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Screening isolates of *Beauveria bassiana* (Bals.) Vuill. for their virulence against larvae of *Helicoverpa armigera* (Hub.) on cotton

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ABSRACT : Nine isolates Bb (PDBC), Bb 4468, Bb 5412, Bb 4521, Bb 5409 Bb 4810, Bb 5411, Bb 5408, and commercial preparation of the entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuill. from Indore Biotech Ltd were bioassayed against second instar larvae of *Helicoverpa armigera* (Hub.) collected from cotton field. All the isolates were found pathogenic causing larval mortality which ranged from 43.3 to 78.3 per cent. The commercial preparation from Indore Biotech Ltd. was found to be the most effective.

Key words : *Beauveria,* bioassay, cotton, *Helicoverpa,* mortality

Cotton is an important fibre crop and *Helicoverpa armigera* (Hub.) is an important pest of cotton. *Beauveria bassiana* (Bals.) Vuill. has wide host range, spanning almost all orders of the class Insecta (Challa *et al.,* 2013). It is one of the most familiar entomopathogenic fungus owing to its wide geographical distribution and host range. The cost of developing a chemical insecticide, the environmental concerns, regulations, etc. are responsible for the interest in this new line of products apart from the fact that the number of promising chemicals is dwindling fast. The increased tempo of development in biopesticides is closely related to the growing awareness and emphasis on integrated pest management (IPM) and sustainable agriculture. Those are compatible with other methods of pest control and can fit into the concept of integrated pest manangment.

The present investigation was carried out in Department of Entomology of CCS Haryana Agricultural University, Hisar during 2006-2007. Nine isolates including one commercial preparation of *B. bassiana* were bioassayed against *H. armigera*. The *B. bassiana* isolates used in the present experiment were Bb (PDBC) from Project Directorate of Biological Control, Bb 4468, Bb 5412, Bb 4521, Bb 5409, Bb 4810 and Bb 5411 from Indian Type Culture Collection (IITC) unit, Division of Plant Pathology, IARI, New Delhi and one *B. bassiana* based commercial preparations from Indore Biotech limited. Bb 4810 and Bb 4521 isolates were isolated from sugarcane borers while rests of the isolates were from *H. armigera* on tomato grown at various places in India. Pure cultures of the fungi were maintained on potato dextrose agar (PDA) slants.

The fungal pathogens were cultured on PDA medium and flasks were incubated for 18-20 days at 25±1 ^{0}C. The fungal conidia were harvested in 10 ml of sterilized distilled water containing 0.05 per cent teepol as wetting agent. The stock suspension was made after filtration of the conidia through double layered muslin cloth. The flasks were shaken well on mechanical shaker for 25 min. The conidial count of this stock suspension was estimated with improved Neubaur haemocytometer under phase contrast microscope at a magnification of 600x. The conidia concentration of the selected isolate was adjusted to $1x10^{7}$ conidia/ml by adding measured quantity of sterilized distilled water.

Average number of conidia/cell was calculated as a mean of conidia counting from the four corners and one central cell. The

concentration of fungal suspension was calculated as per the formula:-

Conidia/ml of suspension = $X \times 2.5 \times 10^5 \times D$

Where ; X = Average number of conidia/big square of haemocytometer

D = Dilution factor

The culture of *H. armigera* was maintained in the laboratory on gram flour based semi-synthetic diet as suggested by Baruah and Chauhan (2001) at a temperature of 27±1 ^{0}C and 60±10 per cent relative humidity. Field collected larvae of *H. armigera* were reared individually in small round plastic vials of size 10x3.5cm till pupation. Pupae were sexed and kept separately. After emergence 10 adults (5 males + 5 females) were released into a rearing jar of size 20x15cm, which served as oviposition chamber for egg laying on the piece of muslin cloth covered over the open mouth of rearing jars and fastened with the help of bands. The jars were kept in the incubator for oviposition at 70-80 per cent relative humidity. After two days, the muslin cloth on which a large number of shining greenish yellow, spherical flat based (dome shaped) eggs were laid singly, was removed and replaced with fresh one.

Muslin cloth carrying eggs was washed thoroughly with 10 per cent formalin solution to avoid or to remove viral contamination. The cloth piece was washed with distilled water to remove formalin effect. Then the cloth was air dried and the eggs were picked up with the help of a camel hair brush and were placed for hatching on a piece of moist white filter paper in the incubator to avoid desiccation. The incubation period of eggs was about 2-3 days at 25-30 ^{0}C. On the third day young caterpillars hatched out from the eggs. Before transferring the neonate larvae directly to semi-synthetic diet, they were transferred for first two days to small cut pieces of young leaves (3×3 cm) and twigs of cotton flower. The average larval period was 16-17 days. Larvae in pre pupal stage stopped feeding and started to bore the cotton plug. At this stage these were transferred to glass battery jars of size 20×15cm containing sand media for pupation.

Second instar larvae (approximately 7-10 mm size) of *H. armigera* were bioassayed for determining the most virulent isolates. Twenty surface sterilized second instar larvae in replication of three were treated with a concentration of 1×10^7 conidia/ml of suspension of each isolate using submersion technique. A set of another 20 larvae in replicates of 3 served as control. The larvae were observed daily for their mortality. Insect body surface contamination technique was adopted for inoculation. The larvae dipped in conidial suspension and were dried for five minutes and transferred to artificial diet. This was done to ensure contact of the conidia with the larval body, as this fungus grows only when it comes in contact with the integument.

All isolates of *B. bassiana* under test were found pathogenic to *H. armigera* (Table 1). The pathogenicity studies showed the differential per cent mortality with respect to different isolates of *B. bassiana.* The per cent larval mortality ranged from 43.3-78.3 per cent.

The commercial preparation taken was found to be most virulent, inflicting larval mortality of 78.3 per cent followed by the isolate Bb 5408 with 66.6 per cent mortality. The isolates Bb 4468 (58.3%), Bb 5411 (58.3%), Bb 5409 (51.6%) and Bb-PDBC (51.6%) were next in terms of pathogenicity. The lowest mortality count of 43.3 per cent mortality was recorded with isolate Bb 4521. No mortality was observed in control.

It is evident from the results that commercial preparation of the fungus from Indore Biotech was significantly highly toxic to *H. armigera* larvae than the remaining isolates of *B. bassiana*, obtained from Indian Type Culture Collection Unit, IARI, New Delhi or Project Directorate of Biological Control, Bangalore.

The isolates from Indian Type Culture

Table 1. Pathogenicity of different *B. bassiana* isolates against second instar larvae of *H.armigera*

Sr. no.	Isolates of *Beauveria bassiana*	Mean per cent mortality
1.	Bb (PDBC)	51.6 (45.9)
2.	Bb 4468Bb	58.3 (49.9)
3.	5412Bb	50.0 (44.9)
4.	4521Bb	43.3 (41.1)
5.	5409Bb	51.6 (45.9)
6.	4810Bb	46.6 (43.0)
7.	5411Bb	58.3 (49.7)
8.	Bb 5408	66.6 (54.7)
9.	Commercial preparation (Indore biotech.)	78.3 (62.2)
	SE (m)	(1.34)
	CD(p=0.05)	(4.0)

Figures in parentheses are arcsin transformed values

Collection Unit (IITCU) and Project Directorate of Biological Control (PDBC) were isolated primarily from *H. armigera* infesting tomato. Perhaps because these were obtained from cadaver of the larvae of *H. armigera*, infecting tomato under varying agroclimatic conditions, the virulence and efficacy of isolates varied. Difference in the level of virulence of isolates may be due to the fact that the microbes undergo selection, recombination and mutation depending upon the ecological situations that ultimately influence their genetic make up. Tyagi *et al.,*(2010) found variations in toxin production in different isolates of *B. bassiana* which could be correlated with the virulence.

Studies conducted by Nguyen *et al.,*(2007) revealed that susceptibility of larvae was negatively associated with age of larvae. Isolates Bb 4810 and Bb 4521 were from sugarcane borers, these may not be as effective against *H. armigera* because of specificity to host. The other possible reason for these cultures being less pathogenic may be their continued sub culturing on artificial media for years. Also *Beauveria* lost its virulence after one year of growth on artificial media. Besides virulence, the mycelial growth, sporulation, germination and toxin production may be reduced progressively due to successive sub culturing on artificial media. Intermittent culturing of isolates of the fungus on natural host is a good mean, therefore, of restoring and maintaining virulence.

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