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Larvicidal and Growth Inhibitory Activities of Entomopathogenic Fungus, *Beauveria bassiana* against Asian Army Worm, *Spodoptera litura* Fab. (Lepidoptera: Noctuidae)

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ABSTRACT

Larvicidal and growth inhibitory activities of ten different isolates of *Beauveria bassiana* from Pulney hills of Western Ghats of Tamil Nadu, India, were evaluated at four different concentrations against the third instar larvae of *Spodoptera litura*. The *B. bassiana* (Bb10) isolate showed maximum larvicidal activity of 68.06%, minimum pupal weight of 183 mg, low number of adult emergence (22.91%) and 100% abnormalities at 10^8 spore mL^{-1} concentration. Similarly, the same isolate Bb10 at all the concentrations tested had statistically significant activity. Dose dependent activities were also noticed in all the tested isolates. Isolate 0 Bb 10 could be a useful alternative to synthetic chemical pesticides.

Key words: Larvicide, adult abnormalities, pupal weight, *Beauveria bassiana*, *Spodoptera litura*

INTRODUCTION

Beauveria bassiana is a virulent, ubiquitous, entomopathogenic and hyphomycete fungus with a very wide range of insect pests (Tanada and Kaya, 1993). It is a resident of soil (Klingen *et al.*, 2002) and has a semelparous life history with a single reproductive episode. This entomopathogenic fungus is the most promising group of biological control agent against insect pests (Lacey and Goettel, 1995) like scarabs and weevils (Keller, 2000) without harming the non target organisms (Goettel and Hajek, 2001). This virulent fungus initially infects and later releases all its infective propagules (conidia) in a single spell after the death of the host insects (Bartlett and Jaronski, 1988; Wraight *et al.*, 2001). Theoretically speaking a single conidium is enough to start infection, but it depends on the hyphal density to ensure a distinct mortality in the host pests under favourable conditions. *B. bassiana* is able to penetrate into the host integument which completely encloses the host body within a chitinous cuticle and develops within the hemocoel. Successful invasion and propagation within a host is determined by the ability of the fungus to enter the hemocoel and to suppress or avoid the host's immune response (Gotz, 1991). A promising and potential strategy would be developed to control the insect pests and at the same

time, to minimize the adverse effects of chemical insecticides on the fungus used as biocontrol agent as well as other microbial agents. Currently, 66 products representing at least 38 taxonomically diverse species or varieties of entomopathogenic fungi have been developed or are being developed (Liu and Li, 2004).

The tobacco cutworm *Spodoptera litura* is a sporadic, polyphagous and cosmopolitan pest. It is a serious pest causing enormous losses to many economically important crops such as cotton, soybean, groundnut, tobacco and vegetables (Qin *et al.*, 2004). The main plant families infested are Cruciferae, Cucurbitaceae and Leguminosae. It has a wide range of hosts, feeding on 112 plant species worldwide, of which 40 species are known from India (Singh *et al.*, 1998). To control these pests the farmers in India have been relying heavily on synthetic pyrethroid insecticides, because of their contact mode of action and quick knockdown effect.

The continuous and indiscriminate use of insecticides over the years has resulted in the development of resistance to certain molecules belonging to different classes of insecticides in different parts of the world (McCaffery, 1998). In course of time the host insects had adapted to the fungus *B. bassiana* and developed effective immune systems composed of both cellular and humoral responses (Boman and Hultmark, 1987) for resisting different microbial agents including *B. bassiana*. To overcome these resistances it is stated that the infection dynamics of a semelparous parasite with clustering of all its (infective) propagules should conform to the 'mass action principle' (Hughes *et al.*, 2004) wherein, infection rate is a linear function of the density of parasites and susceptible hosts. On the contrary, parasites with an iteroparous life history which have repeated spells of reproduction and a serial transmission of their infective units can initiate infection only with a high dose of infective propagules. Such parasites display a so-called 'Allele effect' (Regoes *et al.*, 2002) a phenomenon that is characterized by an invasion threshold for the parasite, i.e., the parasite population can establish an infection only if its native population size exceeds the invasion threshold (Regoes *et al.*, 2002).

With a view to minimize the usage of chemical insecticides and to develop an ecofriendly pesticide, the present study was undertaken to find the efficacy of different isolates of *B. bassiana* against *S. litura*.

MATERIALS AND METHODS

Collection and isolation of the *B. bassiana*: Ten isolates of *B. bassiana* was isolated from coffee berry borer (*Hypothenemus hampei*) which were naturally infected with *B. bassiana*. *H. hampei* infested berries were collected from ten different locations (Table 1) in Pulney hills. The cadaver showing white mycosis, were individually surface sterilized with 0.1% mercuric chloride for one minute and rinsed thrice in sterile water and the specimens were aseptically transferred to Sabouraud dextrose agar supplemented with 0.2% yeast extract (SDYA) and incubated at 25±2°C for 14 days in 12:12 photo period. The fungal isolates were identified based on the identification key out lined by Humber (1997). The established fungal spores were stored in 20% glycerol at -20°C.

Pathogenicity of *B. bassiana* against *S. litura*: The spores were revived on SDYA medium and incubated at 25±2°C until they developed dense sporulation (14 to 15 days). The spores were scraped with spatula and kept in sterile water containing 0.05% of Tween 80. The desired spore concentration was prepared using a haemocytometer. Hundred microlitre of each spore concentration (1x10⁵, 1x10⁶, 1x10⁷ and 1x10⁸) was applied topically on third instar larva using a micro pipette. Larvae treated with 0.05% of Tween 80 in water served as a control. Individually treated larvae were transferred to 90 mm petri dishes and fed with Castor leaves. Four replicates

were maintained for each treatment with 10 larvae per replicate (total n = 40). The dead larvae were transferred to humid chamber and incubated for five days and observed. Only those larvae covered with white mycelia and spores were considered for per cent mortality. The experiment was conducted in laboratory condition (27±2°C) with 14:10 photoperiod and 75±5% relative humidity. Percent mortality was calculated according to Abbott (1925):

$$\text{Abbott's corrected mortality} = \frac{\% \text{mortality in treatment} - \% \text{mortality in control}}{100 - \% \text{mortality in control}} \times 100$$

Pupal weight: After pupation of the treated larvae, their weight were calculated.

Adult emergence: Adult emergence was calculated by subtracting the 84 number of emerging adults from the total number of pupae.

Adult abnormalities: Percentage of adult abnormalities was calculated from the total number of adults emerged.

Statistical analysis: The data related to larvicidal, pupal weight, adult emergence, adult abnormalities were analysed using one way ANOVA. Significant differences between treatments were determined using Tukey's multiple range test (p = 0.05).

RESULTS

The larvicidal activity: In the present study, ten different isolates of *B. bassiana* were tested against *S. litura*.

After 24 h of treatment, no isolate exhibited larval mortality. The mortality was seen on fourth day after treatment. Maximum larvicidal activity of 68.60% was observed in Bb10 followed by Bb6 which showed mortality of 50.27% at 10⁸ spore mL⁻¹ concentration. Statistically significant activity was observed in the isolate Bb10 at all the concentrations. Least activity was observed in Bb7 at 10⁸ spore mL⁻¹ concentration. Bb4 did not reveal any larvicidal activity (Table 2).

Pupal weight reduction: Maximum reduction in pupal weight (183 mg) was observed in Bb10 at 10⁸ spore mL⁻¹ concentration. It was statistically significant from other treatments (Table 3). The

Table 1: *B. bassiana* isolates infected insect host *H. hampei* collected from different locations in Pulney hills of Western Ghats in Tamil Nadu

Isolate code (Bb)	Collected place
Bb1	Thandigudi
Bb2	Solai Kadu
Bb3	N.T.N. Estate
Bb4	Patalankadu
Bb5	Good will
Bb6	N.S.V. Estate
Bb7	Muthiah Estate
Bb8	Manjalparappu
Bb9	Pannerkarantai
Bb10	Kuppammalpatti

Bb: *Beauveria bassiana*

Table 2: Larvicidal activity (%) of *B. bassiana* against *S. litura*

Isolates	Spore concentrations (mL ⁻¹)			
	10 ⁵	10 ⁶	10 ⁷	10 ⁸
1	0.00±0.00 ^a	0.00±0.00 ^c	10.55±0.64 ^{de}	21.11±1.28 ^{ef}
2	8.05±5.39 ^{bc}	15.55±5.13 ^b	26.11±4.49 ^{bc}	34.16±4.19 ^{cd}
3	13.33±5.94 ^{ab}	18.33±4.92 ^b	29.16±6.30 ^b	39.44±4.58 ^c
4	0.00±0.00 ^a	0.00±0.00 ^c	0.00±0.00 ^f	0.00±0.00 ^g
5	13.33±5.94 ^{ab}	16.11±7.05 ^b	26.11±4.49 ^{bc}	42.22±2.56 ^{bc}
6	12.78±4.84 ^{ab}	21.11±1.28 ^b	34.16±4.19 ^b	50.27±7.33 ^b
7	0.00±0.00 ^f	0.00±0.00 ^c	0.00±0.00 ^f	10.55±0.64 ^{de}
8	0.00±0.00 ^f	0.00±0.00 ^c	70.77±5.21 ^{ef}	12.78±4.84 ^f
9	0.00±0.00 ^f	15.83±6.17 ^b	18.33±4.92 ^d	23.61±4.38 ^d
10	21.11±1.28 ^a	34.44±6.84 ^a	55.27±4.09 ^a	68.60±7.38 ^a

Within the columns, Means±SD followed by the same letter do not differ significantly using Tukey 's test at p≤0.05

Table 3: Pupal weight (mg) of *S. litura* after treatment of different isolates of *B. bassiana*

Isolates	Spore concentrations (mL ⁻¹)			
	10 ⁵	10 ⁶	10 ⁷	10 ⁸
1	290.25±4.11 ^{bc}	278.00±6.0 ^{cd}	270.25±5.85 ^{cd}	255.5±3.870 ^e
2	273.50±7.410 ^{de}	268.25±5.85 ^d	260.25±3.09 ^{de}	250.25±2.12 ^{ef}
3	271.75±5.96 ^{ef}	261.00±1.83 ^d	253.50±5.320 ^{ef}	248.00±4.97 ^e
4	309.25±2.75 ^a	306.25±5.38 ^{ab}	299.75±5.43 ^b	296.50±1.730 ^b
5	268.50±3.100 ^{ef}	260.25±3.77 ^d	250.00±3.66 ^{ef}	245.75±1.75 ^f
6	261.75±3.77 ^f	258.75±4.19 ^d	246.25±6.07 ^f	223.00±3.92 ^g
7	283.25±3.30 ^{cd}	279.00±1.14 ^c	273.25±1.89 ^c	271.00±4.240 ^d
8	299.00±2.93 ^b	297.5±2.640 ^b	279.50±3.410 ^c	277.75±3.59 ^c
9	287.75±2.12 ^c	283.25±2.87 ^c	277.50±2.640 ^c	268.50±1.910 ^d
10	240.50±4.930 ^f	219.00±2.94 ^e	206.75±5.85 ^e	183.25±2.75 ^b
Control	312.75±2.36 ^a	312.75±2.36 ^a	312.75±2.36 ^a	312.75±2.36 ^a

Within the columns, Means±SD followed by the same letter do not differ significantly using Tukey 's test at p≤0.05

control pupa showed 312 mg weight which is statistically on par with 10⁵ spores mL⁻¹ concentration of the Bb4. Pupal weight was reduced drastically when treated with higher concentration. The isolates Bb3, Bb5 and Bb6 showed less than 250 mg pupal weight at 10⁸ spores mL⁻¹.

Adult emergence: Adult emergence of *S. litura* was reduced, when the larvae were treated with different isolates of *B. bassiana*. Minimum (22.91%) adult emergence was noticed in Bb10 at 10⁸ spore mL⁻¹ concentration (Table 4). Maximum number of adult emergence (77.5%) was observed in Bb4. Fifty percent reduction was observed in Bb2, Bb3, Bb5, Bb6 and Bb9. Bb10 showed statistically significant activity at all the tested concentrations of the other isolates. Least activity was found in Bb4.

Adult abnormalities: The fungus of *B. bassiana* caused adult abnormalities in *S. litura*. When the pupae emerged to adults; different abnormalities occurred viz., crumpled wing, wingless adults, irregular antennae and small size adults at the different treatments. Cent percent abnormal adults were noticed in Bb10 at 10⁸ spore mL⁻¹ concentration (Table 5). Least abnormalities of 16.66% were

Table 4: Adult emergence (%) of *S. litura* after treatment of different strain of *B. bassiana*

Isolates	Spore concentrations (mL ⁻¹)			
	10 ⁵	10 ⁶	10 ⁷	10 ⁸
1	89.44±0.64 ^a	76.38±4.38 ^{bc}	67.70±5.24 ^{bcd}	56.69±5.12 ^c
2	69.16±7.26 ^b	59.37±6.25 ^{def}	53.57±7.14 ^{def}	48.21±3.57 ^{cd}
3	64.07±6.72 ^b	51.78±3.57 ^{ef}	48.21±3.57 ^{ef}	47.50±5.00 ^{cd}
4	95.00±5.77 ^a	86.38±5.76 ^{ab}	80.00±8.16 ^b	77.50±5.00 ^b
5	72.37±7.22 ^b	59.12±5.08 ^{def}	53.57±7.14 ^{def}	45.00±5.77 ^{cd}
6	70.83±4.81 ^b	50.00±5.83 ^f	44.04±7.89 ^f	37.08±10.57 ^{de}
7	73.88±4.49 ^b	68.33±1.92 ^d	60.55±4.58 ^{de}	55.90±5.11 ^c
8	87.22±4.84 ^a	79.44±1.11 ^{bc}	69.58±3.93 ^{bc}	61.80±4.60 ^{bc}
9	71.11±4.71 ^b	62.99±6.50 ^{de}	54.91±6.07 ^{def}	44.64±3.57 ^{cd}
10	46.42±4.12 ^c	39.76±4.49 ^f	28.75±7.50 ^f	22.91±15.77 ^e
Control	97.50±5.00 ^a	97.50±5.00 ^a	97.50±5.00 ^a	97.50±5.00 ^a

Within the columns, Means±SD followed by the same letter do not differ significantly using Tukey's test at p≤0.05

Table 5: Abnormalities (%) seen in *S. litura* due to the effect of different isolates of *B. bassiana*

Isolates	Spore concentrations (mL ⁻¹)			
	10 ⁵	10 ⁶	10 ⁷	10 ⁸
1	0.00±0.00 ^f	0.00±0.00 ^f	17.05±1.66 ^{de}	23.75±2.05 ^{de}
2	0.00±0.00 ^f	21.25±2.5 ^b	27.08±4.16 ^c	41.66±16.66 ^{bcd}
3	14.16±9.57 ^b	18.75±12.5 ^b	31.25±4.16 ^{bc}	54.16±15.95 ^{bc}
4	0.00±0.00 ^f	0.00±0.00 ^f	12.59±1.30 ^e	25.89±1.78 ^{de}
5	17.14±9.57 ^b	21.66±4.08 ^b	27.08±4.16 ^c	41.66±9.62 ^{bc}
6	12.50±8.33 ^b	27.08±4.16 ^b	37.05±8.03 ^b	62.05±25.00 ^b
7	0.00±0.00 ^f	0.00±0.00 ^f	0.00±0.00 ^f	0.00±0.00 ^f
8	0.00±0.00 ^f	0.00±0.00 ^f	0.00±0.00 ^f	0.00±0.00 ^f
9	0.00±0.00 ^f	0.00±0.00 ^f	23.75±2.5 ^d	31.025±4.16 ^d
10	41.66±9.62 ^a	58.33±9.62 ^a	100.00±0.00 ^a	100.00±0.00 ^a
Control	0.00±0.00 ^f			

Within the columns, Means±SD followed by the same letter do not differ significantly using Tukey's test at p≤0.05

observed in Bb4 at higher concentrations. Isolates Bb7 and Bb8 did not show any malformation. The isolates, Bb1, Bb4, Bb7, Bb8 and Bb9 did not show any abnormality at 10⁵ and 10⁶ spore mL⁻¹ concentrations.

The present study revealed that different isolates exhibited biological activity; among them isolate Bb10 showed the highest larvicidal activity, maximum pupal weight reduction, adult abnormalities and reduction in adult emergence.

DISCUSSION

In the present study *B. bassiana* served as an (bio) insecticidal agent against *S. litura*. *B. bassiana* specimen collected from ten different locations in Pulney hills exhibited larvicidal activity at level ranging from 0-68.60%. The present finding coincides with findings of Sabbour and Sahab (2005) who found that *B. bassiana* exhibited larvicidal activity against *Plutella xylostella*, *Pieris rapae* and *S. exigua*. Similarly other biological agents like *Bacillus thuringiensis* reduced the attack of *S. litura* on groundnut (Krishna *et al.*, 2008). Ahmad *et al.* (2005) reported that soil

derived different microorganism exhibited larvicidal activity against *S. litura*, among them emamectin had maximum larvicidal activity. Similarly *B. bassiana* and *Metarhizium anisopliae*, exhibited larvicidal activity of 98.7 and 85.2%, respectively against *Aproaerema modicella* (Kempraj and Gopalan, 1999). Gillespie (1986) observed that *B. bassiana* exhibited mortality of 85% against *Nilaparvata lugens* at 7th day after treatment. Samuel *et al.* (2009) reported that *B. bassiana* exhibited more than 80% insecticidal activity against *H. hampei*. The fungus, *B. bassiana* at different concentrations of 10^3 , 10^4 , 10^5 , 10^6 , 10^7 and 10^8 mL⁻¹ showed insecticidal activity of 5-33% (Balasubramaniam and Sundaresan, 2009) on *H. hampei*. Jayanthi and Padmavathamma (2001) have reported the individual and combined larvicidal activity of *Bacillus thuringiensis*, nucleopolyhedrovirus and *B. bassiana* against *S. litura*. Maximum larvicidal activity of 90% was observed in *B. thuringiensis*, NPV had 66.67% and *B. bassiana* showed 46.67% and also their combination had 70% in NPV+ *B. bassiana*, NPV+ *B. thuringiensis* showed 90% and *B. bassiana* + *B. thuringiensis* had 80% larval mortality. On the other hand *B. bassiana* and *Nomuraea rileyi* showed more than 90% larval mortality against *H. armigera* (Devi *et al.*, 2003). Hung and Bouicas (1992) reported that *B. bassiana* reduced the population of *S. exigua*.

In the present study it is reported that *B. bassiana* reduced 22.91% adult emergence in host insect, when compared to control which showed 97.50%. Similarly, Anand *et al.* (2009) reported that three different soil born entomopathogenic fungi reduced adult emergence of *S. litura*. Two strains of *B. bassiana* exhibited 50% adult emergence (Ahemed and El-Katatny, 2007). *Galleria mellonella* adult emergence was reduced when the larvae were treated with *B. bassiana* with gamma radiation at increasing concentrations (El-Sinary and Rizk, 2007).

It is also found that isolates of *B. bassiana* treated insect larvae exhibited different abnormalities and this finding is in concordance to reports of Ahemed and El-Katatny (2007) who also observed the abnormalities in *B. bassiana* treated *S. littoralis*. Adult abnormalities of 75-86% were observed in *S. litura*, when they were treated with *B. bassiana* (Malarvannan *et al.*, 2010). Shanthakumar *et al.* (2010) reported that *Nomuraea rileyi* exhibited adult abnormalities against *S. litura*.

In this study different isolates of *B. bassiana* reduced the pupal weight of *S. litura*. Similarly Malarvannan *et al.* (2010) reported that *B. bassiana* reduced the pupal weight of *S. litura* at different concentrations and on the other hand *Nomuraea rileyi* reduced the pupal weight of *S. litura* (Shanthakumar *et al.*, 2010).

CONCLUSION

The present study clearly indicated that *Beauveria bassiana* (Bb10) was a potent biocontrol agent, which can be mass produced and applied in the field, since it showed maximum larvicidal activity, higher abnormalities, reduced pupal weight and adult emergence in *S. litura*.

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