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Screening of Some Mycoinsecticides for the Managing Hairy Caterpillar, *Pericallia ricini* Fab. (Lepidoptera: Arctiidae) in Castor

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ABSTRACT

The effectiveness of oil-based conidia formulation of three indigenous fungal isolates such as *Beauveria bassiana* (Balsamo) Vaillemin, *Verticillium lecanii* (Zimm). Viegas and *Paecilomyces fumosoroseus* (Wize) Brown et Smith (Deuteromycotina: Hyphomycetes) were evaluated against fourth instar larvae of *Pericallia ricini* Fab. (Lepidoptera: Arctiidae) under laboratory conditions by dermal toxicity test. All the three isolates were pathogenic to *P. ricini* at all tested concentrations. Among the three isolates, *P. fumosoroseus* caused lowest mortality (21.67% at 2.7×10^9 spores mL^{-1}), followed by *B. bassiana* (57.33% at 4.1×10^9 spores mL^{-1}) and *V. lecanii* caused highest (97.33% at 3.9×10^9 spores mL^{-1}). LC_{50} values at 96 h was calculated as 9.6×10^8, 1.3×10^8 and 2.36×10^{11} conidia mL^{-1} for *B. bassiana, V. lecanii* and *P. fumosoroseus* respectively. Hence, oil-based formulation of *V. lecanii* can be important castor pest *P. ricini*.

Key words: *Beauveria bassiana, Paecilomyces fumosoroseus, Verticillium lecanii,* fungal-insecticide, oil-based formulation, *Pericallia ricini*

INTRODUCTION

Castor, *Ricinus communis* is one of the cash cultivated in dry lands as monocrop or mixed crop with groundnut, chilly, cotton and cowpea. *Pericallia ricini* Fab. (Lepidoptera: Arctiidae) commonly called as hairy caterpillar or wooly bear is the major pest of castor, gingelly, cotton, country bean, brinjal, drum stick, coccina, banana, calotropis, sunflower, oleander, tea, sweat potato, pumpkin (David and Ananthakrishnan, 2004) and Vanilla (Vanitha *et al.*, 2011). Various mechanical, chemical and botanical (Joseph *et al.*, 2010) control measures have been used to control this pest, however, frequent misuse and abuse of chemical pesticides has led to the problems of pesticides resistance, resurgence and secondary out breaks of the pests besides several environmental hazards (Purwar and Sachan, 2006; Scholte *et al.*, 2006; Jaramillo and Borgemeister, 2006). These constitutes necessitate exploration of newer control strategies such as the use of microbial agents like entomopathogenic fungi against this pest. So far no reports have been available regarding the use of microbial pathogens against *P. ricini*. During larval stage, fourth instar *P. ricini* is causing very severe damage to most of the cash crops cultivated in our region.

Thousands of literatures were available about the usefulness on fungus-based insecticides in pest management. *Beauveria. bassiana* (Balsamo) Vaillemin, *Verticillium lecanii* (Zimm.) Viegas and *Paecilomyces fumosoroseus* (Wize) Brown et Smith (Deuteromycotina: Hyphomycetes) were available locally, easy for mass production, withstand the local climatic conditions, have been used by our farmers in other pest management. We have selected these fungicides for our study (Hazarika and Puzari, 1997; Manjula and Padmavathamma, 1996). *Beauveria. bassiana* is a common soil borne fungi that occurs worldwide and has been reported as a suppressive agent for several insect pests including white flies, aphides, grasshoppers, termites, Colorado potato beetle, Mexican been beetle, caterpillars, Japanese beetle, boll weevil, cereal leaf beetle, chinch bug and fire ants (Gillespie, 1988; Hazarika and Puzari, 1997; Wagner and Lewis, 2000; Sandhu *et al.*, 2001; Castrillo *et al.*, 2003; Mirshekar *et al.*, 2005).

Paecilomyces fumosoroseus an important soil fungi is considered as a very promising biological control agents due to its extensive host range which include diamond black moth, *Plutella xylostella* Linn, Russian wheat aphid, *Diurophis noxia* and silver leaf whitefly, *Bemisia argentifolli* Bellows and Perrung (Cantone and Vandenberg, 1998; Vandenberg *et al.*, 1998; Altre *et al.*, 1999; Cantone and Vandenberg, 1999; Altre and Vandenberg, 2001a, b). *P. fumosoroseus* is also geographically widespread and found to be colonizing many insects in all stages of the life belongs to many orders (Obornik *et al.*, 2000, 2001; Mesquita and Lacey, 2001; Feng *et al.*, 2004).

The fungus, *Verticillium lecanii* attacks many insects and found to be effective against aphids, whitefly and thrips (Gillespie, 1998; Fouorier and Brodeur, 2000). It is well suited for commercialization because it grows on all conventional mycological media (Hall *et al.*, 1982; Zhioua *et al.*, 1999). Ramanujam *et al.* (2002) reported the virulence of *V. lecanii* against *Spodoptera litura* (Fab.) and *Helicoverpa armigera* (Hubner). Various conventional non-synthetic media such as cereals and grains are also suitable for the mass production of all these fungi (Lakshmi *et al.*, 2001). Chitinase produced by all these fungi have lytic activity in the insect chitin at broad range of temperature (5-60°C) and low relative humidity (50%), this conditions are prevailed in South India.

Even though these fungi were used to control various pests, none of the researchers have used these fungi against the lepidopteran hairy caterpillars. Very recently Sahayaraj and Borgio (2010) evaluated the impact of Metarhizium anisopliae (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes) against this pest. With this background we have selected this *B. bassiana, V. lecanii* and *P. fumosoroseus* for our present investigation to find their biological control efficacy against *P. ricini* under laboratory condition.

MATERIALS AND METHODS

Larvae of *P. ricini* were collected from naturally infested castor plantation from the field in an around Palayamkottai, Tirunelveli District, Tamil Nadu, India. *B. bassiana, V. lecanii* and *P. fumosoroseus* were isolated using serial dilution method and identified using micro and macroscopic characters (Goettel and Inglis, 1997). They were sub cultured in petri plates with Potato Dextrose Agar (PDA) medium. The fungal conidia were collected from 14 days old cultures incubated at 27°C by scrapping off with a glass rod. Stock solutions of 4.1×10^{9}, 3.9×10^{9} and 2.7×10^{9} conidia mL^{-1} were prepared for *B. bassiana, V. lecanii* and *P. fumosoroseus* respectively. Four spore concentrations (1.8×10^{6}, 4.0×10^{7}, 1.2×10^{8} and 2.7×10^{9}, for *B. bassiana* 2.1×106, 2.3×10^{7}, 3.0×10^{8} and 4.1×10^{9} for *V. lecanii,* 1.6×10^{6}, 1.9×10^{7}, 2.8×10^{8} and 3.9×10^{9} spores mL^{-1} for *P. fumosoroseus*) were prepared from the stock cultures and water was used as untreated control.

Forty fourth instar larvae (length 2.94±0.23 cm and weight 415±13.92 mg) hairy caterpillar were used for each treatment. Fungal conidial suspensions separately were sprayed on the dorsum of the caterpillar by hand sprayer (Amway product, U.S.A). After 10 minutes they were transferred into a clean plastic container (1 L) containing healthy castor leaf dipped with above prepared spore suspension that served as food for caterpillar. Fungi treated leaf was provided daily for a period of four days and everyday the unconsumed leaf was removed. Ten larvae were maintained in each treatment and also in control with four replicates. The mortality was recorded for every 24 h till 96 h.

Statistical analysis: Larval mortality by each concentration of all the three different entomopathogenic fungi was corrected using Abbott (1925) formula. LC_{50}, Chi-square, regression equation, fiducial limit (lower and higher) were calculated (Abbott, 1925). Percentage mortality values were arcsin transformed and then subjected to ANOVA and Tukey test, the significance was expressed at 5% level. All data analysis was performed with SPSS software (11.5 versions).

RESULTS AND DISCUSSION

The insect pathogenic fungi *B. bassiana, V. lecanii* and *P. fumosoroseus* were isolated using serial dilution method and identified using micro and macroscopic characters (Goettel and Inglis, 1997). All the three tested fungi against *P. ricini* isolate tested showed pathogenicity at different degrees. The extensive control efficacy of *B. bassiana* list of hosts like white flies, aphides, grasshoppers, termites, Colorado potato beetle, Mexican been beetle, lepidopteran caterpillars, Japanese beetle, boll weevil, cereal leaf beetle, chinch bug and fire ants (Hazarika and Puzari, 1997; Castrillo *et al*., 2003; Mirshekar *et al*., 2005) have been available in the literature. Both in laboratory and field condition, *P. fumosoroseus* affects *Plutella xylostella* Linn., *Diurophis noxia* and *Bemisia argentifolli* Bellows and Perrung (Cantone and Vandenberg, 1998; Vandenberg *et al*., 1998; Altre *et al*., 1999; Cantone and Vandenberg, 1999; Altre and Vandenberg, 2001a, b).

Percentage of mortality for each isolates at different conidial concentration against the hairy caterpillar *P. ricini* is presented in Fig. 1. From Fig. 1, it is very clear that mortality was increased when the exposure time increased. The LC_{50} values presented in the Table 1 clearly indicated the superiority of *V. lecanii* over *B. bassiana and P. fumosoroseus*. *V. lecanii* (3.9×10^9 spores mL^{-1}) caused 97.33% mortality at 96 h and its LC_{50} value was 1.3×10^8 spores mL^{-1}. Earlier, Gillespie (1998), Fouorier and Brodeur (2000), Ramanujam *et al.* (2002) and Gindin *et al.* (2001) were reported the biological control efficacy of *V. lecanii* on aphids, whitefly and thrips, *S. litura* and *H. armigera* . The 57.3 and 21.7% of test larvae were due to pathogenicity of *B. bassiana* (4.1×10^9 conidia mL^{-1}) and *P. fumosoroseus* (2.7×10^9 conidia mL^{-1}) at 96 h of incubation and their LC_{50} values were 9.6×10^8 and 2.36×10^{11} spores mL^{-1}. Minimal A spore concentration

Table 1: Impact of *B. bassiana, V. lecanii* and *P. fumosoroseus* on the $LC_{50,}$ lower and upper fiducial limits, Chi Square, p values and regression equation against *Pericallia ricini*

Myco-insecticides	LC_{50}	Fiducial limits lower higher		Regression equation	Chi Square	p value arcsin trasformed
Beauveria bassiana	9.6×10^8	9.6×10^7	4.9×10^9	Y=0.980+2.08x	0.14	0.036432
Verticillium lecanii	1.3×10^8	1.3×10^7	3.5×10^9	Y=1.505+1.80x	3.19	0.023090
Paecilomyces fumosoroseus	2.36×10^{11}	2.3×10^{10}	1.3×10^{16}	Y=2.025-1.83x	0.01	0.090584

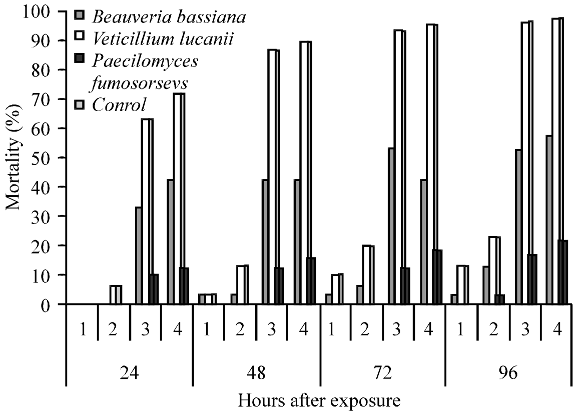

Fig. 1: Mortality caused by *B. bassiana, V. lecanii* and *P. fumosoroseus* at various spore concentrations (1 = 10^6, 2 = 10^7, 3 = 10^8, 4 = 10^9 spores/mL) against *Pericalia ricini*

(10^6 spores mL^{-1}) caused less effect on *P. ricini,* whereas 10^8 spores mL^{-1} caused nearly 50% mortality. Similar results have been reported for *Boophilus microplus* Canestrini (Bittencourt, 2000; Frazzon *et al.*, 2000). It was the general observation of Zhioua *et al.* (1997) which spore density must to reach up to certain threshold for an effective penetration of the cuticle and subsequent causing death of the host. Lower and upper fiducial limits indicate 30 and 90% mortality of *P. ricini* respectively. These values coincided the observations of LC_{50}. For instance, both the lower and upper fiducial limits were minimum and maximum for *P. fumosoroseus* and *V. lecanii,* respectively.

Among the mycopathogens tried against *P. ricini,* the isolates of *V. lecanii* and *B. bassiana* were found to be more virulent, caused significantly higher mortalities, while *P. fumosoroseus* was a weak pathogen. The statistical comparisons were significant by Tukey test at 5% level. *B. bassiana* and *P. fumosoroseus* or *P. fumosoroseus* with *B. bassiana* were significant (P = 0.005; F = 115.7882 and 57.51805) followed by *P. fumosoroseus* and *V. lecanii* (P = 0.000001; F = 42.40260), *B. bassiana* with *V. lecanii* (P = 0.000008; F = 36.60149), V. *lecanii* and *B. bassiana* (P = 0.00011; F = 41.87316) and *V. lecanii* with *P. fumosoroseus* (P = 0.00065; F = 26.27175). Frantz and Mellinger (1998) reported that impact of *B. bassiana* on *Odontotermes brunneus*n was also similar as observed in the present findings. One hundred persent mortality was caused by *B. bassiana* on *Capsium annum*. While the present study revealed that the pathogenicity of *B. bassiana* was moderate against *P. ricini.* Marked correlations analysis was also performed for the percentage mortality caused by *B. bassiana, V. lecanii* and *P. fumosoroseus* to *P. ricini.* The analysis indicates that the comparisons between *B. bassiana* and *V. lecanii* (0.98) was more significant followed by *B. bassiana and P. fumosoroseus* (0.97) and *P. fumosoroseus* and *V. lecanii* (0.96).

The use of biopesticides including entomopathogenic fungi is generally perceived to be ecologically preferable for pest control (Purwar and Sachan, 2006; Scholte *et al.*, 2006; Jaramillo and Borgemeister, 2006). However, biological control carries its own risks (Simberloff and Stilling, 1996), including the potential for damage to non-target organisms. Nontarget effects may be less of a concern when native organisms are used for biological control (Goettel and Hajek, 2001). *B. bassiana, V. lecanii* and *P. fumosoroseus* occurs naturally in soils (Humber, 1992), but they have broad host range, with insect species from seven orders known to be affected (Zimmermann, 1993). Manjula and Padmavathamma (1996) studied the impact of *B. bassiana* to silkworm predators such as *Cheilomenes sexmaculatus*, *Coccinella septempunctata* and *Rhynocoris fuscipus* under laboratory conditions. Latter Haseeb and Murad (1997) reported that C. *septempunctata* was highly susceptible to *B. bassiana* while *Brumoides suturalis* and Syrphids were less susceptible natural enemies. Mesquita and Lacey (2001) have studied the interaction of *Paecilomyces fumosoroseus* and a parasitoid *Aphelinus asychis* and its aphid host. Latter, Nielsen *et al.* (2004) was also studied the compatibility of the pupal parasitoid *Spalangia cameroni* Perkins with *M. anisopliae* under the field trails. Both the studied revealed that fungicides do not affect the natural enemies. Hence it is worthwhile to incorporate them in Integrated Pest Management (IPM) system.

Many workers extensively investigated the field bio-efficacy of the entomopathogenic fungi such as *Beauveria bassiana* (Saxena and Ahamad, 1997; Ramesh *et al.*, 1999; Sandhu *et al.*, 2001; Nahar *et al.*, 2004), *Paecilomyces fumosoroseus* (Shelton *et al.*, 1998; Vandenberg *et al.*, 1998; Altre *et al.*, 1999; Kennedy *et al.*, 2001; Feng *et al.*, 2004) and *Verticillium lecanii* (Jayaraj *et al.*, 1978; Kennedy *et al.*, 2001; Mote *et al.*, 2005). Latin America (Alves and Pereira, 1989) supplies fungal pathogens by high technology methods involving complex equipments and sterile culture condition method in sufficient quantities for markets in its immediate area. These methods are inappropriate for the developing countries, which require production system using cheap, locally available raw materials and equipments (Gopalakrishnan, 2001). Hence study and selection of proper large-scale production technology is essential for this mycoinsecticides to apply in the field conditions in developing countries like India. Wheat bran (Hussey and Tinsley, 1981) and rice grains (Ibrahim and Low, 1993) as a best media for the mass production of *Beauveria bassiana.* Sharma *et al.* (2002) strengthen this finding. Gopalakrishnan *et al.* (1999) tested various cereals, pulses, vegetables, roots, seeds and synthetic medias for the mass multiplication of *Paecilomyces farinosus*. Lakshmi *et al.* (2001) evaluated the whole and broken sorghum, pearl millet and maize for mass culturing of *Verticillium lecanii*. Someway, this mycoinsecticides should be evaluated for their suitability in the field condition against the tested pest.

In conclusion this study portrays *V. lecanii* is a potentially valuable mycopathogen for the management of *P. ricini*. Based on the abiotic nature of India we have selected these myinsecticides. Further necessary work includes obtaining selection of more virulent isolates against a range of pest, findings cost effective and easy ways for the mass production of *V. lecanii* and establishing standard rates and field application techniques.

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