Laboratory evaluation of the entomopathogenic fungi, *Beauveria bassiana* against the Tobacco caterpillar, *Spodoptera litura* Fabricius (Noctuidae: Lepidoptera)

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ABSTRACT

Tobacco caterpillar, *Spodoptera litura* was reared in semi-synthetic diet and the different stages were maintained to conduct bioassay. Entomopathogenic fungi *Beauveria bassiana* was sub-cultured on Potato Dextrose Agar (PDA). Spore suspensions of four different concentrations (2.4×10^7, 2.4×10^6, 2.4×10^5, 2.4×10^4conidia/ml) were prepared from the 15 day old culture of the fungi. A preliminary study on *B. bassiana* against *S. litura* larvae was done. Cypermethrin, neem and untreated (sterile water) were used as controls. The least pupation (43.33%) was observed in larvae treated with the highest spore concentration (2.4×10^7) of *B. bassiana*. A sequential follow up from this assay was done on the resulting pupae and adults, if any. Further treatment of the resultant pupae caused mortality and adult malformation. The healthy moth emergence was least in (2.4×10^4) spore concentration of the treatment, while the fecundity was completely arrested in the highest concentration.

Key words: *Beauveria bassiana, Spodoptera litura,* spore, mortality

INTRODUCTION

The tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is a polyphagous sporadic pest with high mobility and reproductive capacity (Holloway, 1989). It is widely distributed throughout tropical and temperate Asia, Australasia and Pacific Islands (Mohammad Monobrullah and Uma Shankar, 2008). *Spodoptera litura* devastates a large host range of more than 120 host plants (Ramana *et al*., 1988). The major ones include tobacco, cotton, groundnut, jute, lucerne, maize, rice, soybeans, tea, cauliflower, cabbage, capsicum, potato and castor (Sharma and Bisht, 2008).

Several outbreaks of this pest on cotton, tobacco and chillies have been reported in Tamil Nadu (Rao *et al*., 1983) and the economic loss range between 25.8-100% depending upon the crop stage and its infestation level (Dhir *et al*., 1992). Its resistance against almost all the insecticide groups (Armes *et al*., 1997; Kranthi *et al*., 2002) including the new chemistry insecticides like lufenuron (Sudhakaran, 2002) and the adverse effects due to synthetic pesticides on pests and their subsequent impacts to ecological imbalance (Zadoks and Waibel, 1999) demands sustainable alternatives (Parmar, 1993). Microbial pesticides are one such alternative to tackle insecticide resistant population of *S. litura* (Pawar and Borikar, 2005).

Fungal pathogen particularly, *Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii* and *Nomuraea rileyi* have been found to be promising in the control of several agricultural pests (Lingappa *et al*., 2005). *B. bassiana* is a hyphomycete insect-pathogenic fungus in the subdivision Deuteromycotina which occurs worldwide. Over 200 species of insects in nine orders, mainly Lepidoptera and Coleoptera have since been recorded as hosts (Li and Yang, 1988). It is found naturally on some plants and in soils and is regarded as a safe biopesticide (Uma Devi *et al.*, 2008).

B. bassiana was found effective against *S. litura* (Gopalakrishnan and Narayanan, 1989), the sweet potato weevil, *Cylas formicarius*, the termite *Odontotermis brunneus* and *O. obesus* (Khader Khan *et al.*, 1993). The efficacy of *B. bassiana* against the diamondback moth, *Plutella xylostella* in greenhouse and the field proved that the fungal spores significantly affected larval survival, indicating the potential for using the entomopathogen against this pest (Vandenberg *et al.*, 1998).

Laboratory bioassays of *B. bassiana* against several life stages of the pine beauty moth, *Panolis flammea* resulted in high mortality in the fifth instar larvae. Similarly, *B. bassiana* sprayed against the field populations of the grasshopper, *Melanoplus sanguinipes* (Fabricius) resulted in high rates of population decline (Askary *et al.*, 1998).

Efficacy of *B. bassiana* against the larvae, pupae, and adult females of the Mexican fruit fly, *Anastrepha ludens* (Loew) showed high levels of mortality were obtained for adult flies (Daglish, 1998). Some of the major economic insect pests that are susceptible to this fungus are European corn borer, *Ostrinia nubilalis*; codling moth, *Laspeyresia pomonella*; Japanese beetle, *Popillia japonica*; Colorado potato beetle, *Leptinotarsa decemlineata* chinch bug, *Blissus leucopterus*; and the European cabbageworm, *Pieries brassicae* (Tanada and Kaya, 1993).

Though *B. bassiana* has been studied against various insect pests in both green house and field, an in-depth literature survey proves that the fungi have not been studied in detail against *S. litura*. Hence, the present study aimed to explore the pesticidal activity of the fungi against the target pest.

MATERIALS AND METHODS

Collection and rearing of pest

S. litura larvae were collected from infested castor plants from Kannivadi in Dindigul District., Tamil Nadu, India. The larvae collected from castor were maintained in the laboratory at 22 ± 2°C and 70 – 75 % relative humidity (RH). The larvae were reared both on castor and semi-synthetic diet in individual containers to prevent contamination (Santharam, 1985).

Fungal source

Beauveria bassiana culture was obtained from Project Directorate of Biological Control (PDBC), Bangalore.

Bioassay

Spore suspension was prepared from 15 days old culture of *B. bassiana* on PDA medium. The fungal surface was scraped using a sterile loop with 10 ml of sterile distilled water having 0.02% Tween 80 as a wetting agent (Rombach *et al.*, 1986). The suspension was then filtered through sterile muslin cloth to eliminate the medium (Sasidharan and Varma, 2005). Spore concentration of the filtrate was determined using a Neubauer Hemocytometer. This served as the stock suspension. Different spore concentration was prepared by adding sterile 0.02% Tween 80 in distilled water. Spore suspension of *B. bassiana* at four different concentrations, 2.4×10^7, 2.4×10^6, 2.4×10^5 and 2.4×10^4 spores/ml was prepared and tested for its efficacy on third instar larvae, pupae and adults of *S. litura*.

Growth inhibition of larvae (Hafez *et al.*, 1994)

For bioassay, spraying method was adopted. Nine ml of different spore concentrations of *B. bassiana* was sprayed against *S. litura* larvae. Ten larvae were used per replication. The larvae were treated with sterile distilled water and 0.006 % (v/v) of neem product and cypermethrin. These three served as positive control. After treatment, the larvae were allowed to feed on semi-synthetic diet. Each treatment was replicated thrice. Growth parameters namely larval duration (days), larval length, larval weight and pupation (%) were recorded.

Growth inhibition of pupae (Hafez *et al.*, 1994)

Four different spore concentrations of *B. bassiana* with three replications each were used for infecting the pupa of *S. litura*. The pupae were sprayed with 10 ml of respective fungal spore suspensions using hand atomizer. The pupae were treated with sterile distilled water and 0.006 % (v/v) neem product and cypermethrin. These three served as positive control. The growth of surviving pupa was recorded up to adult emergence for the parameters such as pupal duration (days), pupal weight (mg), pupal length (cm) and adult emergence (%).

Adult longevity, fecundity and egg hatchability

(Malarvannan, 2004)

Healthy adults were released into mud pots at 1:1 male-female ratios. Cotton swabs dipped in 10% honey treated with 1 ml of the test fungi served as treatment. The experiment was performed using four different spore concentrations of the test fungi, *B. bassiana*. Cypermethrin, Neem and Untreated served as controls. Adult longevity (days), fecundity (numbers) and hatchability (%) were recorded. Triplicates were maintained for each treatment and the data were analyzed statistically using Agres package version 4 and SPSS version 9.

RESULTS AND DISCUSSION

Larval growth

Among the different fungal concentrations, the least pupation was noticed in 2.4×10^7 and 2.4×10^4 (43.33%) as against 100% in untreated (Table 1). The variation between different treatments was significant. In addition, the fungal growth was observed on the larvae (Plate 1), which confirms the efficacy of the biocontrol agent. The larval mortality was observed 3-7 days after the fungal treatment. With entomophthoralean fungi, unicellular yeast-like cells with chitinous walls (hyphal bodies) spread throughout the insect obtaining nutrients, leading to the death of the host by physiological starvation about 3-7 days after infection (Shah and Pell, 2003).

The larval length ranged from 2.7– 3.9 cm in the different treatments. The least (2.7 cm) was recorded in 2.4×10^5 spore concentration as against the highest in the untreated

Table 1. Effect of *B. bassiana* on growth of *S. litura* larvae

Treatments		Larval growth parameters			
		Larval length (cm)	Larval weight (mg)	Larval duration (days)	Pupation (%)
Control	Untreated	3.9^{c}	396.2^{c}	7.9^{d}	100.0^{b}
	Cypermethrin	3.0^{a}	340.5^{a}	7.3^{a}	36.7^{a}
	Neem Plus	3.1^{b}	346.2^{a}	7.8^{ab}	40.0^{a}
B.bassiana (spores/ml)	2.4 x 10^{4}	2.8^{a}	361.2^{ab}	7.6^{bc}	43.3^{a}
	2.4 x10^{5}	2.7^{a}	357.0^{ab}	7.7^{bc}	46.7^{a}
	2.4 x 10^{6}	2.9^{a}	366.9^{b}	7.3^{a}	50.0^{a}
	2.4 x 10^{7}	3.0^{a}	367.5^{b}	7.5^{ab}	43.3^{a}
CD (P = 0.05)		0.3	29.1	0.32	22.2

Each value mean of triplicate, Different letters in each column differ significantly (5%) by LSD

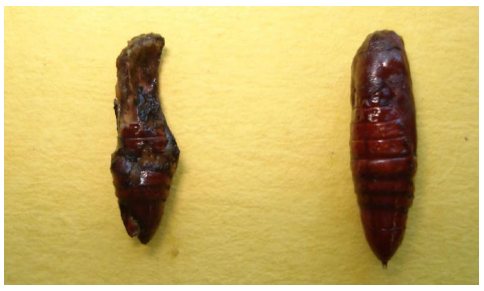
(3.9 cm) (Table 1). Similarly, among the fungal treatments, the larval weight was least (357.0 mg) in 2.4 × 10^{5} treatment compared to untreated 396.2 mg (Table 1).The larval duration was 7.3 days in 2.4 × 10^{6} as against the untreated (7.9 days) (Table 1).

Plate 1. Effect of *B. bassiana* on the growth and development of *S. litura* larva.

Pupal growth

The pupal weight ranged from 163.5 to 205.1 mg. Comparison between the different fungal treatments revealed that least pupal weight (184.1 mg) was recorded in 2.4 x 10^{5} spore concentration/ml as against the untreated (205.1 mg) (Table 2). Malformed pupal stages were observed in the fungal treatments (Plate 2).The results varied significantly at 5% level (Table 2). In general there was not much variation in the pupal length. It was least (1.3 cm) in 2.4 x 10^{5}, 2.4 x 10^{6} and 2.4 x 10^{7} spore concentrations which was on par with cypermethrin (1.3 cm). This suppressive effect may be due to the inhibitory action on mitochondrial respiration by affecting the NADH- Cytochrome C-reductase and complex-I of insect mitochondria (Londershausen *et al*., 1991).

The pupal duration was 8.0 days in 2.4 × 10^{5} as against the untreated (8.5 days) (Table 2). In contrast, the pupal duration was prolonged in *B. bassiana* treated pupae of *Phthorimaea operculella* compared with the control (Hafez *et al.*, 1994). Among the different treatments, healthy moth emergence was severely affected in larvae treated with cypermethrin (0%) followed by neem (11.1%) and *B. bassiana* at 2.4×10^{4} (13.1%) (Plate 3). The highest percentage (80.0%) of healthy moth emergence was recorded in larvae maintained on normal diet (Table 2). Significant reduction in pupation with larval–pupal intermediates (due to phagodepression and difficulty in moulting) was observed (Tables 1-2).



a b

a- deformed pupa; b- healthy pupa

Plate 2.Activity of *B. bassiana* on *S. litura* pupa

Table 2. Effect of *B. bassiana* on growth of *S. litura* pupae

Treatments		Pupal growth parameters			Adult emergence	
		Pupal Weight (mg)	Pupal Length (cm)	Pupal Duration (Days)	Healthy (%)	Malformed /Dead Pupa (%)
Control	Untreated	205.1^{c}	1.5^{c}	8.5^{b}	80.0^{d}	20.0^{d}
	Cypermethrin	167.6^{a}	1.3^{a}	0.0^{a}	0.0^{a}	100.0^{a}
	Neem	163.5^{a}	1.4^{b}	9.0^{b}	11.1^{b}	88.89^{ab}
B.bassiana (spores/ml)	2.4 x 10^{4}	186.3^{b}	1.4^{b}	8.7^{b}	13.1^{b}	86.6^{ab}
	2.4 x10^{5}	184.1^{b}	1.3^{a}	8.0^{b}	36.1^{c}	63.9^{c}
	2.4 x 10^{6}	204.5^{c}	1.3^{a}	8.5^{b}	27.8^{c}	72.2^{bc}
	2.4 x 10^{7}	189.3^{b}	1.3^{a}	8.5^{b}	25.0^{c}	75.0^{bc}
CD (P = 0.05)		23.6	0.04	5.9	23.0	23.1

Each value mean of triplicate, Different letters in each column differ significantly (5%) by LSD

The decrease in the juvenile hormone titre and its associated disturbances in oogenesis, larval-pupal and pupal-adult moults are interpreted as an interference with moulting hormone pools (Rembold *et al.*, 1982). Decrease in juvenile hormone influences the storage proteins and fat which are highly essential for metamorphosis, moulting and reproduction (Palli and Locke, 1987; Koul and Isman, 1991).

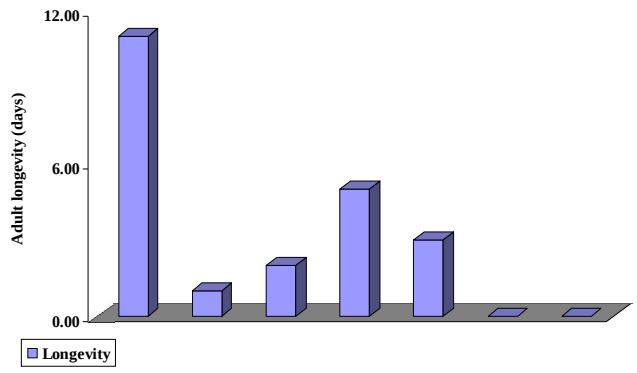
a b

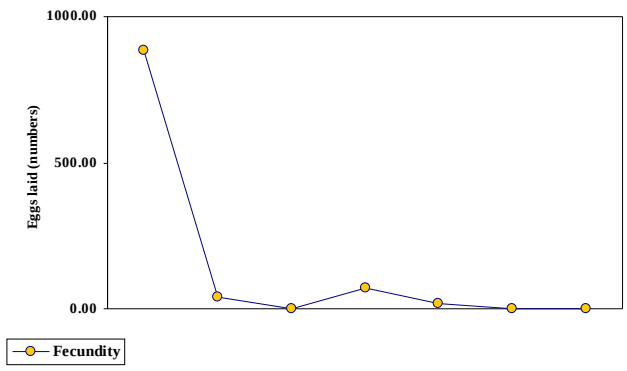
a- malformed adult; b- healthy adult

Plate 3. Effect of *B. bassiana* on *S. litura adult*

Adult longevity

In general, the longevity of adult varied from 0 to 11 days between treatments. With the honey solution the adults lived longer (11 days) (Figure 1). In fungal treatments, an early adult mortality (0.0 days) was observed with 2.4×10^7, followed by cypermethrin (2.0 days). The longevity of adult males of *Phthorimaea operculella* was reduced to 9.3 days at 16.5×10^8 conidia/ml of *B. bassiana* as compared to 12.9 days in the control (Hafez *et al.*, 1994).





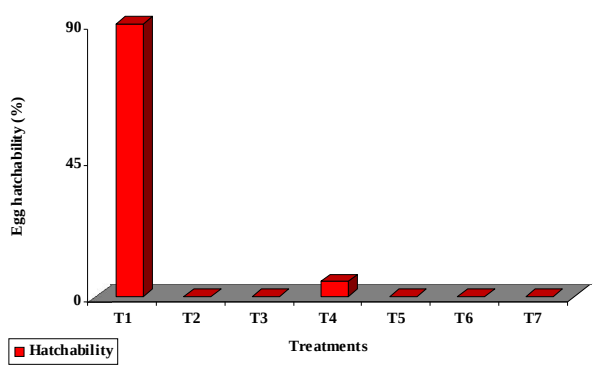


Figure 1. Effect of *Beauveria bassiana* on growth of *S. litura* adults

T1- Control (10% honey solution); T2- cypermethrin; T3- neem plus; T4- 2.4×10^4; T5 – 2.4×10^5; T6- 2.4×10^6; T7- 2.4×10^7

Fecundity

In general, there was a wide variation in the fecundity among different treatments. Few treatments *viz.*, 2.4×10^7, cypermethrin and neem arrested the fecundity completely. The red palm weevil adults, *Rhynchophorus ferrugineus* when treated with *B. bassiana* increased egg mortality and reduced their hatchability. The total percentage mortality of eggs and hatched larvae was 80-82% (Gindin *et al.*, 2006).

Egg hatchability

The egg hatchability was suppressed in most of the treatments. The highest hatchability percentage (90.0) was recorded with normal control, whereas it was nil in 2.4×10^7 spore concentration, cypermethrin and neem. Khodadad *et al*. (2007) reported similar results of remarkable effects of *M. anisopliae*, *B. bassiana* and *L.*

psalliotae on the egg hatchability (%) and reproductive efficiency of *Rhipicephalus* (Boophilus) *annulatus*.

The experimental results proved that the biopesticides, particularly microbial pesticides can be used as an alternate control method in combating the pest. Its wide application as a biological pesticide could be taken up after exploring its toxicity and field trials.

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