

Original Research Article

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Efficacy of *Beauveria bassiana* on Different Larval Instars of Tobacco Caterpillar (*Spodoptera litura* Fab.)

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ABSTRACT

Efficacy of *Beauveria bassiana* to different larval stages of *Spodoptera litura* using 1%, 2%, 3%, 4% and 5% in 2.3×10^6 conidia/ml were undertaken in laboratory conditions. Observation was recorded on 24 hrs, 48 hrs and 72 hrs in the laboratory of Entomology Department, SHUATS, Allahabad. Virulence potential of the entomopathogenic fungi varied with different larval stages of *Spodoptera litura*. All the treatments resulted in significantly higher mortality than control. The results revealed that percent mortality was quite high in earlier instars as compared to later instars. The highest dose of 5% 2.3×10^6 conidia/ml brought 91.66, 90.00, 88.33, 78.77, 66.11 and 49.99 percent mortality in 1st, 2nd, 3rd, 4th, 5th and 6th instar respectively as compared to only 11.66, 07.21, 01.10, 00.00, 01.11 and 00.00 percent mortality in control of respective stages. Thus, higher the dose of *Beauveria bassiana* higher will be the mortality of tobacco caterpillar.

Keywords

Beauveria bassiana, Entomopathogenic fungus, Mortality, *Spodoptera litura*.

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Introduction

Tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is a polyphagous sporadic pest with high mobility and reproductive capacity (Holloway, 1989). It is one of the most destructive pest of cauliflower, groundnut, cotton, tomato, cabbage and other cruciferous crops (Anand *et al.*, 2009). It passes through 5-6 overlapping generations annually (Sasidharan and Varma, 2005; Kumar and Chapman, 1984) and if not controlled timely, it may result in huge crop losses ranging from 25.8-100 per cent in various parts of India (Ahmad *et al.*, 2005). For the management of this pest,

insecticide use is most widely practiced (Kaur *et al.*, 2011). Although effective in reducing pest population in short term, these chemicals have little long term regulatory impact on pest population and often cause unwanted environmental side effects (Ummidi *et al.*, 2013). Entomopathogenic fungi as biological agents show promise in reducing insect pest populations and damage in different agro ecosystems (Inglis *et al.*, 2001). An attractive feature of these fungi is that infectivity is by contact and the action is through penetration (Nadeau *et al.*, 1996). The most important fungal pathogens are *Metarhizium* spp.,

Beauveria spp. (Ummidi and Vodlamani, 2014). *Beauveria bassiana*, the most common and ubiquitous fungal entomopathogen is known to be highly potent for the control of insects belonging to various orders. Among these *B. bassiana* (Balsamo) Vuillemin (Ascomycota: Hyphocreales) is a facultative pathogen with wide host range (Armes *et al.*, 1997). This fungus has potential to control over 70 insect pests belonging to different orders particularly lepidopteron pests (Kaur *et al.*, 2011). The main objective of this study is to measure the efficacy of *B. bassiana* to control different larval instar using different concentration of *B. bassiana* in Laboratory.

Materials and Methods

Isolation of Fungus

Beauveria bassiana was obtained from cadavers of *Indarbela quadrinotata* collected from guava orchard of SHIATS, Allahabad. The fungus mycelia on the surface of dead *Indarbela* was isolated with the help of sterile needle and was inoculated on petriplates poured with SDA (composition). The inoculated petriplates were incubated in incubator (30°C±2 82% RH) for 7 days. Pure culture was obtained through the series (2-3) of subcultures of the original inoculation on SDA (Sabouroud Dextrose Agar).

Identification of fungi

When sporulation took place, the saprophytic contaminants were rejected, and morphological examinations of the desired fungi were carried out. Lacto-phenol blue was used for the microscopic visualisation of the fungus, and glycerol was used as a mounting agent. Mycotaxonomic keys were used for the identification of the fungus. Slides were prepared to check the colony of *Beauveria bassiana*. The fungi were identified using a light microscope the sample stains with lectophenol and aniline blue for mounting

between the slides and fungi were identified by their mycelia growth; spores characters and pigmentation of culture media (Aneja, 2005).

Bioassay

Spore suspension was prepared from 15 days old culture of *B. bassiana* on SDA 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 five different concentrations, 1, 2, 3, 4 and 5% of 2.4×10^7 conidia /ml were prepared and tested for its efficacy on 1st, 2nd, 3rd, 4th and 5th instar larva, of *S. litura*. Three replicates of 20 larvae each with a control was run for each dose. Observations were recorded 24, 48 and 72 hrs. All experiments were conducted in BOD at 27±1°C and 70±5 per cent RH. The results were analyzed both spastically and by observing morphological abnormalities. Fungal infections in treated larvae were determined by microscopic examinations.

Rearing of *Spodoptera litura*

Spodoptera litura larvae were collected from infested SHUATS fields of Allahabad district, Uttar Pradesh for rearing in the laboratory. Larvae were transferred into plastic bowls and fed with cabbage leaves (15 cm in diameter). To avoid crowding, 20 larvae were taken per bowl sealed with muslin cloth for aeration and kept at 26°C, at a photoperiod of 12h + 12h light and dark regimes. Cabbage leaves were changed daily. After pupation the pupae were

kept in plastic boxes, half filled with sterile moist sand for maintaining RH. After 5 to 6 days, the emerged moths were transferred into cages 30 cm³ volume having bouquets of cabbage leaves with petioles dipped in water in a conical flask. The adults were fed on artificial nectar (20% honey, 0.4% Vit. E and Vit. B complex 0.5%). Masses of eggs appear in light brown color and egg patches were covered with felt of pale brown scales on the cabbage leaves. Egg patches were collected carefully along with a piece of leaf and kept in sterile boxes. Boxes containing egg patches were incubated at 25-28°C for hatching. Larvae that emerge from the eggs after 3 to 5 days were reared at 26°C. Young larvae were fed with fresh leaves of cabbage. The larvae obtained from a single egg patch were used for each experiment. Second generation larvae were used for conducting pathogenicity experiments in order to get homogeneity. Larvae of same age group, (1st, 2nd, 3rd, 4th, 5th and 6th instar) were chosen for experimentation.

Results and Discussion

In the 1st instar, larval mortality ranged from 56.10, 65.44, 77.44, 82.21 and 91.66 per cent, it increased with the increase in their concentration i.e. 1%, 2%, 3%, 4% and 5% in 2.3x10⁶ conidia/ml. The highest mortality was observed in 5% concentration (91.66 %)

which was not significant to 4 and 3 per cent concentration but was significant to rest of the treatments. Mortality range of 2nd instar larvae were 48.88, 61.66, 73.88, 80.55 and 90.00 per cent, it also increased with the increase in their respective concentration. Significant differences were observed between all the treatments. The highest per cent mortality rate i.e. 90.00% was observed in 5% concentration which significant with rest of the concentrations. In 3rd instar, the larval mortality ranged was observed from 33.88 to 88.33 per cent at different concentrations. All treatments were found significant to each other and the highest mortality was in 5 per cent concentration with 88.33 per cent mortality.

The fungus exerted considerable pathogenicity of 78.99 per cent mortality to the 4th instar larvae at 5% concentration which was having significant difference with rest of the concentrations. All the treatments were significant to each other except 2 and 3% which were not significant to each other but were superior over control.

The cumulative mortality steadily increased with the increase in concentrations of the treatments to reach 66.66 per cent mortality at 5% in 5th instar larvae. Here all the treatments were significant to each other and superior over control.

Table.1 Mean percent mortality of different larval instar of *S. litura* due to *B. bassiana* at different concentrations

Treatment/instar	Per cent Mortality (%)					
	1 st instar	2 nd instar	3 rd instar	4 th instar	5 th instar	6 th instar
1%	56.10 ^c	48.88 ^e	33.88 ^e	37.21 ^d	24.44 ^c	22.21 ^c
2%	65.44 ^{bc}	61.11 ^d	56.10 ^d	47.77 ^c	39.99 ^d	34.44 ^b
3%	77.77 ^{ab}	73.88 ^c	62.77 ^c	53.88 ^c	47.22 ^c	38.29 ^b
4%	82.21 ^{ab}	80.55 ^b	74.99 ^b	64.44 ^b	54.44 ^b	49.99 ^a
5%	91.66 ^a	90.00 ^a	88.33 ^a	78.77 ^a	66.66 ^a	49.99 ^a
Control	11.66 ^d	07.21 ^f	01.10 ^f	00.00 ^e	01.11 ^f	00.00 ^d
CD(0.05)	19.48	23.92	21.29	26.17	18.21	14.85
S.Ed(±)	40.96	42.07	44.38	37.43	31.78	29.56

In the 6th instar, larva mortality ranged from 22.21 to 49.99 per cent in different concentrations. The highest mortality was observed in 4 and 5 per cent concentration but was at par to each other. 2 and 3 percent concentration also observed higher mortality as compared over control but were also at par to each other (Table 1).

Present investigation displayed that all the larval stages of *Spodoptera litura* were not equally susceptible to the infection of *Beauveria bassiana* but the fungus was found more pathogenic to early instar. Higher susceptibility to younger stages and reduced susceptibility to older stages if considered together reveal that the younger stages when treated die at once due to their very small and soft body parts. Younger larval stages thus have an increased probability of acquiring an infection. Late larval stages, on the other hand, have reduced food intake and a thicker cuticle and thus it is less likely that spores would enter or penetrate their body. The present studies are conformity with the findings of Hung and Boucias (1992) who testing the fungi against the leaf worm and beet army worm showing that the fungus *B. bassiana* (Balsamo), Vuillemin caused a high mortality to the insect at earlier stages. Similarly, Gundannavar *et al.*, (2006) recorded Susceptibility of *H. armigera* (Hubner) to *B. bassiana*. Asi *et al.*, (2013) reported virulence potential of the entomopathogenic fungi varied with different biological stages of the *Spodoptera litura*. Prasad and Veerwal (2012) reported that different doses exerted variable effects on larvae, pupae and adults ranging from general mortality, prolongation in developmental period and various morphological abnormalities in mosquitoes.

From the critical analysis of the present findings it can be concluded that *Beauveria bassiana* tested was pathogenic on all

Spodoptera litura larval stages. The young larval stages are the most vulnerable concentration 6% in 2.3×10^6 conidia/ml is showed highest mortality of insect. Hence, fungal spore were developed with a strong emphasis on protecting the environment and consumer from harmful effect of poisonous chemical pesticide.

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