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Research Article

Efficacy of local isolates of *Beauveria bassiana* against *Spodoptera litura* (F.) (Lepidoptera: Noctuidae)

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ABSTRACT: Isolates of the entomopathogenic mitosporic ascomycete, *Beauveria bassiana* were evaluated against *Spodoptera litura* using leaf spray method. Mortality of 66.67, 73.33 and 80.0% with respect to the isolates Bb_{02}, Bb_{09} and Bb_{10}, respectively, was obtained four days after treatment. LC_{50} of the isolates was $2.1x10^6$, $3.6x10^7$ and $1.2x10^7$ conidia ml^{-1} for Bb_{02}, Bb_{09} and Bb_{10}, respectively. The LT_{50} value for Bb_{02} and Bb_{09} was 4.8 days, whereas it was 4.0 days for Bb_{10} @10^8 spore mL^{-1}. The germination percentage was 79.83, 88.33 and 95.53% for Bb_{02}, Bb_{09} and Bb_{10}, respectively Bb_{10} isolate was the most virulent with potential for the management of *S. litura*.

KEY WORDS: *Beauveria bassiana*, *Spodoptera litura*, germination, LC_{50}, LT_{50}, bioassay

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INTRODUCTION

Tobacco caterpillar, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae), is a polyphagous pest with high mobility and reproductive capacity (Holloway, 1989). It is widely distributed throughout tropical and temperate Asia, Australia and Pacific islands (Monobrullah and Uma Shankar, 2008). The major host plants for *S. litura* include tobacco, cotton, groundnut, jute, lucerne, maize, rice, soybeans, tea, cauliflower, cabbage, capsicum, potato, castor, etc. (Sharma and Bisht, 2008). Several outbreaks of this pest on cotton, tobacco and chillies have been reported in Tamil Nadu (Rao *et al*., 1983). Outbreak of this pest has been attributed to resistance to insecticides, favorable weather conditions, cyclonic weather and heavy rainfall after a long dry spell (Thanki *et al*., 2003).

The entomopathogenic fungus, *Beauveria bassiana* displays a broad host range. More than 200 insect species from nine orders of insects, mainly Lepidoptera and Coleoptera have been recorded as hosts (De la Rosa *et al.,* 2000). *B. bassiana* spore is found naturally on some plants and soils and is regarded as a safe biopesticide (Uma Devi *et al*., 2008). Malarvannan *et al*. (2010) found 56.67% pupation reduction in *S.litura* with $2.4x10^7$ spore ml^{-1} concentration. The aim of this work was to evaluate the efficacy of *B. bassiana* isolates from different geographical regions of Tamil Nadu against *S. litura* in the laboratory.

MATERIALS AND METHODS

Beauveria bassiana (Bb_{02}, Bb_{09} and Bb_{10}) isolates used in this investigation were isolated from different regions of Madurai and Dindigul District, Tamil Nadu and routinely grown on potato dextrose agar (PDA). The plates were incubated at 26°C for 10-14 days and stored in a refrigerator. All the fungal isolates were sub-cultured once in three weeks. To maintain the virulence, after sub-culturing all three fungal isolates were passed through host insect and re-isolated for further studies.

Preparation of spore concentrations of the fungal isolates

Three fungal isolates were cultured in potato dextrose agar (PDA). The plates were incubated at 26°C for 10 days. After sporulation, aerial conidia were harvested by flooding the plate with sterile deionized water (dH_2O) containing 0.02% Tween-80. Conidial spore suspensions were prepared and conidial count determined using a haemocytometer. All the suspensions were adjusted to a concentration of $1.5x10^8$ conidia ml^{-1} from which lower concentrations were prepared by serial dilution technique for bioassay studies.

Laboratory bioassays

Bioassays were conducted with different isolates of *B. bassiana* against *S. litura* (third instar) larvae in the laboratory with conidial suspensions containing $1.5x10^8$

conidia ml^{-1}. Twenty 3rd instar *S. litura* larvae were released on a castor leaf sprayed with the fungal suspension and transferred into a glass jar. The jars were covered with a muslin cloth. *S. litura* larvae sprayed with 0.02% of Tween-80 were maintained as control. Three replications were maintained for each treatment. Mortality counts were taken at 24, 48, 72 and 96 h after inoculation.

Germination test

Sterile glass microscope slides (Hyline Enterprises Limited) with a drop of PDA on each end were prepared. Aliquots of 0.1 ml of each isolate were thinly spread over the PDA surface and the slides were placed in sterile Petri dishes (15mm in height and 100mm in diameter), wrapped with parafilm (American Can) and incubated at 28°C. Conidial germination was assessed after 24 and 48h. The conidia were viewed under a microscope at 100x. Each conidium was considered germinated when the germ tube was equal to at least half of the long axis of the conidium. All the conidia in the field of view were counted to obtain at least a total of 300 conidia for each replicate (Moore *et al*., 1993). The experiment was repeated three times.

Statistical Analysis

Per cent mortality data was transformed in arc sin and subjected to one-way analysis of variance (ANOVA) using PAST software. Lethal Concentration (LC_{50}) was calculated by using Statistical Packages for Social Sciences 10 (SPSS 10). Median lethal time (LT_{50}) was calculated by $\sum$ (days$_n$ x infected larvae$_n$) /total of infected larvae (Beron and Diaz, 2005).

RESULTS AND DISCUSSION

The per cent mortality of *S. litura* at different time intervals is presented in Table 1. The highest cumulative mortality of 80.0% was recorded against *S. litura* with Bb_{10} isolates at 1.5x10^8 spore ml^{-1}, whereas Bb_{09} and Bb_{02} resulted in 73.33 and 60.0% mortality, respectively. Spore germination of 79.83±0.16, 88.33±0.33 and 95.53±0.01% with respect to Bb_{02}, Bb_{09} and Bb_{10} (Table 2).

The dose–response data were consistent with the model as evidenced by the goodness-of-fit statistics, which were significant. Bb_{02} had the lowest LC_{50} (2.1x10^6 spores ml^{-1}). Based on the fiducial limits, its susceptibility was not significantly different from Bb_{09} and Bb_{10} (Table 2). Mycosis in *S. litura* became evident after death when cadavers were covered with diffuse hyphal growth and spores. The above study demonstrates the pathogenicity of *B. bassiana* isolates against *S. litura*. As stated by Boucias *et al.* (1988) and Fernandez *et al.* (2001), direct spraying enhances conidia lodging within cuticular folds thereby facilitating attachment, germination, and penetration. Mycosis and sporulation in cadavers appeared

Table 1. Effect of *Beauveria bassiana* (Bb_{02}, Bb_{09} and Bb_{10}) isolates on *Spodoptera litura* at different time intervals

Bna Strains	Dose (Conidia ml^{-1})	% Cumulative Mortality (Mean ± SD) days after treatment			
		Hours			
		24	48	72	96
Bb_{02}	1.5x10^5	0.00±00	6.67±0.09	40.00±0.47	53.33±0.81
	1.5x10^6	0.00±00	13.33±0.94	46.67±094	53.33±0.94
	1.5x10^7	0.00±00	6.67±0.09	40.00±0.47	60±0.94
	1.5x10^8	6.67±0.09	13.33±0.94	60±0.942	66.67±0.8
Bb_{09}	1.5x10^5	0.00±00	20.00±0.94	40.00±0.94	46.67±0.94
	1.5x10^6	0.00±00	20.00±0.94	53.33±0.94	60.00±0.94
	1.5x10^7	0.00±00	26.67±0.81	53.33±0.94	66.67±0.81
	1.5x10^8	6.67±0.09	40.00±0.94	53.33±0.94	73.33±0.08
Bb_{10}	1.5x10^5	0.00±00	6.67±0.94	33.33±0.81	40.00±0.94
	1.5x10^6	0.00±00	26.67±0.81	53.33±0.94	66.67±0.81
	1.5x10^7	0.00±00	40.00±0.94	60.00±0.94	66.67±0.81
	1.5x10^8	6.67±0.09	26.67±0.81	73.33±0.08	80.00±0.94
Control	(0.02% Tween 80)	0.00±00	0.00±00	0.00±00	0.00±0

Each value is the mean of three replications

Table 2. Germination percentage, LC_{50}, intercept, regression coefficient and Chi square of *Beauveria bassiana* isolates against *Spodoptera litura*

Bb isolate	% Germination (24hrs) (Mean ± SE)	LC_{50} value (Lower – Upper Fiducial limit)	Intercept/SE	Regression Co-efficient/SE	Chi square
Bb_{02}	79.83± 0.167	$–2.1x10^6$ ($0.17x10^7$ – $5.28x10^8$)	–9.640	–1.3045	9.903 (P = 0.007)
Bb_{09}	88.33±0.333	$3.6x10^7$ ($5.56x10^7$ – $3.15x10^8$)	–16.630	2.486	63.55 (P = 0.00)
Bb_{10}	95.53±0.0167	$1.2x10^7$ ($7.1x10^7$ – $2.78x10^8$)	–18.017	0.748	139.87 (P = 0.000)

Table 3. Median lethal time of *Beauveria bassiana* isolates causing 50% mortality of *Spodoptera litura* larvae

Isolate of *Beauveria bassiana*	Median lethal time (Days)			
	10^5	10^6	10^7	10^8
Bb_{02}	7.0	7.0	7.0	4.8
Bb_{09}	0.0	5.4	5.83	4.8
Bb_{10}	0.0	5.4	3.42	4.0

to depend on exposure method, conidial concentrations and temperature.

Virulence of an isolate depends upon the interaction of the host, the pathogen, and the environment (Santiago-Alvarez *et al.,* 2006; Thomas and Elkinton, 2004; De Faria and Wraight, 2007; Poprawski and Jones, 2000; Casadevall and Pirofski, 1999). The difference in pathogenicity may be due to the susceptibility of larval stages of *S. litura* to tested isolates. Third and fourth instar larvae appeared to be less susceptible to fungal infection than second instars (Osborne *et al.,* 1990). Compared to Nirmala *et al.* (2006) who found LT50 value of 3.17 days and Hesketh *et al*. (2008) who recorded LT_{50} value of 3.31 for *Verticillium lecanii* against *Aphis fabae*, in the present investigation, Bb_{10} needed 3.42 and 4.0 days to cause 50% mortality of *S. litura* population at $1.5x10^7$ and $1.5x10^8$ conidia ml^{-1}, respectively. LC_{50} and LT_{50} values of Bb_{10} indicate its higher virulence against *S. litura.* It can be used as a potential biocontrol agent after field experiments for the management of *S. litura.*

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