Comparative vulnerability of *Helicoverpa armigera* (Hubner) larvae to selected entomopathogenic fungi

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**Abstract**

The larvae of *Helicoverpa armigera* (Hubner), (Lepidoptera: Noctuidae), a polyphagous pest affecting different crops in India, were treated with variable concentrations of conidia of two soil isolates and two commercially available entomopathogenic fungi belonging to two species, *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) in a laboratory. The suspension of conidia (10^9 conidia/ml) collected from Sabouraud dextrose agar media with yeast extract (SDAY) plates resulted in the highest mortality (98.3%) with *Beauveria bassiana* (PSC-13) and the lower mortality (75%) with *Metarhizium anisopliae* (Ag-CM). Using the larval immersion method, the values of LC50 and LC90 reveal that *Beauveria bassiana* was the most virulent species while *Metarhizium anisopliae* was the less virulent species in between the entomopathogenic fungi used in bioassay. The local strain of *Beauveria bassiana* (PSC-13) and *Metarhizium anisopliae* (PSC-11) was more virulent than the tested commercial strain.

**Keywords:** Entomopathogenic fungi, *Helicoverpa armigera*, Biocontrol, Bioassay, *Beauveria bassiana*, *Metarhizium anisopliae*.

**Introduction**

The legume pod borer or cotton bollworm *Helicoverpa armigera* (Hubner), (Lepidoptera: Noctuidae) is a cosmopolitan polyphagous pest of more than 182 plant species including cotton, pigeon pea, chickpea, peas, cowpea, field beans, tomato, sorghum, groundnut, tobacco, maize and a range of vegetables, fruit crops and trees species (Gowda, 2005) [12]. A chemical insecticide is the main pest control agent in developing countries like India, currently increasing cases of resistance reduces the vulnerability of *H. armigera* to many synthetic chemical insecticides reported throughout the world (Ahmad et al. 1997; Gunning et al. 1998; Martin et al. 2000) [3, 13, 18]. Entomopathogenic fungi are one of the alternatives of chemical insecticide to manage the *H. armigera*. The entomopathogenic fungi are well known for environment safety and pest selectivity (Jayaraj et al. 1989) [17]. Entomopathogenic fungi having unique advantages to control insect-pest because they are capable to attack all four stages of insect pest (Ferron 1978) [10]. Biopesticides are more efficacious (70-90%) as compare to chemical insecticides (20-50%) (Ansari et al., 2007) [6]. In the genus *Beauveria, B. bassiana* (Balsamo) Vuillemin is recognized as a common species with a broad ecological host range of more than 700 arthropod species, which covers most orders of the class Insecta (Feng et al., 1994) [9] (Hajek, 2000) [14].

The objective of this study was to evaluate the effectiveness of entomopathogenic fungi for the control of *H. armigera* larvae. The effectiveness of several species of entomopathogenic fungi *B. bassiana, M. anisopliae* commercial as well as local strain were evaluated. Further, the best-performing strain of each tested species was used to characterize.

**Materials and Methods**

**Rearing of Helicoverpa armigera.**

The larvae of the *H. armigera* were collected in 2018 from chickpea farms of the Patna City area in Bihar, India. To avoid cannibalism larvae are kept separately in sterile plastic containers. For feeding of larvae, following the procedures of Abbasi et al. (2007) [1] with minor modification. The artificial diet consists of chickpea flour 171g., 5.7g. active yeast, 7.1g. agar, 2.9g. methyl-para-hydroxy-benzoate, 0.86g. Sorbic acid, 2g. Ascorbic acid, 1.42g.
Vitamin mixture for insects, 40mg. Streptomycin sulphate, and 2ml Formaldehyde in 3.5 litres of distilled water. Larvae were kept in the sterile plastic container on a layer of tissue paper supplemented with a prepared diet.

The emerged adults were transferred to a rearing cage and fed on cotton buds soaked with 10% honey for oviposition. To avoid mortality due to unhygienic conditions, the rearing chamber was cleaned, and fresh food was provided daily. Rearing was carried out in a controlled chamber at 25 ± 2 °C, 70 ± 5% relative humidity with a photoperiod of Light (L) 16: Dark (D) 8. Third instar larvae were used in experiments.

**Preparation of Conidial suspension and viability test**
Conidia of entomopathogenic fungi were harvested by scraping the medium surface by inoculation loop needle and transferred to a test tube containing 1 ml of 0.1% Tween 80. Homogenize the conidia using a Vortex mixer. Conidial concentration was determined by counting using a Neubauer haemocytometer (Alves & Moraes, 1998). Using serial dilution method 10^4 – 10^9 conidia/ml were prepared using distilled water containing Tween 80 (0.1% v/v). The viability of conidia was tested by plating 200 µl conidial suspension (containing 1 × 10^6 conidia ml⁻¹) on SDAY medium and incubated at 25 ± 2 °C and 65 ± 5% RH in dark for 24 hrs. The germination was checked by staining with lactophenol cotton blue and viewed under the microscope (Nikon, x450) (Goettel and Inglis, 1997). [11].

**Bioassay Methodology**
The bioassay technique was used to evaluate the virulence of entomopathogenic fungi. 2 isolates of *B. bassiana* and 2 isolates of *M. anisopliae* were used in the experiment. *Helicoverpa armigera* third instar larvae were dipped into six different spore concentrations (10^4 – 10^8 spore ml⁻¹) for 10 sec. as described by Goettel and Inglis (1997) [11]. For control larvae were dipped into 0.1% Tween 80 solution. Larvae were air-dried by allowing them to freely crawl in laminar airflow for 5-10 minutes and transferred to sterile plastic vials containing a freshly prepared diet. The plastic vials were kept in a BOD incubator at 25 ± 2 °C, 65 ± 5 °C RH. Mortality of larvae and conidial sporulation were examined daily for 15 days. The dead larvae were transferred to a sterilized petri plate having wet cotton to stimulate sporulation. Twenty larvae were used in each treatment and experiments were repeated three times.

**Statistical Analysis**
Correct mortality percentage was calculated by applying Abbott’s formula (Abbott 1925) [2] and before analysis, mortality percentage was transformed and LC₅₀ and LC₉₀ values were determined by the Probit analysis using Microsoft excel-2016 software.

**Results**
*B. bassiana* was more virulent than *M. anisopliae* against third instar larvae of *H. armigera* at different concentrations. However, at 1×10⁸ spore ml⁻¹, all the strain of *B. bassiana* and *M. anisopliae* shows a high degree of mortality after 15 days. Significantly 98.3% virulence was recorded in *B. bassiana* (PSC-13) with LC₃₀ value (1.8 × 10⁶ spores ml⁻¹) followed by *M. anisopliae* PSC-11 (85.00%) with LC₃₀ value (5.07 × 10⁸ spores ml⁻¹) after 15 days of treatment (Table 2).

The experimental data reveal that there was a minimum threshold conidial concentration required for the higher mortality which varied from species to species and even within two isolates of the same species. Minimum LC₃₀ and LC₉₀ were observed with *B. bassiana* (PSC-13) followed by *M. anisopliae* (PSC-11).

The value of LC₃₀ and LC₉₀ reveals that *B. bassiana* (PSC-13) was the most virulent fungal strain followed by *M. anisopliae* (PSC-11). Lowest Possible virulent among the four strains of two species used in the bioassay was *M. anisopliae* (Ag-CM) whereas *B. bassiana* (Ag-CB) had virulence of intermediate level.

**Table 1:** Source of Entomopathogenic fungi used in Bioassay

| Entomopathogenic fungi (Strain) | Source | Location |
|---------------------------------|--------|----------|
| *Beauveria bassiana* (PSC-13)   | Soil   | Patna Science College, Bihar, India |
| *Beauveria bassiana* (Ag-CB)    | Agrizone-Green Beauveria | Bio-Pesticide Pollachi, Tamil Nadu, India |
| *Metarhizium anisopliae* (PSC-11) | Soil | Patna Science College, Bihar, India |
| *Metarhizium anisopliae* (Ag-CM) | Agrizone-Green Met | Bio-Pesticide Pollachi, Tamil Nadu, India |

**Table 2:** LC₅₀ and LC₉₀ of the four fungal isolates used in bioassay against the third instar larvae of *H. armigera*

| Isolate                 | LC₅₀      | LC₉₀      |
|-------------------------|-----------|-----------|
| *B. bassiana* (PSC-13)  | 1.8 × 10⁸  | 8.4 × 10⁸  |
| *B. bassiana* (Ag-CB)   | 7.5 × 10⁸  | 2.72 × 10⁹ |
| *M. anisopliae* (PSC-11)| 5.07 × 10⁸ | 1.9 × 10⁹  |
| *M. anisopliae* (Ag-CM) | 1.09 × 10⁷ | 7.5 × 10⁷  |
Fig 1: Percent mortality of *H. armigera* larvae 15 days post-incubation with a conidial suspension of (a) *B. bassiana* (PSC-13) (b) *B. bassiana* (Ag-CB) (c) *M. anisopliae* (PSC-11) (d) *M. anisopliae* (Ag-CM) as a function of conidial concentration. The percentage mortality values show the mean of three replicate experiments.

**Microscopic View of dead larvae**
Photographs of larvae showing mycosis indicated that the presence of profusely growing mycelia of respective fungi used in bioassay (Figure 2). The characteristic white mycelia appeared on the surface of *H. armigera* larvae when treated with *B. bassiana*, while green mycelia appeared on the surface of larvae when treated with *M. anisopliae*. The *B. bassiana* shows higher mycosis (up to 82%) as compared to *M. anisopliae* (up to 62%).

Fig 2: Third instar larvae of *H. armigera* showing mycosis when treated with (a.) *B. bassiana* (PSC-13) (b.) *M. anisopliae* (PSC-11).

**Features of Entomopathogenic fungi recovered from infected larvae**
The pure culture of all entomopathogenic fungi used in bioassay was recovered from the dead larvae on SDAY media. The recovered isolates showed the respective mycelial and conidial characteristics of entomopathogenic fungi used in the bioassay. Very few contaminations also appeared during entomopathogenic fungi culturing which was insignificant.

**Discussion**
Several local strains of *B. bassiana* have been reported to be pathogenic to *H. armigera* (Sandh et al. 2001). The present study shows that local isolates of *B. bassiana* (PSC-13) and *M. anisopliae* (PSC-11) were more virulent than commercial strain *B. bassiana* (Ag-CB) and *M. anisopliae* (Ag-CB) under
laboratory conditions. This may be due to successive sub-
culturing and preservatives used for long life. As per the 
report of Ana et al. (2018) [17], the fall armyworm, Spodoptera 
frugiperda (J.E Smith) (Lepidoptera: Noctuidae) is highly 
susceptible to B. bassiana isolates causing 100% larval 
mortality at 10⁶ conidia/ml.

The B. bassiana fungal isolate PSC-13 had the highest 
virulence against the third instar larvae of H. armigera 
because it had a lower LC₅₀ and LC₉₀ value. These results 
were compared with those of Swathi et al. (2017) [21] say 
that the lower the LC₅₀ value, the highest (100%) larval 
mortality of H. armigera from B. bassiana (strain-4). Various 
research shows that B. bassiana and M. anisopliae express insecticidal 
activity against Rhynchophorus ferrugineus (El Kichaoui, 
Abu & El-hindi, 2017) [8] H. armigera (Douro et al. 2012) [7], 
Bemisia tabaci biotype B (Mascarin et al. 2013) [19], 
Spodoptera exigua (Wright et al. 2010) [22] and Plutella 
xylostella (Xia et al. 2013) [23].

According to the study by Quesada, Moraga et al. (2006) [19] 
the efficiency of the entomopathogenic fungi began clearly 
after 48 hrs. of inoculation and the hyphae penetrated the 
tegument, trachea, and epithelial cell. Entomopathogenic 
fungi sporulation on cadavers is a key factor for proliferation 
and disease spread within pest population (Hajek et al. 1994) 
[15], (Inglis et al. 2001) [16].

In this study, high mortality level (98.3%) was achieved for 
H. armigera third instar larvae treated with 10⁶ spores/ml. of 
B. bassiana (PSC-13) at 15 days of treatment under 
laboratory. For effects on larvae, an adequate concentration of 
the pathogens is required. It also reveals that there is an 
increase in mortality with increment in dosage of entomopathogenic fungi. Biopesticides could be promising 
biological control agents against H. armigera larvae as an 
alternative to chemical insecticides in the leguminous plant 
including chickpea. Further efforts to develop more potent 
biopesticides for controlling H. armigera in chickpeas are 
required to screen new, possibly more virulent isolates.

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References
1. Abbasi B, Ahmed K, Khalique F, Ayub N, Liu H, Kazmi 
S, et al. Rearing of African bollworm, Helicoverpa 
armigera, on a tapioca-based artificial diet. Journal of 
Insect Science 2007;7:1-7.
2. Abbott WS. A method of computing the effectiveness of 
an insecticide. J Econ Entomol 1925;18:265-267.
3. Ahmad M, Arif MI, Attique MR. Pyrethroid resistance of 
Helicoverpa armigera (Lepidoptera: Noctuidae) in 
Pakistan. Bulletin of Entomological Research 1997;87:343-347.
4. Alves SB, Moraes SA. Quantificação de inóculo de 
patógenos de insetos. In S. B. Alves (Ed.), Controle 
microbiano de insetos (2nd ed., pp. 765–778). Piracicaba:
FEALQ (chap. 23) 1998.
5. Ana M, Maria DL, Jorge EL, Ma CD. High virulence of 
Mexican entomopathogenic fungi against fall armyworm, 
Spodoptera frugiperda (J.E. Smith) (Lepidoptera: 
Noctuidae). Journal of Economic Entomology 2018;112(1):99-107.
6. Ansari MA, Shah FA, Whittaker M, Prasad M, Butt TM. 
Control of western flower thrips (Frankliniella 
occidentalis) pupae with Metarhizium anisopliae in peat 
and peat alternative growing media. Biological Control, 2007;40:293-297.
7. Douro KO, Djegui D, Glitho IA, Tamo M. Sensitivity of 
Helicoverpa armigera (Hübner) (Lepidoptera: 
Noctuidea) to the entomopathogenic fungi, Metarhizium 
anisopliae and Beauveria bassiana in laboratory. Journal of 
Agriculture & Biological Science 2012;7(12):1007-
1015.
8. El-Kichaoui AY, Abu AB, El-Hindi MW. Isolation, 
molecular identification and under lab evaluation of the 
entomopathogenic fungi Metarhizium anisopliae and 
Beauveria bassiana against the red palm weevil, R. 
ferrugineus, in Gaza Strip. Advances in Microbiology 
2017;7:109-124.
9. Feng M, Poprawski T, Khachatourians GG. Production, 
formulation, and application of the entomopathogenic 
fungus Beauveria bassiana for insect control: current 
status. Biocontrol Sci Technol 1994;4(1):3-34
10. Ferron P. Biological Control of Insect Pests by 
Entomogenous Fungi. Annual Review of Entomology, 
1978;23:409-442.
11. Goettel MS, Inglis GD. Fungi: Hyphomycetes. In L. A. 
Lacey (Ed.), Manual of techniques in insect pathology 
(pp. 213–247). San Diego, CA: Academic Press 1997.
12. Gowda C.L.L. Helicoverpa-The Global Problem. In: 
Sharma H.C. (editor) Heliothis/Helicoverpa Management 
Emerging Trends and Strategies for Future Research. 
Science Publishers, Inc. Enfield, USA 2005.
13. Gunning RV, Moores GD, Devonshire AL. Insensitive 
acetylcholinesterase causes resistance to 
organophosphates in Australia Helicoverpa armigera 
(Hu‘ner) (Lepidoptera: Noctuidae). Pesticide Science 
1998;54:319-320.
14. Hajek AE, Butler L. Predicting the Host Range of 
Entomopathogenic Fungi. In: Follen P.A., Duan J.J. (eds) 
Nontarget Effects of Biological Control. Springer, 
Boston, MA 2000.
15. Hajek AE, Leger RJ. Interactions between fungal 
pathogens and insect hosts. Annual Review of 
Entomology 1994;39:293-322.
16. Inglis GD, Goettel MS, Butt TM, Strasser H. Use of 
Hyphomycetous fungi for managing insect pests. In: Butt 
TM, Jackson C, Magan N (Eds.) Fungi as biocontrol 
agent progress, problems, and potential. Wallingford, 
UK: CABl Publishing 2001, 23-70.
17. Jayaraj S, Rabindra RJ, Narayanan K. Development and 
use of microbial agents for control of Heliothis spp. 
(Lepidoptera: Noctuidae) in India. In: King EG, Jackson 
RD, editors. Proceedings of the workshop on biological 
control of Heliothis: Increasing the effectiveness of 
natural enemies. 11_15 November 1985, New Delhi, 
India. Far Eastern Regional Research Office, US 
Department of Agriculture 1989, 483-503.
18. Martin T, Ochou GO, Hala N’Klo F, Wassal J, Waisayre 
M. Pyrethroid resistance in cotton bollworm Helicoverpa 
armigera in West Africa. Pest Management Science 
2000;56:549-554.
19. Mascarin GM, Kobori NN, Quintela ED, Delalbera I. 
The virulence of entomopathogenic fungi against Bemisia 
tabaci biotype B (Hemiptera: Aleyrodidae) and their 
conidial production using solid substrate fermentation. 
Biological Control 2013;66:209-218.

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20. Quesada-Moraga E, Carrasco-Diaz JA, Santiago-Alvarez C. Insecticidal and antifeedant activities of proteins secreted by entomopathogenic fungi against *Spodoptera littoralis* (Lep: Noctuidae). Journal of Applied Entomology 2006;130:442-452.

21. Swathi P, Ganga P, Visalakshy N, Das SB. Potentiality of *Beauveria bassiana* strains against *Helicoverpa armigera* through laboratory bioassay. Journal of Entomology & Zoology 2017;5(3):463-467.

22. Wraight SP, Ramos ME, Avery PB, Jaronski ST, Vandenberg JD. Comparative virulence of *Beauveria bassiana* isolates against lepidopteran pests of vegetable crops. Journal of Invertebrate Pathology 2010;103(3):186-199.

23. Xia J, Huang Z, Hu Q. Histopathological study of *Plutella xylostella* infected by three entomopathogenic fungal species. Advances in Entomology 2013;1(2):15-19.

24. Zimmermann AL. The ‘Galleria bait method’ for detection of entomopathogenic fungi in soil. Journal of Applied Entomology 1986;102:213-215.