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Pathogenicity of *Beauveria bassiana* aginst second instar larvae of *Spodoptera litura* and Compatibility with insecticides

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Abstract
An investigation entitled "cultural and morphological characterizations of *Beauveria bassiana*" was carried out during 2018-2019. Influence of different spore concentrations *viz*., 10^5, 10^6, 10^7, 10^8 and 10^9 spores per ml were tested for the pathogenicity of *B. bassiana* against second instar larvae of *Spodoptera litura*. Similarly compatibility of *B. bassiana* was evaluated with commonly used insecticides *viz.*, spinosad 45 SC, imidachloprid 200 SL, chloropyriphos 20 EC and indoxacarb 14.5 SC. In terms of larval mortality, 10^7 spores per ml was recorded highest mortality of *S. litura* followed by 10^8 spores per ml, least larval mortality was observed at 10^5 spores per ml after 3, 5, 7 and 10 days of treatment. Compatibility of *B. bassiana* against commonly used insecticides in plant protection was tested. Imidachloprid were non-toxic to *B. bassiana*, as no significant reduction in radial growth was noticed. The insecticide chloropyriphos recorded 77.20 per cent inhibition of growth and was toxic to *B. bassiana*.

Keywords: Biological control, *Beauveria bassiana*, *Spodoptera litura*, compatability

Introduction
Entomogenous fungi are potentially the most versatile biological control agents, due to their wide host range that often results in natural epizootics. An attractive feature of these fungi is that infectivity is by contact and the action is through penetration (Nadeau *et al*. 1996) [4]. These fungi comprise a heterogenous group of over 100 genera with approximately 750 species, reported from different insects. Many of these offer a great potential in pest management. The most important fungal pathogens are *Metarhizium spp*., *Beauveria spp*., *Metarhizium rileyi*, *Lecanicillium lecanii* and *Hirsutella* spp.
The availability of *B. bassiana* virulence as myco-insecticide or pathogen which naturally survives in the field is the first step in development of controlling agents to biological elements. Virulence isolate can be obtained through selection using one to three characters such as biological, molecular or phenotypic in colony morphology. Selection of fungus isolate according to phenotypic characters in colony morphology is easier and more affordable compared with that of biological and molecular characters. Various isolates of *B. bassiana* indicate that phenotypic character in colony morphology is correlated to virulence isolates (Afandhi *et al*. 2012) [2].

Materials and Methods
The present investigation on "Cultural and Morphological Characterizations of *Beauveria bassiana*" was conducted during 2018-2019 in the Laboratory of Plant Pathology Section and Department of Agricultural Entomology, College of Agriculture, Nagpur. In this experiment, various material were used and methods adopted during the entire course of investigation are described here.

Source of culture
The pure culture of fungus *Beauveria bassiana* was isolated from naturally infected larvae of lepidopteran pests on different field conditions from different places.

Test insect
Spodoptera litura was used as test insect. The larvae were collected from cotton, soybean and castor crops from Agronomy and Entomology field of Agriculture College, Nagpur and they were reared in the laboratory on artificial diet.

The F1 generation larvae of second instar were used for studies.

Pathogenicity studies

To test the pathogenicity of B. bassiana against S. litura simultaneously five different concentrations were evaluated. The test concentrations taken up for the study include (Fatima *et al*. 2017) [3]. 105, 106, 107, 108 and 109 spores per ml and control was maintained.

The second instar larvae of S. litura were taken separately in sterile petriplates lined with filter paper and were directly sprayed with 2 ml spore suspension using hand atomizer. For each concentration, three replications were maintained with 10 larvae in each replication. Untreated control was maintained simultaneously by spraying with only 0.02 per cent tween-20 in sterile distilled water. After air drying, the treated larvae were placed in petriplates that were carefully reared by providing fresh castor leaves daily to *S. litura*. The experiment was conducted at room temperature of 25 °C and relative humidity of 95 per cent. The efficacy of *B. bassiana* in terms of pathogenicity to *S. litura* was evaluated and data were recorded on per cent larval mortality (from date of inoculation to final instar). The larval mortality on incubation for each subsequent day up to 10th day after treatment were recorded. From these observations, per cent cumulative larval mortality after 3, 5, 7 and 10th days was calculated.

Compatibility of *B. bassiana* with selective insecticides

The compatibility of four commonly used insecticides *viz*. spinosad, imidachloprid, chloropyriphos and indoxacard on variety of field crops were tested against *B. bassiana* by adopting poison food technique (Rajanikantha *et al*. 2010) [5]. The insecticides were tested at recommended dose concentrations. For each treatment 100 ml sabouraud dextrose agar medium was taken in 250 ml conical flask and autoclaved at 1.04 Kg/cm2 for 20 minutes. The specified concentration of insecticide was added at lukewarm temperature and mixed thoroughly by shaking the flask. The poisoned medium was poured into three petriplates and allowed to solidify. Test fungus culture was cut into 5 mm disc from the periphery of 10 day old pure culture with sterilized Cork borer and transferred to the centre of each of the plates containing poisoned medium. Controls were maintained by placing fungal disc in medium without insecticides. All the plates were incubated at 25° C. The entire procedure was carried out under aseptic conditions. The diameter of fungal colony was measured seven days after inoculation and per cent inhibition over the control was calculated.

Results and Discussion

Table 1: Mean cumulative per cent mortality of 2*nd* instar larvae of *Spodoptera litura* with *Beauveria bassiana* at different concentration

Conc. of spore/ml	Per cent cumulative mortality of 2nd instar larvae of *S. litura* days after treatment (DAT)*			
	3 Days**	5 Days***	7 Days***	10 Days***
10^9	10.00 (3.16)	28.33 (32.15)	45.00 (42.13)	71.66 (57.79)
10^8	11.66 (3.41)	40.00 (39.23)	66.66 (54.73)	81.66 (64.64)
10^7	18.00 (4.24)	71.66 (57.87)	78.33 (62.25)	92.33 (73.92)
10^6	3.33 (1.82)	16.66 (24.08)	26.66 (31.04)	36.66 (37.26)
10^5	0.00 (00)	10.00 (18.43)	20.00 (26.56)	31.66 (34.24)
Control	0.00 (00)	0.00 (00)	0.00 (00)	0.00 (00)
SE (m)±	1.42	2.04	2.04	1.89
CD (P=0.01)	5.84	8.36	8.36	7.76

*Average of three replications,
** Figure in parenthesis are square root transformed value
*** Figure in parenthesis arearcsin transformed value

The effect of different spore concentrations of *B. bassiana* on the second instar of *S. litura* was evaluated and were presented in the (table 1). Perusal of the data obtained in the experiment carried out for studying pathogenicity of *B. bassiana* against *S. litura* indicated that, significant increase in per cent mortality when compared to untreated (0.0 per cent) was recorded at 10^7, 10^8, 10^9, 10^6 and 10^5 spores per ml was 92.33, 81.66, 71.66, 36.66 and 31.66 per cent respectively. Highest larval mortality of 92.33 per cent was noticed in the treatment where concentration of *B. bassiana* was 10^7 spores per ml which was significantly different from untreated control (0.0 per cent), followed by 81.66 per cent at 10^8 spores per ml concentration. Least larval mortality of 31.66 per cent was noticed at a test concentration of 10^5 spores per ml. Mortality of 36.66 and 71.66 per cent was observed when *B. bassiana* was used at 10^6 and 10^9 spores per ml concentration, respectively. This was evidenced by the fact that 1×10^7 spores per ml concentration of *B. bassiana* in general was found promising in terms of increased per cent larval mortality, reduced pupation and reduced per cent adult emergence of both *S. litura* and *P. xylostella*. Fatima *et al.* (2017) [3].

Compatibility of *B. bassiana* with selective insecticides

Table 2: Effect of selective insecticides on radial mycelial growth of *Beauveria bassiana*

Sr. No.	Treatment	Conc. (%)	Radial mycelial growth after 7 days in (mm)*	Percent growth inhibition
1	Imidacloprid 200 SL	0.0045	29	27.25
2	Spinosad 45 SC	0.018	24	40
3	Chloropyriphos 20 EC	0.05	9.12	77.20
4	Indoxacarb 14.5 SC	0.0145	15.66	60.85
5	Control	-	40	-
SE(m)±			0.59	
CD (P=0.01)			2.52	

The isolate of *B. bassiana* were found compatible with the insecticide imidacloprid when compared to other insecticides spinosad, chloropyriphos and indoxacarb by recording 27.25 per cent of least inhibition of growth. Chlorpyriphos was found to be highly incompatible with *B. bassiana* and exhibited high inhibition of growth with 77.20 per cent (table 2). The results concur with the findings of Rajanikanth *et al.* (2010) [5] reported that the *B. bassiana* was compatible with imidacloprid. Chlorpyriphos was found to be incompatible with all the strains. According to Abidin *et al.* (2017) [1] reported that the highest conidial production of the fungi was triggered by imidacloprid 0.5 x DF. Based on compatibility calculation, imidacloprid 0.5 x DF worked with *B. basiana* (BI: 67.77).

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