Studies on the influence of *Beauveria bassiana* on survival and gut flora of groundnut caterpillar, *Spodoptera litura* Fab.

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ABSTRACT

Laboratory studies using the spore of the fungus *Beaueria bassiana* were carried out at different concentrations to assess its influence on the survival of the larvae of groundnut caterpillar, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) as well its impact on the larval gut microflora. In the contaminated food bioassay, a spore density of 10^9 spores/ml caused 100% larval mortality while LC_{50} value was found to be $0.5x10^6$ spores / ml. The heterotrophic bacterial population and the generic composition in the digestive tract of the larvae treated with the entomopathogenic fungi were analyzed. Nine species of bacterial genera *Bacillus* sp., *Proteus* sp., *Enterobacter* sp., *Salmonella* sp., *Pseudomonas* sp., *Escherichia* sp., *Klebsiella* sp., were identified in the digestive tract. The ingestion of fungal spores eliminated three genera of bacteria in the digestive tract

Key words : Entomopathogenic fungi, *Spodoptera litura, Beauveria bassiana,* gut microflora heterotrophic bacteria.

INTRODUCTION

The successful use of microbial control in agricultural crops suggests that there may be beneficial fitness effects of naturally occurring insect diseases to the plant (Elliot *et al.,* 2000). Various pathogenic organisms such as viruses, bacteria, protozoa, and nematodes and most fungi have shown promise for use in biological control (Benjamin *et al.,* 2002). In general, entomopathogenic fungi have a strong potential as viable control agents for many insect pests (Hafez *et al.,* 1998). Quite a good number of entomopathogenic fungi have been effective in laboratory and field tests and *Beauveria bassiana* and *Metarhizium anisopliae* had been successfully used in the field (Michael *et al.*, 1987).

At present there is an increasing interest in the use of entomopathogenic fungi for insect pest control. Among the many entomopathogenic fungi, isolates of *Beauveria bassiana* Balsomo Vuillemin were found to be highly pathogenic to many insect pests such as whiteflies, aphids, grasshoppers, termites, Colorado potato beetle, Mexican bean beetle, Japanese beetle, boll weevil, cereal leaf beetle, bark beetle, lygus bugs, chinch bug, fire ants, European corn borer, codling moth and Douglas fir tussock moth. It occurs in the soil as a saprophyte (Hoffman and Frodsham, 1993). *B. bassiana* belongs to the class Hypomycetes under the division Deuteromycetes. The colonies are moderately growing, spreading, and woolly, powdery or mealy in texture, white to yellowish white or occasionally pinkish in colour in nutrient agar plates. Conidiogenous cells are hyaline; flask shaped with a long a zigzag - appearing rachis bearing lateral conidia. Conidia are hyaline, 1 - celled and globose to ovoid with a length of about 3.5 mm. In the present study, experiments were conducted to find out the bio - efficacy of the fungus *B. bassiana against* groundnut caterpillar, *Spodoptera litura* Fab. as well as its influence on the gut microflora.

MATERIALS AND METHODS

Collection and maintenance of pest

Neonates and early instar larvae of *S. litura* were collected from groundnut fields in Kadayam Panchayat at Tirunelveli district, Tamil Nadu, India. The collected larvae were maintained on fresh clean plastic jars with fresh leaves at 30^0C and 70% relative humility (Sanjrani *et al.,* 1989). The larvae were fed with fresh leaves of groundnut. In each plastic jars 5 numbers of pests with same age and size groups were introduced.

Fungal collection and maintenance

The isolate MTCC - 2028 of *Beauveria bassiana* was obtained from the Microbial Type Culture Collection Centre, Chandigarh. The culture was maintained in potato carrot agar medium. The fungal strain was also maintained in a potato dextrose agar medium. The conidial spores of *B. bassiana* were mass-cultivated employing rice grains. Rice husk supported the proliferated growth of this fungus because it is rich in nutrients like lignin (20 - 47%) and cellulose (30 - 45%) etc. The rice grains (75g) were

filled in small bottles, and autoclaved for 30 minutes at 121^{0}C and 15 psi. Then the fungal inoculum (2 ml) was added to Rice husk medium (Mazumder *et al.*, 1995) and stored at 27 - 30^{o}C for 12 days for the mass production of *B. bassiana*.

Preparation and standardization of spore suspensions

Spore suspensions of *B. bassiana* were prepared by adding 5ml of 0.2% (v/v) dispersing agent (Tween 80) into each of the test tube slants were containing the fungal culture. After adding Tween 80, the spores were scraped off from the agar surface by using stirring glass rod and the spores were suspended in Tween 80. The spore suspension was collected in a sterile test tube, filtered through cheese cloth to remove hyphal debris.The concentration of spores in the final suspension was determined using haemocytometer count. From this initial spore suspension, 10^2, 10^3, 10^4, 10^5, 10^7, 10^8 and 1x 10^9 conidia / mL of spores were prepared.

Bioassay

The larvae of *S.litura* were fed with groundnut leaves dipped in *B. bassiana* spore suspension containing 10^2, 10^3, 10^4, 10^5, 10^7, 10^8 and 10^9 conidia/mL prepared in 0.02% of Tween 80 (Yeo *et al.*, 2003). The dipped leaves with different spore concentrations were air-dried. Then the leaves were used to feed the larvae. The control larvae were fed with untreated groundnut leaves.LC_{50} value was calculated by the method of Reed and Muench (1938).

Physiological grouping of bacterial strains

After the experimental regimes, the control and spore treated larvae were brought to the laboratory in living condition for counting the gut microflora. The larvae were sacrificed and the gut was aseptically dissected out for studying bacterial population. All instruments used to dissect out the gut tissues were thoroughly sterilized to avoid any contamination. The aseptically excised gut tissues (1g) were placed in separate sterile Petri plate. Microbial analysis was made by pour plate method. One gram of dissected gut tissue was homogenized separately using sterile 1% peptone water. Then homogenates were made to 100 ml using 1% sterile peptone water. Further serial dilution was done using 9 ml of same 1% sterile peptone water. One ml aliquots of serially diluted homogenates were taken out into a sterile Petri plates. About 20 mL sterile molten agar of different type's *viz.*, starch agar, casein agar, gelatin agar, and tween agar were poured aseptically in serial Petri plates. Different physiological group of bacteria perform metabolic activities by different enzymes. Based on the production of extra cellular enzymes, the bacterial strains belong to different groups were identified. Bacterial strains taken from gut tissues in control and spore treated larvae were thus tested for the presence or absence of amylolytic, gelatinolytic, caseinolytic and lipolytic bacterial groups. The percentage occurrence of various groups of bacteria was recorded for different samples

Generic composition of bacterial strains

The generic level identification was carried out by adopting the scheme described in the Bergey's manual of determinative bacteriology (1984 - 89). The bacterial strains isolated from the gut of control and treated samples were analyzed for their amylolytic, cellulolytic, caesinolytic and lipolytic activities. (Meenakshi Sundaram *et al.*, 1998)

RESULTS AND DISCUSSION

In the present study, 50% (LC_{50}) mortality was observed at a density of 10^5 spores/ml of *B. bassiana*. The maximum mortality (100 - 75%) was observed at a density of 10^8 spores / ml as against the minimum (25%) at density of 10^4 spores / ml. In the present investigation, LC_{50} value was found to be 0.5 x 10^6 spores /ml of *B. bassiana*. The total heterotrophic bacterial population in the digestive tract of control and treated larvae was found to be 1.21 and 0.52 x 10^6 CFU / g respectively. The heterotrophic bacterial population of the digestive tract decreased, when the pests were treated with fungal pathogen *B. bassiana* (Sreeramakumar *et al.*, 2002). The bacterial count in the digestive tract of the treated larvae was 1.24 x 10^6 CFU/g. A total number of 20 bacterial isolates were identified both in control and treated larvae. The bacterial genera in the digestive tract of the control larvae were *Bacillus* sp., *Proteus* sp., *Enterobacter* sp., *Salmonella* sp., *Pseudomonas* sp., *Escherichia coli* and *Klebsiella* sp. Among various bacterial genera isolated, *Bacillus* (40%) and *Proteus* (20%) were found to be the dominant flora followed by *Enterobacter* (10%), *Salmonella* (10%), *Pseudomonas* (10%), *E. coli* (10%), and *Klebsiella* (5%).

Table 1. Physiological grouping of bacterial strains isolated from digestive tract of *S. litura*.

Source	Physiological grouping				Total No. of bacterial strain tested
	Cellulolytic	Amylolytic	Caesinolytic	Lipolytic	
Control	15 (75%)	5 (25%)	10 (50%)	8 (40%)	20
Treated	14 (70%)	4 (35%)	12 (60%)	10 (50%)	20

Table 2. Fungal density in the digestive tract of *S. litura* treated with *B. bassiana*

Source	Dilutions	Fungal population (CFU/g)					
		Control			Treated		
		T_1	T_2	Average	T_1	T_2	Average
Digestive system	10^5	5	3	4	120	100	110
	10^6	0	0	0	6	12	9
		4×10^5 CFU/g			110×10^5 CFU/g		

In the gut of the *S. litura* a considerable reduction in occurrence of various bacterial genera was observed. As observed in control, the digestive tract microflora of the treated pest was dominated by *Bacillus* (55%) followed by *Proteus* (25%), *Pseudomonas* (15%) and *E. coli* (15%). The bacterial genera such as *Enterobacter sp.*, *Salmonella sp.*, and *Klebsiella sp.*, which occurred in control larvae were found to be absent in the treated pest. The reason for this may be that these bacteria were eliminated by the fungal colonization in the digestive tract of *S. litura* treated with fungus. With regard to the enzymatic activities, higher occurrence of cellulolytic bacteria strains (75%) followed by caesinolytic (50%), amylolytic (25%), and lipolytic (40%) bacteria was observed (Table 1).

The fungal density in the digestive tract of the control larvae was lesser when compared to treated population (4×10^5 CFU/g.) (Table 2). The fungal density in the digestive tract of treated larvae was 110×10^5 CFU/g. Thus it may be concluded that B. bassiana was found to be highly pathogenic to *S. litura* as well and it influenced the incidence and the activity of larval gut microflora.

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