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RESEARCH ARTICLE

# Bioefficacy of *Beauveria bassiana* (Balsamo) against third instar larvae of *Spodoptera litura* (Far.)

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## SUMMARY

Among the microbial agents, the first diseases in insects were cause by fungus that has conspicuous macroscopic growth on the surface of their host. All most all insects order is susceptible to fungus diseases. Entomopathogenic fungi are associated with insects living in diverse habitats, including fresh water, soil, soil surface and aerial locations. Laboratory studies were undertaken to evaluate the bioefficacy of *Beauveria bassiana* against 3rd instar larvae of *Spodoptera litura*. *B. bassiana* was identified, isolated and maintained from field collected cadaver of lepidopteron larvae. White colour spore were taken in sterilized plate and white colonies from these plates were transferred aseptically in culture slant containing PDA media. *Spodoptera litura* was reared in artificial diet. Spore suspension of three different concentrations 0.1, 0.125 and $0.2\times10^8$ were prepared by serial dilution. When these concentrations of spores were applied on 3rd instar larvae of *S. litura*, it increases the percentage of mortality. The fungal preparation @ $0.2\times10^8$ spore/ ml caused 80% (maximum) mortality of *Spodoptera litura* followed $0.125\times10^8$ spore/ ml (73.3%) and $0.1\times10^8$ spore/ml (46.6%). The minimum mortality was observed under control *i.e.* 23.3%. Thus, as the number of spore increased, per cent mortality also increased. Hence, fungal spore were developed with a strong emphasis on protecting the environment and consumers from harmful effects of poisonous chemical pesticide.

**Key Words :** Bioefficiency, Larvae, Instar

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Insects, constituting the largest class in animal kingdom and whole living world, are the friend as well as foes of human being. But unfortunately the role of insects as harmful organism is much wider than the beneficial. The tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is a polyphagous sporadic pest with high mobility and reproductive capacity (Holloway, 1989). It is serious of various economically important crops such as cotton, groundnut, chilli, tobacco, castor and pulses etc. Chemicalization is known to destabilize the ecosystem by destroying the rich fauna of predators, parasitoids, pollinators, soil builders, birds, lizards, small animals as well as earthworms and microbial diversity.

In view of these side effects, the necessity for sustainable crop production through eco-friendly pest management technique is being largely felt in the recent times. Of the several microbial pathogens *viz.*, bacteria, fungi, viruses, protozoan and entomopathogenic nematodes, only a few have been studied systematically for their usefulness.

Entomopathogenic fungi as biological agents show promise in reducing insect pest populations and damage in different agro ecosystems (Inglis *et al*., 2001). Many of these offer a great potential in pest management. 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.,

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*Verticillium lecanii* and *Hirsutella* spp.

In culture *B. bassiana* grows as white mould. On most common culture media, it produces many dry, powdery conidia in distinctive white spore balls. Each spore ball is composed of a cluster of conidiogenous cells. The condiogenous cells of *B. bassiana* are short and ovoid, and terminate in a narrow apical extension called a rachis. The conidia is single celled, haploid and hydrophobic.

The major issues involved in mass production and utilization of mycopathogens are selection of effective strains, development of cost effective methods for mass rearing, development of effective methods for storage and shipment, and production of effective formulation. Environmental factors like temperature, humidity and sunlight play a profound role on the field persistence of entomopathogenic fungi.

Thus, conservation and periodic enhancement of efficacy of biological control agents will help in crop protection and in producing agricultural commodities free from pesticide residues. There is a general feeling that, the development and spread of biological control will empower the poor farmers to manage their pest problems in an eco-friendly ways.

*B. bassiana* was found effective against *Spodoptera litura* (Gopalakrishnan and Narayanan, 1989). *B. bassiana* were studied in both laboratory and field on various insect pests, but only few investigations has been done against *Spodoptera litura*. Thus, present investigation focus on bioefficacy of *B. bassiana* against 3rd instar larvae of *Spodoptera litura*.

## MATERIAL AND METHODS

**Collection of samples:**

Extensive and repeated survey for the occurrence of the entomopathogenic fungus in instructional farm of B.C.K.V, Jaguli, Nadia, West Bengal, India was made. The fungi were isolated from cadavers of lepidopteron larvae showing white colour of sporulation, infesting pea crop.

**Identification of fungus :**

For identification of fungus (*B. bassiana*), mycelium present on the body of insects were separated with the help of needle and taken on a slide. Lacto phenol was added on the slide and microscopy was done.

**Isolation of fungi from insect cadever and maintenance:**

Infested insects collected from field in sterilized small glass vial and bought in laboratory for isolation. White colour mass of spore was taken in sterilized distilled water and diluted further with distilled water and plated with potato dextrose peptone yeast extract agar media with streptomycin sulphate as antibacterial antibiotic.

Plates were incubated after solidification at 28±1°C in BOD incubator for 7-10 days. After growth of white colonies on plate's small bits of the colonies were transferred aseptically in culture slant containing same media (PDA) and incubated at above mentioned conditions. Cultures were maintained in the above medium in refrigerator and transferred periodically. After sufficient growth fungus structure were observed under microscope for confirmation. For detail studies slides were prepared following slide culture technique.

**Mass rearing of *Spodoptera litura:***

The test insect was maintained in the laboratory by rearing the larvae, collected from castor leaves. Rearing was done in cages (40×40×60 cm) where leaves of castor were provided for egg laying. The eggs were laid on clusters and the eggs masses are covered with buff- coloued hairs. When larvae were transformed into pupa they were shifted into soil filled jars for adult emergence. The emerged moths were collected and transfer into another sterile jars. Brown paper strips were hanged for egg laying and moths were provided cotton pieces soaked with 10 per cent honey solution for feeding. Paper strips carrying egg masses were collected and transferred to fresh plastic containers and kept in BOD incubator at 27±1°C and 70% RH .On hatching the larvae were transferred into plastic box containing artificial diet were provided daily in the morning.

**Preparing sterile water with 0.075% Tween-80 and application of spore suspension:**

Sterile water with tween-80 was used to prepare suspension with hydrophobic spores of *B.bassiana*. 0.075ml of tween-80 was mixed with 200ml of sterilized distilled water to prepare stock solution and shaken thoroughly. The solution was then poured into a sterilized conical flask.50ml of stock solution was taken in a sterilized conical flask and mixed with culture media, taken from the slant. The mixture was shaken for 15min until the spores were separated from the media. The spore suspension was filtered using muslin cloth. The numbers of spores were counted under heamocytometer. Three different concentrations *i.e.* 0.1, 0.125 and $0.2\times10^8$spore/ml were prepared by serial dilution. 5ml of each concentrations were sprayed in Petri plates. 5ml of sterilized distilled water containing tween- 80 solution was sprayed as a control. The 3rdinstar larvae of *Spodoptera litura* were released inside the Petri plates @10 larvae / plate for about 1 hour. The larvae were then transferred to other Petri plates with artificial diet as food. Mortality was recorded daily up to 15 days, starting from 24hour after treatment.

## RESULTS AND DISCUSSION

The bioefficacy of *Beauveria bassiana* against the third instar larvae of *Spodoptera litura* was studied in laboratory, using three different concentrations 0.1, 0.125 and $0.2\times10^8$ spore/ml. The mortality of the larvae was recorded daily up to 15 days after treatment. A linear relation between per cent mortality and dose concentrations was observed. Per cent

**Table 1 : Effect of *B. bassiana* on 3rd instar of *Spodoptera litura***

| Sr. No. | Treatments | | No. of replication | % mortality |
|---|---|---|---|---|
| 1. | Control | Untreated | 3 | 23.3% |
| 2. | *B.bassiana* | $0.1\times10^8$ | 3 | 46.6% |
| | (spore/ml) | $0.125\times10^8$ | 3 | 73.3% |
| | | $0.2\times10^8$ | 3 | 80% |

mortality increased from 46.6 to 80% as the dose was increased from $0.1x10^8$ spore /ml to $0.2 x10^8$ spore /ml as compared to 23.3 % in control.

It is clear from above result when 3rd instar larvae of *Spodoptera litura* were treated with three concentrations *i.e.* 0.1, 0.125 and $0.2 \times10^8$ spore/ml of fungus the data (Table 1) showed linear relationship between dose concentration and mortality. As the number of spore increased, per cent mortality also increased.

The pathogenicity and bioefficacy of *B. bassiana* has been evaluated by few authors. Ekesi *et al*. (2000) evaluated the entomopathogenecity of *B.bassiana* and *M.anisopliae* isolates to the apterous adult of the cowpea aphid, *Aphis craccivora* and recorded 58-91%, 64-93% and 66-100% mortality by 3 isolates of the two fungi, respectively after 7 days of treatment. Pandey (2003) investigated the pathogenicity of *B. bassiana* in eggs and pupae of the lepidopteron pest, *Spodoptera litura*, *Spilosoma oblique* and *Helicoverpa armigera*. *Spodoptera litura* eggs were more susceptible to the biological control agents than those of the other pests. EI-sufty *et al.* (1982)and Hung and Boucias (1992) tested the fungi against the leaf worm and beet armyworm, the results showed that the fungus *Beauveria bassiana* (Balsamo) Vuillemin causes a higher mortality to the insect pests. Sabbour and sahib (2005) also observed that *Beauveria bassiana* (Balsamo) was highly effective against *P.xylostella*, *P.rapae* and *S.exigua* and percentage of infestation reached to 20 and 21 per cent after 90 days of treatment. .When they were allowed to feed on artificial diet contaminated with $3x10^3$ or $4.5x10^3$ spores.

*Beauveria bassiana* spores when come in contact with cuticle, they germinate and grow directly through it to inner body of host insect. Here fungus proliferated inside host tissue, produces toxins and nutrients, eventually kills it. Once fungus has killed its host, it grows back out through softer portion of cuticle, covering the insect with a layer of white molds. This downy mold produces million of new infective spore. The cuticle is the first barrier to infection by fungi. Hence, rapid and direct penetration of the cuticle is important for virulence (Pekrul and Grula, 1979). The insect procuticle is primarily chitin fibrils embedded in a protein matrix and penetration appeared to involve both mechanical and enzymatic components (Charnley and St. Leger, 1989; St. Leger *et al*., 1988).

Hence, it is concluded, that results obtained for treatment of the 3rd instar larvae of *Sodoptera litura* (Hubner) treated with *Beauveria bassiana* (Balsamo) may be mass multiplied for the management of the insect pest population and eco-friendly. This fungus can also be included successfully in IPM.

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