Evaluation of *Beauveria bassiana* and *Metarhizium anisopliae*, for the management of *Helicoverpa armigera* (Hubner)

AJEET KUMAR AND CHOUDHARY SHARFUDDIN

J. Mycopathol. Res. 60(1) : *91-97, 2022;*
ISSN 0971-3719

Department of Botany,
University of Calcutta,
Kolkata 700 019, India

J. Mycopathol. Res. **60(1)** : 91-97, 2022; (ISSN 0971-3719)

Evaluation of *Beauveria bassiana* and *Metarhizium anisopliae*, for the management of *Helicoverpa armigera* (Hubner)

AJEET KUMAR AND CHOUDHARY SHARFUDDIN
Plant Pathology and Microbiology Laboratory, Department of Botany, Patna University, Patna-800005, Bihar

Received : 04.12.2021 Accepted : 28.01.2022 Published : 28.03.2022

The old-world bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), is a destructive pest of chickpeas and pigeon pea that has proven difficult to control using traditional methods. In the present investigation the virulence of 6 local isolates of *Beauveria bassiana* and *Metarhizium anisopliae* against third instar larvae of *H. armigera* was evaluated. Two isolates of *B. bassiana*, PSC-13, PSC-14 and two of *M. anisopliae* strain PSC-11 and PSC-12 were most effective against third instar *H. armigera* at 10^9 conidia/ml. At this rate, *B. bassiana* was more virulent to the third instar *H. armigera* than *M. anisopliae*. Among the *B. bassiana* isolates, PSC-13 had the highest virulence; i.e., LC_{50} (1.8×10^6 conidia/ml). Finally, it wasobserved that PSC-13 isolate show potential as biological control agents of *H. armigera* larvae. Further research should focus on the effectiveness of *Beauveria* and *Metarhizium* under field conditions.

Key words : *Beauveria bassiana*, *Metarhizium anisopliae*, biocontrol, bioassay, *Helicoverpa armigera*

INTRODUCTION

India is the largest producer of chickpea, representing 70% and 73% of the world's share of the crop and production area (FAOSTAT 2020). It is India's largest legume with 30% of the total crop area, 37% of the pulse production and 43% of the total pulse consumption. Yield losses caused by biotic factors like insect pests, pathogens, and weeds are a major stumbling block to enhanced agricultural production. These losses amount to 68% of potential production, with insect pests accounting for nearly 18% of all crop losses. More than 20,000 species of field and store pests are thought to destroy one-third of the world's food output, worth more than $100 billion per year, with the poor world suffering the greatest losses around 43 % (Oerke, 2005).The old-world bollworm or legume pod borer, *Helicoverpa armigera* (Hubner)is the world's most destructive and polyphagous insect of many agricultural crops causing severe damage and greater loss in a wide range of food, fibre, oil, vegetables, fodder, horticultural, ornamental, aromatic and medicinal plants (Moral Garcia, 2006).

*Correspondence: iajeetji@gmail.com

It has been reported that *H. armigera* has infected more than 182 plant species of 45 families, including 40 dicots and 5 monocots (Sharma and Ortiz, 2002). In India, total population of *Helicoverpa* is represented by three species *H. armigera* (99.2 %), *Heliothis peltigera* (Denis & Schiffermuller) (0.6 %) and *H. assulta* (Guenee) (0.2 %).

The continuous use of the chemical pesticide led to the emergence of resistance in *H. armigera.* In Integrated Pest Management programmes, it is essential to limit the use of insecticides and promote natures enemies of insect pests. To get the best combination of natural enemies and insecticides, evaluating the effects of numerous insecticides on natural enemies is essential. Chemical controls are restricted using insecticides because of broad toxicity for pests, natural enemies as well as human and environment in the management of agricultural systems.

Entomopathogenic fungi have unique advantages over other microbial control agent because they are able to infect all the stages of insects. EPF are one of the components of most terrestrial ecosystems and play a key role in controlling insect population (Jackson *et al.* 2000, Goettel *et al.* 2010).

MATERIALS AND METHODS

Rearing of Helicoverpa armigera

The initial stock of larvae of the *H. armigera* were collected in 2017-2018 from chickpea and pigeon pea farms of the Patna and Nalanda district of Bihar, India. To avoid cannibalism larvae are kept separately in sterile containers capped with perforated mesh to exchange the air. For feeding of larvae, were offered natural diet like caster leaves, chickpea plant and semi-synthetic diet following the procedures of Abbasi *et al*. (2007) with minor modification. The semi-synthetic diet consists of chickpea flour (100 g), active yeast (30 g), agar (15 g) , methyl-para-hydroxy-benzoate (2 g), Sorbic acid (1 g), Ascorbic acid (3g). Vanderzant Vitamin solutions(8ml) for insects, Streptomycin sulphate (40mg), Wesson's salt mix (7g) and Formaldehyde (2ml) in 750ml 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. Paper strips carrying egg masses were collected and transferred to fresh plastic containers and kept in BOD incubator. 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 27 ± 2 °C, 70 ± 5% relative humidity with a photoperiod of Light (L) 16: Dark (D) 8. Third instar larvae were used in experiments.

Isolation and Selection of Entomopathogenic fungi

Entomopathogenic fungi were isolated from the soils of 15 different location using the *Galleria* bait method (Zimmermann, 1986). The infected *Galleria mellonella* (Greater wax moth) larvae were selected (trapped EPF) were cultures on Sabouraud dextrose agar Yeast media SDAY (dextrose 40g, peptone 10 g, agar 15 g, yeast extract 10 g for 1000 ml of media. Chloramphenicol 125 mg/litre was used to prevent bacterial growth. The plates were incubated at 25 ± 2 °C with 65 ± 5% relative humidity for 15-20 days in the dark (Goettel and Inglis, 1997).

Preparation of Conidial suspension and viability test

Surface of a 14-day old culture was gently scratched with inoculation needle and transferred to containers containing 5ml sterile Tween-80 solution (0.1%v/v). The concentration of conidia in stock suspensions was determined by direct counting using Neubauer haemocytometer. Using serial dilution method 10^4 – 10^9 conida/ml were prepared using distilled water containing Tween 80 (0.1% v/v). (Quesada-Moraga *et al.,* 2006). Conidial viability was tested according to Goettel and Inglis (1997). Three droplets of a diluted suspension 1×10^7 conidia ml^{-1} were placed on medium and incubated at 25 ± 1°C and 65 ± 5% in dark for 24 hours. After staining with lactophenol cotton blue, germination was checked under microscope (Nikon × 450). Only spores with a germ tube were considered to have germinated.

Bioassay

The bioassay technique was used to evaluate the virulence of entomopathogenic fungi. Twenty larvae of *H. armigera* (third instar) were transferred into the plastic container, containing 250 ml. of tap water with five different fungi conidial concentrations (1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8, and 1×10^9 conidia/ml) was introduced to bioassay containers for 10 sec. as described by Goettel and Inglis (1997) An equal number of controls were set up simultaneously using non chlorinated tap water. 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.Larval mortality was calculated in a percentage using the following formula:

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduce}} \times 100$$

$$\text{Correct Percentage Mortality} = 1 - \frac{n \text{ in } T \text{ after treatment}}{n \text{ in } C \text{ after treatment}} \times 100$$

where 'C' is the control mortality and 'T' is the treated mortality

Statistical Analysis

Larval mortality data were corrected using formula given by Abbott (1925). LC_{50} and LC_{90} lethal time required to kill 50% and 90% of *H. armigera* larvae were calculated by Probit analysis with a reliable interval of 95% using the Microsoft excel 19.

RESULTS

The fungi isolated from the cadaver (dead *Galleria*) used as bait were found to be isolates of *Beauveria* and *Metarhizium*. These were considered most efficient bio control agents and were selected for further studies.

Table 1. Names, host, collection region and strain code of entomopathogenic fungi isolated from Patna, Bihar**.**

Name of Fungus	Source/Host	Collected region	Strain Code
Beauveria bassiana	Soil	Patna Science college, Patna	PSC-13
Beauveria bassiana	Soil	B.N College, Patna	PSC-14
Beauveria bassiana	Soil	Chickpea field, Patna	PSC-15
Metarhizium anisopliae	Soil	Bairiya, Patna	PSC-10
Metarhizium anisopliae	Soil	Patna Science college, Patna	PSC-11
Metarhizium anisopliae	Cadaver	Karanpura, Patna	PSC-12

Table 2. Morphological Characteristics of Isolated Fungi

Entomopathogenic fungi	Colour of colony	
	Above media	Reverse of plate
Beauveria bassiana	Whitish, later yellowish or occasionally reddish	Uncoloured, or yellowish to pinkish
Metarhizium anisopliae	Khaki colour or light Greenish with yellow shad	Uncoloured or yellowish

Beauveria bassiana

B. bassiana was cultured on Sabouraud Dextrose Agar medium. It produced white coloured mycelial colony, later yellowish or occasionally reddish in colour above the media. While it appears uncoloured or yellowish to pinkish colour below or reverse of plate. Many dry powdery conidia in distinctive white spore balls were found. Each ball was composed of a culture of conidiogenous cells. The conidiogenous cells of *B. bassiana* were short ovoid and terminate in a narrow apical extension called rachis. The rachis elongates after each conidium was produced, resulting a zig zag extension. The conidia were single celled haploid and hydrophobic.

Metarhizium anisopliae

M. anisopliae was cultured on Sabouraud Dextrose Agar medium. It produced light green coloured with yellow shade like Khaki colour mycelial colony above the media. While mycelial colony appear uncoloured or yellowish colour below the plate or reverse of the plate. Many dry powdery conidia in distinctive dark green spore balls. Each ball was composed of cluster of conidiogenous cells. The conidiogenous cells of *M. anisopliae* were long ovoid (like rice) and terminate in a narrow apical extension called rachis. The rachis elongates after each conidium was produced, resulting a zig zag extension. The conidia were single celled haploid and hydrophobic.

For the six isolates of entomopathogenic fungi the percent mycosis value followed the pattern of larval

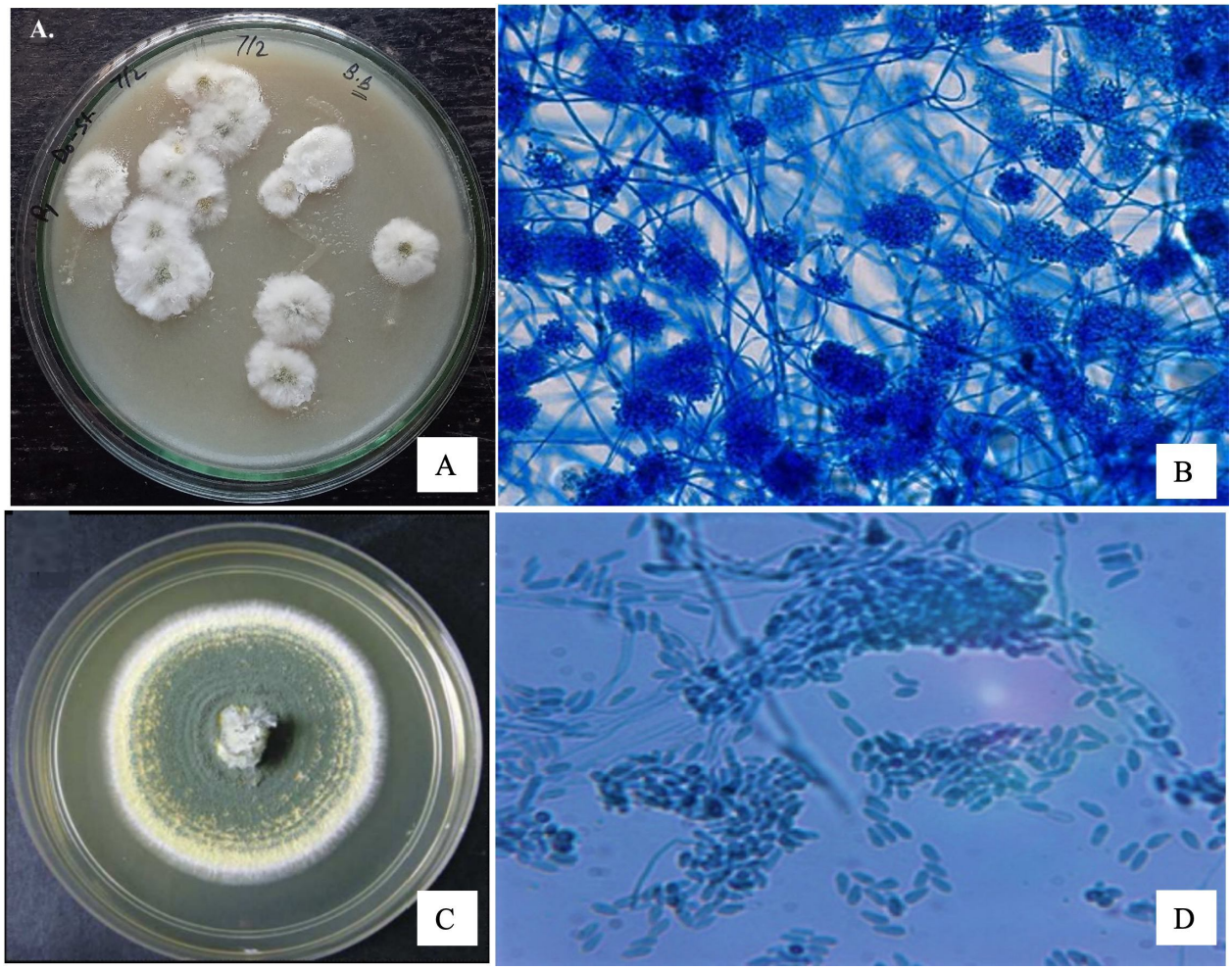

Fig.1(A) *B. bassiana* culture plate on SDAY media, (B) Microscopic view of *B. bassiana* under the microscope (×450), (C) Pure culture of *M. anisopliae* and (D) Microscopic views of *M. anisopliae* under the microscope (×450).

Table 3. Virulence of *B. bassiana* and *M. anisopliae* isolates against the 3rd instar larvae of *H. armigera* in laboratory condition

Fungal Species	Isolates	Mortality (%)	Mycosis (%)
B. bassiana	PSC-13	98.3± 2.88	90± 10
	PSC-14	85± 5	75 ± 5
	PSC-15	80± 10	75± 10
M. anisopliae	PSC-10	70± 5	65 ± 5
	PSC-11	85± 5	85 ± 10
	PSC-12	85± 5	80± 10

Note: After 15 days of treatments at 10^9 conidia/ml. concentration (Mean ± SE).

mortality (Table 3). In all cases, the median lethal concentration (LC_{50}) increased with a decrease in conidial concentrations. Thus, the LC_{50} value of *B. bassiana* (PSC-13) have low value 1.82 $\times 10^6$ spore/ml. among the six isolates of *B. bassiana* and *M. anisopliae.* At the same time, it showed high (90%) mycosis (Table-3).

Table 4: LC_{50} and LC_{90} values of six local isolates used in bioassay against the third instar larvae of *H. armigera*

Fungal Isolate	LC_{50}	LC_{90}
B. bassiana (PSC-13)	1.82×10^6	8.47×10^7
B. bassiana (PSC-14)	7.91×10^6	1.16×10^9
B. bassiana (PSC-15)	7.94×10^6	1.73×10^9
M. anisopliae (PSC-10)	1.13×10^7	3.46×10^9
M. anisopliae (PSC-11)	5.06×10^6	1.90×10^9
M. anisopliae (PSC-12)	7.27×10^6	1.35×10^9

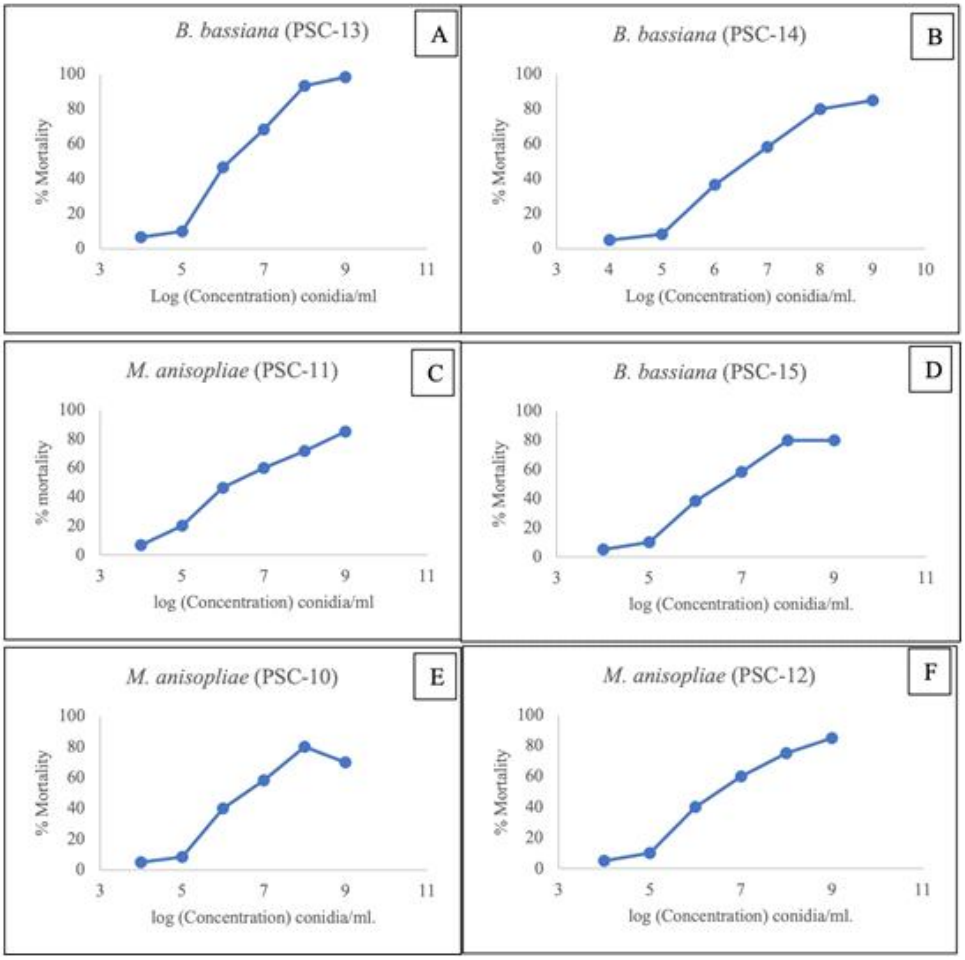

Fig. 2. Percent mortality of *H. armigera* 15 days post inoculation with conidial suspension of (A.) *B. bassiana* (PSC-13), (B.) *B. bassiana* (PSC-14), (C.) *B. bassiana* (PSC-15), (D.) *M. anisopliae* (PSC-10), (E.) *M. anisopliae* (PSC-11) and (F.) *M. anisopliae* (PSC-12) as a function of conidial concentration. Values given are mean of three replicates ±SD.

Bioassay

B. bassiana was more virulent than *M. anisopliae* against third instar larvae of *H. armigera* at different concentrations. However, at 1×10^9 spores ml^{-1}, all the strains of *B. bassiana* and *M. anisopliae* showed a high degree of mortality after 15 days. Significantly 98.3% virulence was recorded in *B.*

bassiana (PSC-13) with LC_{50} value (1.82×10^6 spores ml) followed by *M. anisopliae* PSC-11 (85.00% virulence) with LC_{50} value (5.06×10^6 spores ml) after 15 days of treatment (Table 4). The experimental data revealed 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 LC_{50} and LC_{90} were observed with *B. bassiana* (PSC-13) followed by *M. anisopliae* (PSC-11). The value of LC_{50} and LC_{90} revealed that *B. bassiana* (PSC-13) was the most virulent fungal strain followed by *M. anisopliae* (PSC-11). Lowest Possible virulent among the six strains of two species used in the bioassay was *M. anisopliae* (PSC-10) with LC_{50} value 1.13×10^7 conidia/ml. followed by *B. bassiana* (PSC-15) with LC_{50} value 7.94×10^6 conidia/ml. whereas *B. bassiana* (PSC-14) and *M. anisopliae* (PSC-12) and virulence of intermediate level having LC_{50} value 7.91×10^6 and 7.27×10^6 respectively.

DISCUSSION

Bioassay of isolated entomopathogenic fungi against the third instar larvae of *H. armigera* suggest that all the isolates of entomopathogenic fungi infected the third instar larvae of *H. armigera* in a dose dependent manner. *B. bassiana* (PSC-13) was more infective and resulted in maximum average percent mortality among the six isolates of *B. bassiana* and *M. anisopliae* under investigation. *B. bassiana* (PSC-13) had the highest virulence against the third instar larvae of *H. armigera* because it had a lower LC_{50} and LC_{90} values. Based on the previous reports, it is believed that virulence of both *B. bassiana* and *M. anisopliae* could be due to the following factors: (a) one or more virulence factors such as chitinases, Pr1 and Pr2 proteases, etc. as already shown by EST analysis (Freimoser *et al*. 2003), (b) the occurrence of collagenous protective coat which enables fungi to overcome the innate immunity of insects when the fungus comes into contact with hemolymph (Wang and Leger 2006), and (c) the occurrence of adhesins MAD1 on the surface of conidia for attachment to the cuticle of insects (Wang and Leger 2007).

B. bassiana and *M. anisopliae* are virulent, according to Jarrahi and Safavi (2016) and Kalvnadi *et al*. (2018), producing significant larval mortality and having negative effects on *H. armigera* biological parameters, respectively. Wraight *et al*. (2010) compared the virulence of *B. bassiana* isolates against lepidopteran insect pests, discovering that *S. exigua* and *Helicoverpa zea* were susceptible to fungal infection, while *S. frugiperda* was the least susceptible, indicating that there is a huge variation among the different host and fungal species used in pathogenicity testing.

The ANOVA, average percent mortalities and the LC_{50} and LC_{90} values gives enough evidence to speculate that *B. bassiana* (PSC-13) and *M. anisopliae* (PSC-11) are more virulent among the six isolates of entomopathogenic fungi. Based on our *in vitro* experimental findings, we suggest that *B. bassiana* (PSC-13) and *M. anisopliae* (PSC-11) could be developed as biological control agents against *Helicoverpa armigera* in IPM programs.

ACKNOWLEDGMENTS

Thankful to the Plant Pathology and Microbiology laboratory, Department of Botany, Patna University, Patna for all supports.

REFERENCES

Abbasi, B., Ahmed, K., Khalique, F., Ayub, N., Liu, H., Kazmi, S., Aftab, M. 2007**.** Rearing of African bollworm, Helicoverpa armigera, on a tapioca-based artificial diet. *J. Insect Sci.* **7**: 1–7.

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ.Entomol.***18**: 265–267.

FAOSTAT 2020.https://www.fao.org/3/cb1329en/online/cb1329en. html

Freimoser, F. M., Screen, S., Bagga, S., Hu, G., St. Leger, R. J. 2003**.** Expressed sequence tag (EST) analysis of two subspecies of *Metarhizium anisopliae* reveals a plethora of secreted proteins with potential activity in insect hosts. *Microbiology* **149**: 239-247.

Goettel M.S, Inglis G.D.1997. Fungi: Hyphomycetes. In: *Manual of techniques in insect pathology* (L.A.Lacey,Eds.) Academic Press, pp 213-247.

Goettel, M.S., Eilenberg, J., Glare, T. R. 2010. Entomopathogenic fungi and their role in regulation of insect populations, pp. 387-431. In:*Insect Control Biol. Ans Synth. Agents*.(L.I. Gilbert, D.S. Gill, Eds.), Academic Press, San Diego, CA.

Jackson, T. A., Alves, S. B.,Pereira, R. M. 2000.Success in Biological Control of Soil-dwelling Insects by Pathogens and Nematodes. In : *Biological Control: Measures of Success* (G.Gurr, S.Warren, Eds.), Springer, Dordrecht, pp 271–296.

Jarrahi, A., Safavi, S. A. 2016**.** Fitness costs to *Helicoverpa armigera* after exposure to sub-lethal concentrations of *Metarhiziumanisopliae*sensulato: Study on F1 generation. *J. Invert.Pathol.***138**: 50–56.

Kalvnadi, E.,Mirmoayedi, A., Alizadeh, M., Pourian, H. 2018. Sub-lethal concentrations of the entomopathogenic fungus, *Beauveria bassiana* increase fitness costs of *Helicoverpaarmigera* (Lepidoptera: Noctuidae) offspring. *J. Invert.Pathol.***158**:32–42.

Moral Garcia F. J. 2006. Analysis of the spatiotemporal distribution of *Helicoverpa armigera* (Hübner) in a tomato field using a stochastic approach. *Biosystems Engineering* **93**: 253-259.

Oerke, E.C. 2005. Crop losses to pests.*The J. Agricult. Sci.***144**: 31-43.

Prasad, A., Syed, N. 2010. Evaluating prospects of fungal bio pesticide bassiana (Balsamo) against *Helicoverpaarmigera* (Hubner): Anecosafe strategy for pesticidal pollution. *Asian J. Exp. Biol. Sci***. 1**: 596-601.

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

Sharma, H.C., Ortiz, R. 2002. Host plant resistance to insects: an eco-friendly approach for pest management and environment conservation. *J. Environ. Biol*. **23**: 111-135.

Wang C., Leger S. 2006. A collagenous protective coat enables *Metarhizium anisopliae* to evade insect immune responses. *Proc. Natl. Acad. Sci.* USA **103**:6647–6652.

Wang C., Leger S. 2007. The MAD1 adhesin of *Metarhizium anisopliae* links adhesion with blastospore production and virulence to insects; the MAD2 adhesin enables attachment to plants. *Eukaryotic Cell* **6**:808–816.

Wraight, S. P., Ramos, M. E., Avery, P. B., Jaronski, S. T., Vandenberg, J. D. 2010. Comparative virulence of *Beauveria bassiana* isolates against lepidopteran pests of vegetable crops. *J. Invert.Pathol.* **103**:186–199.

Zimmermann, A.L. 1986. The 'Galleria bait method' for detection of entomopathogenic fungi in soil. *J. Appl.Entomol.***102**: 213–215.