS2 + MIS7, T2: MIS2 + MIS7 + *Pongamia pinnata* oil (0.5%); T3: 0.08% Tween 80.
DBT = day before treatment; DAT = days after treatment; RI: reduction of infestation,

ANOVA

Source of variation	Sum of squares	df	Mean square	*F*	Significance
Location	1.097	1	1.097	0.183	0.675
Treatment	1.090	3	0.363	0.061	0.980
Location * treatment	0.214	3	0.071	0.012	0.998
Error	95.966	16	5.998		
Total	712.144	24			

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II, respectively, whereas T1 recorded a density of 4.80 and 4.01 larvae. Both T2 and T1 differed significantly from control (T3) which had a larval density of 7.81 and 7.90 larvae per plant in locations I and II, respectively. After 7 days of treatment, significant difference in reduction of infestation was recorded between T2 and T1 in both locations. Larval densities of 3.82 and 2.57 were recorded in the case of treatment T2, while T1 recorded a densities of 3.34 and 3.25 larvae per plant in the two locations, respectively. Larval densities of 8.27 and 7.46 larvae per plant were recorded in the untreated control (T3). The average number of larvae per plant based on observations from both the locations showed significant difference in the reduction of infestation between treatments. Treatment T2 recorded 56.26% reduction of infestation, while 51.60% reduction was observed in treatment T1 (Table 3).

Most of the studies reported on management of *H. puera* mainly involved the use of HpNPV, and some works using insecticides, *B. thuringiensis*, *B. bassiana* and botanicals were also documented. The infection of NPV in *H. puera* under field conditions was reported by Sudheendrakumar et al. (1988). Laboratory and field studies on NPV infection of *H. puera* were carried out by Ahmed (1995).

4. Conclusions

The results of this study suggest the prospects of using the entomopathogenic fungus, *M. anisopliae* for the control of the Teak Defoliator. The results further indicate the suitability of combining the isolates and incorporating *P. pinnata* oil for application of the pathogen to enable more effective management of the defoliator. Since the virulence of the isolate depends on environmental factors, the growth requirements of the formulated pathogen must be attuned to the habitat of the target insect. The key question that arises with the use of fungi in field is the long term storage and viability. Further studies that elucidate mechanisms that conserve the viability and efficacy after long term storage and field persistence are essential for the successful application of these formulations in field.

Acknowledgements

The authors are grateful to the Department of Biotechnology, New Delhi for providing financial support to carry out this work. Thanks are also due to the Director, ATREE and Director, IWST, Bangalore for providing facilities to undertake the study. The permission granted by the PCCF Karnataka and PCCF Kerala to undertake survey in the states is also acknowledged.

References

Ahmed SI. 1995. Investigations on the nuclear polyhedrosis of teak defoliator, *Hyblaea puera* (Cram) (Lep., Hyblaeidae). J Appl Entomol. 119(5):351–354.
Beeson CFC. 1941. The ecology and control of the forest insects of India and the neighboring countries. New Delhi: Government of India. p. 767.
Burgess HD, Hussay NW. 1971. Microbial control of insects and mites. New York: Academic Press. 861 p.
Deepa B, Remadevi OK. 2007. Efficacy of plant products against the teak defoliator *Hyblaea puera* Cramer (Lepidoptera: Hyblaeidae). Pest Manage Hort Ecosyst. 13(1):68–70.
Finney DJ. 1971. Probit analysis. Cambridge (UK): Cambridge University Press.
Gouli S, Gouli V, Skinner M, Parker B, Marcelino J, Shternshis M. 2008. Mortality of western flower thrips, *Frankliniella occidentalis*, under influence of single and mixed fungal inoculations. J Agric Tech. 4(2):37–47.
Henderson CF, Tilton EW. 1955. Tests with acaricides against the brown wheat mite. J Econ Entomol. 48:157–161.
Jackson MA, Dunlap CA, Jaronski ST. 2010. Ecological considerations in producing and formulating fungal entomopathogens for use in insect biocontrol. Biocontrol. 55(1):129–145.
Javaregowda, Naik LK. 2007a. Seasonal incidence of Teak Defoliator, *Hyblaea puera* Cramer (Hyblaeidae: Lepidoptera) in Uttara Kannada District of Karnataka. Karnataka J Agric Sci 20(1):153–154.

Javaregowda, Naik LK. 2007b. Antifeedant properties of tree born oil seeds (TBO's) against Teak Defoliator, *Hyblaea puera* Cramer. Karnataka J Agric Sci. 20(1):183–184.
Javaregowda, Naik LK. 2008a. Key mortality factors of Teak Defoliator, *Hyblaea puera* Cramer (Hyblaeidae: Lepidoptera). Karnataka J Agric Sci. 21(4):519–523.
Javaregowda, Naik LK. 2008b. Efficacy of nuclear polyhedrosis virus (HpNPV) against Teak Defoliator, *Hyblaea puera* Cramer (Hyblaeidae: Lepidoptera). Karnataka J Agric Sci. 21(4):452.
Kalia S, Lal RB. 2000. Efficacy of three varietals toxins of *Bacillus thuringiensis* tested against some important forest insect pests of multipurpose forest tree species. Ind Forester. 126 (1): 62–66.
Loganathan J, David PMM. 2000. Laboratory and field evaluation of *Bacillus thuriengiensis* Berliner products against the teak defoliator *Hyblaea puera* Cramer. Insect Sci Appl. 20 (1):61–65.
Mahmoud MF. 2009. Pathogenicity of three commercial products of entomopathogenic fungi, *Beauveria bassiana*, *Metarhizum anisopilae* and *Lecanicillium lecanii* against adults of Olive Fly, *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae) in the laboratory. Plant Prot Sci. 45(3):98–102.
Mathew G, Sudheendrakumar VV, Mohanadas K, Nair KSS. 1990. An artificial diet for the teak defoliator, *Hyblaea puera* Cramer (Lepidoptera: Hyblaeidae). Entomon. 115(3&4):159–163.
Nair KSS. 1988. The teak defoliator in Kerala, India. In: Berryman AA, editor. Dynamics of forest insect populations: pattern, causes, implications. New York: Plenum Press. p. 267–289.
Nair KSS, Mohandas K. 1996. Early events in the outbreak of the teak caterpillar, *Hyblaea puera*. Int J Eco Env Sci. 22:271–279.
Nair KSS, Sudheendrakumar VV, Verma RV, Chacko KC, Jayaraman K. 1996. Effect of defoliation by *Hyblaea puera* and *Eutectona machaeralis* (Lepidoptera) on volume increment of teak. Paper presented at: IUFRO Symposium on Impact of Diseases and Insect Pests on Tropical Forests. Proceedings of the IUFRO Symposium on Impact of Diseases and Insect Pests on Tropical Forests; 23–26 November; Peechi, Kerala.
Rajak RC, Agarwal GP, Khan AR, Sandhu SS. 1993. Susceptibility of teak defoliator (*Hyblaea puera* Cramer) and teak skeletonizer (*Eutectona machaeralis* walker) to *Beauveria bassiana* (Bals.) Vuill. Ind J Exp Biol. 31(1):80–82.
Roychoudhury N, Dadwal VS. 2010. Occurrence of Entomogenous fungi, *Beauveria bassiana* and *Fusarium oxysporum* on teak leaf skeletonizer, *Eutectona machaeralis* and their pathogenicity. World J Zool. 5(3):200–204.
Sandhu S, Rajak RC, Agarwal GP. 1993. Microbial control agents of forest pests of Jabalpur. Ann For. 1(2):136–140.
Sudheendrakumar VV, Mohammed Ali IM., Varma R V. 1988. Nuclear Polyhedrosis Virus of the teak defoliator Hyblaea *puera*. J Invert Pathol. 51(3):307–308.
Sudheendrakumar VV, Nair KSS, Biju B, Sajeev TV, Mohammed Ali MI, Varma RV, Mohandas K. 1996. Field trials of Nuclear Polyhedrosis Virus for the control of the teak defoliator, *Hyblaea puera* (Lepidoptera: Hyblaeidae), in Kerala, India. Paper presented at: IUFRO Symposium on Impact of Diseases and Insect Pests on Tropical Forests. Proceedings of the IUFRO Symposium on Impact of Diseases and Insect Pests on Tropical Forests; 23–26 November; Peechi, Kerala.
Tewari DN. 1992. A monograph on teak. Dehra Dun: IBD.
Vongxomphou T. 2001. Population dynamics and life cycle of teak defoliator *Hyblaea puera* Cramer (lepidoptera: hyblaeidae) in Laos people's democratic republic. MSc thesis, School of Graduate Studies, Universiti Putra Malaysia.
Zimmermann G. 2007. Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. Biocont. Sci Technol. 17(9):879–920.

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