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

I. Joseph, D. Edwin Chellaiah and A. J. A. Ranjit Singh

**ABSTRACT**

Laboratory studies using the spore of the fungus *Beauveria 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 as 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, holl 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 Hypomyces 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°C and 70% relative humidity (Sanjrani et al., 1989).

**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°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°C for 12 days for the mass production of B. bassiana.

Preparation and standardization of spore suspensions
Spor 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 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, 10³, 10⁴, 10⁵, 10⁷, 10⁸ and 10⁹ conidia/mL of spores were prepared.

Bioassay
The larvae of S. litura were fed with groundnut leaves dipped in B. bassiana spore suspension containing 10⁵, 10⁴, 10³, 10², 10¹, 10⁰ and 10⁰ 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. LC₅₀ 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 plates. Microbial analysis was made by pour plate method. One ml aliquots of serially diluted homogenates were taken out into a sterile 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.

Percentage occurrence of various groups of bacteria was recorded for different samples

<table>
<thead>
<tr>
<th>Source</th>
<th>Physiological grouping</th>
<th>Total No. of bacterial strain tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cellulolytic</td>
<td>Amylolytic</td>
</tr>
<tr>
<td>Control</td>
<td>15 (75%)</td>
<td>5 (25%)</td>
</tr>
<tr>
<td>Treated</td>
<td>14 (70%)</td>
<td>4 (35%)</td>
</tr>
</tbody>
</table>

Table 1. Physiological grouping of bacterial strains isolated from digestive tract of S. litura.
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 x 10^5 CFU/g.) (Table 2). The fungal density in the digestive tract of treated larva was 110 x 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.

**REFERENCES**


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

<table>
<thead>
<tr>
<th>Source</th>
<th>Dilutions</th>
<th>Fungal population (CFU/g)</th>
<th>Control</th>
<th>Treated</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestive system</td>
<td>10⁵</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>120</td>
</tr>
<tr>
<td>Digestive system</td>
<td>10⁶</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Digestive system</td>
<td>4 x 10⁵ CFU/g</td>
<td>110 x 10⁵ CFU/g</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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