Pathogenicity of *Beauveria bassiana* (Deuteromycotina : Euteromycotina : Hyphomycetes) strains on *Spodoptera litura* (Fab.)

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ABSTRACT

The pupae of *Spodoptera litura* (Fab.), (Lepidoptera: Noctuidae), a polyphagous pest affecting common crops in Indian subcontinent, were treated with different isolates of conidia of an entomopathogenic fungi *Beauveria bassiana* (SBT#11and SBT#16) under laboratory conditions. Both strains of *B.bassiana* were highly pathogenic causing 100% mortality in *S. litura* which is conidial concentration dependent. SBT#11 was more virulent with a LT_{50} of 5.1 and 6.0 days in laboratory for SBT#16 and SBT#11 respectively. Fungal sporulation was observed in 87 % of the insect cadaver in the treated group while no sporulation was observed in the control. This study indicates that dry conidia of both isolates are pathogenic to *S. litura* and could be used as potential biological control agents for the tested insect.

Key words: *Spodoptera litura*, entomopathogenic fungi, *Beauveria bassiana*

INTRODUCTION

In inundative applications of microbial control agents, combination treatment with two entomopathogens offers an attractive biorational strategy. If the two entomopathogens complement each other, or act synergistically, a beneficial effect can be obtained. One of the limitations of fungi as microbial control agents is that, each species and strains within a species are usually efficacious against a particular pest. The tobacco cutworm *Spodoptera litura* (Fab.) is regarded as a major destructive pest of subtropical and tropical agricultural crops. *S. litura* (Fab.) (Lepidoptera: Noctuidae), the common cutworm, is an economically serious and polyphagous pest in India. Control currently relies mainly on the application of various classes of chemical insecticides including carbamates, pyrethroids and organophosphates (Liburd *et al.*, 2000). It is recognized that widespread continuous use of these chemical insecticides causes environmental problems and leads to the development of insect resistance. Microbial insecticides such as entomopathogenic fungi can provide an alternative, more environmentally friendly option to control this insect pest. The indiscriminate use of chemical pesticides is assuming a serious cause of concern to human health and environment safety. A viable alternative to chemical pesticides is integrated pest management.

The entomopathogenic fungus *B. bassiana* is a promising and extensively researched biological control agent that can suppress a variety of economically important insect pests (Coates *et al*., 2002; McGuire *et al*., 2005). Sporadic occurrences of *B. bassiana* have been observed (Uma Devi *et al.*, 2003). *B. bassiana* has a wide host range, but differences in both host specificity and virulence among isolates has been reported (Ferron *et al*., 1991). However, it is increasingly being realized that this fungus is rather a generalist, with no strict host specificity (Rehner and Buckley, 2005). Earlier infection of a single *Spodoptera litura* larva with *B. bassiana* and *Nomuraea rileyi* (Hypocreales) fungi has also been observed (Uma Devi *et al*., 2003). Moreover Purwar and Sachan (2005) studied the impact of different isolate such as Pantnagar isolates and IMTECH strains on *B. bassiana* and *Metarhizium anisopliae* on *S. litura* and *Spilarctia obliqua*. Uma Maheswara Rao *et al*. (2006) also studied the impact of *B. bassiana* on *S. litura* in relation to different temperature. Several B. *bassiana*- based mycoinsecticide currently registered or under commercial development for agricultural pests management (Hajek *et al*., 2001). Here fore, the biological control of *S. litura* by a potential mycoinsecticide of *B. bassiana* is reported in this paper.

MATERIAL AND METHODS

Collection and maintenance of insect

Spodoptera litura larvae were collected in the cotton field of Peddakorapadu, Guntur district. Larvae were maintained in 17 cm x 21 cm x 25 cm cages under laboratory conditions (16L: 8D photoperiod, 25 ± 1^{0}C temperature, and 70-75% RH).

Table 1. Mean cumulative percent mortality of *S.litura* infested with *B .bassiana*

Isolate Name	Days After Treatment							
	4	5	6	7	8	9	10	11
SBT # 11	38 ± 8.7^c	56 ± 8.8^c	72 ± 6.8^c	88 ± 7.2^c	98 ± 4.2^c	100 ± 0^c	100 ± 0^b	100 ± 0^b
SBT # 16	27 ± 5.2^b	44 ± 12.5^b	54 ± 5.1^b	69 ± 4.3^b	84 ± 6.8^b	87 ± 6.2^b	97 ± 4.8^b	100 ± 0^b
Control	1 ± 3.1^a	1 ± 3.1^a	1 ± 3.1^a	1 ± 3.1^a	1 ± 3.1^a	1 ± 3.1^a	1 ± 3.1^a	1 ± 3.1^a

Mean followed by the same letter within days (columns) are not significantly different at 5 % level

Fungal isolates

Beauveria bassiana fungal isolates were originally isolated from the *Spodoptera litura*, collected in cotton growing regions of Guntur district, Andhra Pradesh. The isolated strains of *B. bassiana* were grown in Sabouraud Dextrose Agar (SDA) with yeast extract (2g/L) (SDAY) at 24 ± 1°C. From this sample, two isolates were selected and they were labeled as SBT#11 and SBT#16. For bioassays, conidia were harvested from eight-day-old cultures. Aqueous conidial suspensions were collected in 10 ml sterile distilled water with 0.01% Tween-80 (Sigma Aldrich) to reduce surface tension. Conidia were counted in a Neubauer chamber and dilutions were made to a final concentration of 1×10^8 conidia/ml and stored at 4^0C until use.

Treatment

Fresh cotton leaves cleaned and wiped and their stalks wrapped in cotton plugs dipped in water were placed in the glass jar to serve as food for the larvae. For each treatment batch, 30 second/ third/forth (check???) instar larvae were chosen at random. Larvae were introduced on the surface of the leaves. The fungal inoculum (10 mL/ insect of aqueous suspension of 10^8 conidia/ml with 0.02 percent Tween 80) was sprayed on the larvae. Four replications were maintained for each treatment. Control category was sprayed with 0.02 percent Tween 80 alone. The treated larvae were monitored daily for mortality. The glass jars were cleaned off litter every day and fresh cotton leaves were placed. Mortality was recorded on forth day after treatment until eleventh day. The fungal pathogen expressed on each dead larva was identified and recorded.

Data analysis

Mortality was recorded in the control (untreated) category. Percentage mycosis was estimated as proportion of dead insects that showed mycosis. The mortality and mycosis values were arcsine percent square root transformed and the means were back-transformed (Gomez and Gomez, 1984). Mortality data was subjected to ANOVA with test temperatures and treatment types (respective fungal isolates with control) as the main effects. The LT_{50} was determined by linear regression in which percentage of mortality with each treatment was plotted against time for the days of evaluation. Analysis of variance (ANOVA) was carried out, followed by means separations by Tukey's test ($P < 0.05$) (SAS Institute 1998). The means of mortality are presented with the original data. The LT_{50} was determined by probit analysis in the program ED_{50} plus v1.0.

RESULTS AND DISCUSSION

The strains SBT#11 and SBT#16 of *B. bassiana* caused 100% mortality on *S. litura* larvae under laboratory condition. The mortality data showed that from the fourth days to 10, strain SBT#11 of *B. bassiana* was statistically more pathogenic than strain SBT#16 ($F = 115.89$; $df = 2, 27$; $P = 0.0001$). On 11th day, 100% mortality was recorded by both strains of the fungus. All the cadavers of *S. litura* showed sporulation of the fungus, indicating that death was caused by infection of the fungi under laboratory conditions.

The LT_{50} occurred at 5.1 day in the laboratory condition for strain SBT#11, and at 6 day for strain SBT#16. Though LT_{50} value was slightly lower for strain SBT#16, but it eventually killed 100% of *S.litura* larvae within 11 days. Fungal sporulation was first observed primarily on the mouth parts, thorax and eventually spreading to other parts of the insect cadaver. Samuels *et al.* (1989) from their study noted that an LT_{50} longer than 14 d indicated non-pathogenicity. The *Beauveria bassiana* has the potential to control over 70 insects pest of various crops, and appears to be innocuous to most non-target organisms (Feng *et al.*, 1994). Purwar and Sachan (2005) reported that in general, *B. bassiana* was more virulent than *M. anisopliae* to *Spodoptera* spp. Tanada and Kaya (1993) argue that Infection and sporulation of several entomopathogenic fungi are influenced by environmental factors, especially temperature and humidity, and to lesser extent photoperiod. In this study, daily survival rate was observed to decrease in the treated compared to control i.e., when 100%of the infected specimen had died at the end of the experiment than control and also noted the difference between the LT_{50} values for the treated and control groups. The results indicate the potential for utilization of entomopathogenic fungi to control insect pest, *S. litura*. Further research will be conducted in different formulation and field trials to evaluate the effectiveness of *Beauveria bassiana* for controlling *Spodoptera litura* and other pests of crops.

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REFERENCES:

Coates, B. S., Hellmich, R. L., Lewis, L.C. 2002. Allelic variation of a *Beauveria bassiana* (Ascomycotina: Hypocreales) minisatellite is independent of host range and geographic origin. *Genome*, **45**: 1: 125 - 132.

Feng, M. G., Poprawski, T. J., Khachatourians, G. G. 1994. Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: current status. *Bioscience Control Technology*, **4**: 3 - 34.

Ferron, P., Fargues, J., Riba, G. 1991. Fungi as microbial insecticides against pests. In Handbook of applied mycology: Humans, animals and insects (Arora, D.K., Mukherji, K.G. and Drouhet, E. eds.), New York, Marcel Dekker. **2**: 665 - 705.

Gomez, A. K. and Gomez, A. A. 1984. Statistical procedures for agricultural research. Singapore: John Wiley & Sons, Inc.

Hajek, A. E, Wraight, S. P. and Vanderberg, J. D. 2001. Control of arthropods using pathogenic fungi, In: Bioexploitation of filamentous fungi (Pointing, S.B. and Hyde, K. D. eds.), *Fungal diversity Research Series.* **6**: 309 - 347.

Liburd, O. E., Funderburk, J. E. and Olson, S. M. 2000. Effect of biological and chemical insecticides on *Spodoptera* species (Lepidoptera : Noctuidae) and marketable yields of tomatoes. *Journal of Applied Entomology*, **124**:19 - 25.

McGuire, R.M., Mauricio Ulloa, Young-Hoon Park and Neal Hudson. 2005. Biological and molecular characteristics of *Beauveria bassiana* isolates from California *Lygus hesperus* (Hemiptera: Miridae) populations. *Biological Control*, **33:** 307 - 314.

Purwar, P. and Sachan, G. C. 2005. Biotoxicity of *Beauveria bassiana* and *Metarhizium anisopliae* against *Spodoptera litura* and *Spilarctia oblique*. *Annals of Plant Protection Sciences*, **13**: (2).

Rehner, S.A. and Buckley, E. 2005. A *Beauveria* phylogeny inferred from nuclear ITS and EF1-a sequence: evidence for cryptic diversification and links to Cordyceps teleomorphs. *Mycologia*, **97**: 84 - 98.

Samuels, K. D. Z., Eale, J.B.H. and Llewellyn, L. 1989. Characteristics relating to the pathogenicity of *Metarhizium anisopliae* toward *Nilaparvata lugens*. *Journal of Invertebrate Pathology*, **53**: 25-31.

Tanada, Y., and Kaya, H. K. 1993. *Insect Pathology.* San Diego, CA, USA: Academic Press, 666 **PP**.

Uma Devi., K, Murali Mohan, C.H., Padmavathi., J, Ramesh K. 2003. Susceptibility to fungi of cotton boll worms before and after a natural epizootic of the entomo pathogenic fungus Nomuraea rileyi (Hyphomycetes). *Biocontrol Science and Technology,* **13:** 367 - 371.

Uma Mahaeswara Rao, C., Uma Devi, K. and Albar Ali Khan, P. 2006. Effect of combination treatment with entomopathogenic fungi *Beauveria bassiana* and *Nomuraea rileyi* (Hypocreales) on *Spodoptera litura* (Lepidoptera: Noctuidae). *Biocontrol Science and Technology*, **16**(3): 221 – 232.

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