# IN VITRO BIO-EFFICACY OF ENTOMOPATHOGENIC FUNGI BEAUVERIA BASSIANA (BALS.) VUILL., AGAINST GRAM POD BORER, HELICOVERPA ARMIGERA HUBNER ON CHICKPEA

# Yaspal Singh Nirala\*, V.K. Dubey, J.L. Ganguli and R.K.S. Tiwari

Department of Entomology, College of agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur-492012, Chhattisgarh, India Email: <u>ypsnirala@gmail.com</u>

Received-15.06.2017, Revised-26.06.2017

**Abstract:** The present study was conducted at Bio-control lab, Department of Entomology, College of agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur during 2015-16 and 2016-17. The results of *in vitro* experiments revealed that the 2- $3^{rd}$  instars larvae of *Helicoverpa armigera* susceptible to different doses of *Beauveria bassiana*. Mortality of larvae was started after 2-3 day of treatment. Among the doses of *B. bassiana*  $T_4$  (*B. bassiana* @5000g/ha) 0.00-75.00% show the maximum mortality followed by  $T_3$  (*B. bassiana* @3000g/ha) 0.00-45.00%,  $T_2$  (*B. bassiana* @2500g/ha) 0.00-32.00% and  $T_1$  (*B. bassiana* @2000g/ha) 0.00 to 15.00% but superior than control  $T_7$  (0.00%) in both the year.

Keywords: Chickpea, Helicoverpa armigera, Beauveria bassiana

#### INTRODUCTION

Thickpea (Cicer arietinum L.) belongs to the family Leguminosae and commonly known as Chana, Bengal gram or Garbanzo, is mainly used for human consumption and also a small proportion forms the part of animal and poultry feed. It has one of the highest nutritional compositions as of any dry edible legume and is not reported to contain any specific major anti-nutritional factors. In India, during 2014-15 Bengal gram production area was 8250.5 (In '000 Hectare), production 7331.8 (In '000 Tonne) tones and productivity 889 (In Kg./Hectare). In Chhattisgarh during 2014-15 the production area was 280.6 (In '000 Hectare), production 267.6 MT (In '000 MT) and productivity 1035 (In Kg./Hectare) (Anonymous, 2016). The potential seed yield of about 5 t/ha has been reported in chickpea. However, the realized seed yield hovers around 850 kg/ha which has stagnated over the years. Series of biotic and abiotic stresses reduce the yield and yield leaving room only stability marginal improvements and the key biotic constraint is the pod borer, Helicoverpa armigera Hubner.

The gram pod borer, Helicoverpa armigera Hubner, (Lepidoptera: Noctuidae) is a major threat to intensive agriculture (Sigsgaard, 2002). It is one of the most destructive and cosmopolitan insect pests of field crops worldwide and is highly polyphagous causing severe damage to a wide range of food, oil, fodder, vegetables, horticultural, ornamental, aromatic and medicinal plants (Neoliya, 2007, Kontsedalov et al. 2012). Yield losses due to this pest in chickpea may range from 70 to 95% (Prakash et al., 2007). Due to its wide host range, production of multiple generations per year, high fecundity, migratory behavior and pronounced resistance to many insecticides, the control up to desired level has become difficult (Mccaffery et al. 1998). Moderate

to high level of resistance to conventional insecticides such as (chlorinated hydrocarbons, organophosphates, carbamates and pyrethroids) as well as to neonicotinoids pesticides and Insect Growth Regulator (IGR's) has been reported in field populations of H. armigera (Nauen Bretschneider 2002). Chemical control is the most commonly used method in insect pest management. Due to adverse effects on non-target organisms, toxicity to mammals and birds and the risk of environmental pollution, chemical control measure should be replaced by the other environmentally friendly control methods to refrain from consumption and other ways it can be replaced (Fields, 1998). Therefore, with the current urgent and conflicting goals of reduced pesticide usage while maintaining adequate agricultural production, microbial control agents with selectivity and a low environmental impact could become ideal components of integrated pest management programs (IPM) in this century (Lacey and Goettel, 1995). The entomopathogenic fungus has been used for biological control of pests to reduce pesticide usage. More than 700 fungal species from about 90 genera are pathogenic to insects (Hong, 2003). Under natural conditions, these pathogens are frequent and often cause natural mortalities of insect populations.

The entomopathogenic hyphomycetes fungi have great potential as biological control agents against insect pests and are used as an important component in integrated pest management systems, particularly Beauveria bassiana (Balsamo) Vuillemin, Metarhizium anisopliae (Metch) Sorok, Verticillium lecanii Zimmeman and Nomuraea rileyi (Farlow) Samson have been found to be promising in the control of several agriculturally important insect pests and are facultative pathogens (Lingappa et al., 2005). Among the entomopathogenic fungi B. (Balsamo) Vuill, (Deuteromycetes: bassisna

\*Corresponding Author

Hyphomycetes) belonging to sub division Deuteromycotina grows naturally in soils throughout the world and acts as a parasite on various arthropod species, causing white muscardine disease. Over 200 species of insects in nine orders, mainly lepidoptera, coleoptera and hemiptera have been recorded as hosts (Li and Yang, 1988). B. bassiana are characterized by having conidiophores consisting of whorls and dense clusters of sympodial, short and globose or flask shaped conidiogenous cells with apical denticulate rachis and one celled conidia. This fungal biopesticide receives more attention because of its more eco-safe, eco-friendly environmental control measures. Its spores may be formulated and applied in a similar way as chemical pesticides and. therefore, could be adopted as a new technology. This includes oil based, dust, powder formulations and ultralow volume application, cheap to produce and may provide low cost control (Langle, 2006).

## MATERIAL AND METHOD

#### Rearing of the insects

Gram pod borer, *Helicoverpa armigera* (Hubner) larvae were collected from nearby fields and brought into the laboratory for further rearing. Larvae were reared individually in plastic tube/plastic disposal cup with cap/plastic petriplates (size,9cm diameter)/glass petriplates (size, 9 cm diameter) on artificial diet (Krishnareddy and Hanur, 2015, Ahmed *et al.* 1998) to get pure line culture. Freshly emerged larvae from pure line culture were used for experimental purpose (Namasivayam, *et al.* 2015).

#### **Experimental Protocols**

The experiment was conducted with 7 treatments including control and in each replication 10 larvae (2<sup>nd</sup> - 3<sup>rd</sup> instars) were used. The experiment was replicated four times. Commercial formulation of 1.15% WP ( $1x10^8$  cfu/g min) of *B. bassiana*, was used in this experiment. The observations were recorded at 24 hrs interval upto eight (8) days. All experiments were conducted in BOD having optimum temperature and humidity (25 $\pm$  2  $^{0}$ C and 70+10% RH). Calculated amount (ml) of each fungal suspension  $(1x10^8 \text{ cfu/g})$  was taken in petridish/plastic beaker 1000 ml size 15cm length and 12cm diameter and 2<sup>nd</sup>- 3<sup>rd</sup> instars larvae was treated by leaf dip-sprays method. One to two pieces of chickpea leaves cut into 5 cm-7 cm pieces were dipped in fungal conidial suspension contaminated both sides of the leaf surfaces then left on a laboratory bench to allow to dry for 2-3 min under aseptic conditions in laminar air flow.

Ten larvae were then placed on the conidia treated leaves in each petridish then also spray conidial suspension on larvae using a potters sprayer (agriculture hand sprayer) and allowed to feed for 24 h, after which the remains of the leaf material and frass were carefully removed. The larvae were then provided with unsprayed leaves. The control larvae

(10 larvae per exposure) were sprayed sterile distilled water (Tefera and Pringle, 2003, Elizabeth *et al.*, 2008, Senthamizhlselvan, 2010). The dead larvae were surface sterilized by sodium hypochlorite and placed on petridish lined with moist filter paper. These petridishes were incubated at  $25 \pm 2^{\circ}$ C and  $70\pm10\%$  RH to encourage fungal growth and sporulation in order to confirm infection of microbial agents on the larvae which showed mycelial growth were considered to have died of infection and only those counts were used to compute the pathogenicity of microbial agents. Slides was prepared by taking spores from dead larvae and observed under microscope to study its morphology for confirmation.

## RESULT AND DISCUSSION

#### First Year

In vitro bio-efficacy of B. bassiana against H. armigera Hubner on Chickpea year 2015-16 presented (table 1 Fig 1) revealed that at 1 day after treatment among the treatment T<sub>6</sub> (Profenophos 50% EC @1000ml/ha) 100.00 % show the superior mortality over the other treatment, followed by T<sub>5</sub> (Neem oil Azadirechtin 0.03% @5000ml/ha) 62.00 % which is not on par with T<sub>4</sub> (B. bassiana @5000g/ha) 0.00%. Among the doses of B. bassiana show 0.00 % mortality similar to control T<sub>7</sub> 0.00 %. At 2<sup>nd</sup> day among the treatment T<sub>6</sub> (Profenophos 50% EC @1000ml/ha) 100.00 % show the superior mortality over the other treatment, followed by T<sub>5</sub> (Neem oil Azadirechtin 0.03%@5000ml/ha) 72.50 % which is not on par with T<sub>4</sub> (B. bassiana @5000g/ha) 5.00%. Among the doses of B. bassiana T<sub>4</sub> (B. bassiana @5000g/ha) 5.00% show the maximum mortality but another treatment  $T_3$ ,  $T_2$  and  $T_1$  showed similar to control  $T_7$  0.00%. At 3<sup>rd</sup> day among the treatment  $T_6$ (Profenophos 50% EC @1000ml/ha) 100.00 % show the superior mortality over the other treatment, followed by T<sub>5</sub> (Neem oil Azadirechtin 0.03%@5000ml/ha) 85.00 % which is not on par with T<sub>4</sub> (B. bassiana @5000g/ha) 15.00 %. Among the doses of B. bassiana T<sub>4</sub>(B. bassiana @5000g/ha) 15.00% show the maximum mortality followed by T<sub>3</sub> (B. bassiana @3000g/ha) 0.00 % and T<sub>2</sub>(B. bassiana @2500g/ha) 0.00 % and T<sub>1</sub> (B. bassiana @2000g/ha) 0.00 %. In this day the percent mortality of treatment  $T_2$  and  $T_1$  was similar to control  $T_7$  (0.00%).

At  $4^{th}$  day among the treatment  $T_6$  (Profenophos 50% EC @1000ml/ha) 100.00 % show the superior mortality over the other treatment, followed by  $T_5$  (Neem oil Azadirechtin 0.03% @5000ml/ha) 90.00 % which is not on par with  $T_4$  (B. bassiana @5000g/ha) 25.00%,  $T_3$  (B. bassiana @3000g/ha) 15.00% and  $T_2$  (B. bassiana @2500g/ha) 15.00%. Lower percent mortality recorded in  $T_1$  (B. bassiana @2000g/ha) 10.00 % but superior than control. At  $5^{th}$  day among the treatment  $T_6$  (Profenophos 50% EC @1000ml/ha) 100.00 % and  $T_5$  (Neem oil Azadirechtin 0.03% @5000ml/ha) 100.00 % show the superior

mortality over the other treatment, which is on par with each other. Among the doses of B. bassiana T<sub>4</sub> (B. bassiana @5000g/ha) 30.00% show the maximum mortality followed by T<sub>3</sub> (B. bassiana @3000g/ha) 15.00% and  $T_2$  (B. bassiana @2500g/ha) 15.00%. Lower percent mortality recorded in T<sub>1</sub> (B. bassiana @2000g/ha) 10.00 % but superior than control. At 6<sup>th</sup> day among the treatment T<sub>6</sub> (Profenophos 50% EC @1000ml/ha) 100.00 % and T<sub>5</sub> (Neem oil Azadirechtin 0.03% @5000ml/ha) 100.00 % show the superior mortality over the other treatment, which is on par with each other. Among the doses of B. bassiana T<sub>4</sub>(B. bassiana @5000g/ha) 60.00% show the maximum mortality followed by T<sub>3</sub> (B. bassiana @3000g/ha) 30.00% and  $T_2$  (B. @2500g/ha) 20.00%. Lower percent bassiana mortality recorded in T<sub>1</sub> (B. bassiana @2000g/ha) 15.00 % but superior than control  $T_7$  (0.00 %).

At 7<sup>th</sup> day among the treatment T<sub>6</sub> (Profenophos 50% EC @1000ml/ha) 100.00 % and  $T_5$  (Neem oil Azadirechtin 0.03% @5000ml/ha) 100.00 % show the superior mortality over the other treatment, which is on par with each other. Among the doses of B. bassiana T<sub>4</sub> (B. bassiana @5000g/ha) 75.00% show the maximum mortality followed by T<sub>3</sub> (B. bassiana @3000g/ha) 40.00% which is on par with  $T_2$  (B. bassiana @2500g/ha) 30.00%. Lower percent mortality recorded in T<sub>1</sub> (B. bassiana @2000g/ha) 15.00 % but superior than control  $T_7$  (0.00 %). At  $8^{th}$ day among the treatment T<sub>6</sub> (Profenophos 50% EC @1000ml/ha) 100.00 % and  $T_5$  (Neem oil Azadirechtin 0.03% @5000ml/ha) 100.00 % show the superior mortality over the other treatment, which is on par with each other. Among the doses of B. bassiana T<sub>4</sub> (B. bassiana @5000g/ha) 75.00% show the maximum mortality followed by T<sub>3</sub> (B. bassiana @3000g/ha) 45.00% which is on par with  $T_2$  (B. bassiana @2500g/ha) 32.00%. Lower percent mortality recorded in T<sub>1</sub> (B. bassiana @2000g/ha) 15.00 % but superior than control  $T_7$  (0.00 %).

## Second year

In vitro bio-efficacy of B. bassiana against H. armigera Hubner on Chickpea year 2016-17 presented (table 2 Fig 2) revealed that at 1 day after treatment among the treatment T<sub>6</sub> (Profenophos 50% EC @1000ml/ha) 100.00 % show the superior mortality over the other treatment, followed by T<sub>5</sub> (Neem oil Azadirechtin 0.03% @5000ml/ha) 57.00 % which is not on par with T<sub>4</sub> (B. bassiana @5000g/ha) 0.00%. Among the doses of B. bassiana show 0.00 % mortality similar to control T<sub>7</sub> 0.00%. At 2<sup>nd</sup> day among the treatment T<sub>6</sub> (Profenophos 50% EC @1000ml/ha) 100.00 % show the superior mortality over the other treatment, followed by T<sub>5</sub> (Neem oil Azadirechtin 0.03% @5000ml/ha) 77.50 % which is not on par with T<sub>4</sub> (B. bassiana @5000g/ha) 5.00%. Among the doses of B. bassiana T<sub>4</sub> (B. bassiana @5000g/ha) 5.00% show the maximum mortality but another treatment  $T_3$ ,  $T_2$  and  $T_1$  showed similar to control  $T_7$  0.00%. At  $3^{rd}$  day among the treatment  $T_6$  (Profenophos 50% EC @1000ml/ha) 100.00 % show the superior mortality over the other treatment, followed by  $T_5$  (Neem oil Azadirechtin 0.03%@5000ml/ha) 90.00 % which is not on par with  $T_4$  (B. bassiana @5000g/ha) 15.00%. Among the doses of B. Bassiana  $T_4$  (B. bassiana @5000g/ha) 15.00% show the maximum mortality which is on par with  $T_3$  (B. bassiana @3000g/ha) 5.00% and  $T_2$  (B. bassiana @2500g/ha) 5.00%. The lower percent mortality recorded  $T_1$  (B. bassiana @2000g/ha) 0.00 % similar to control  $T_7$  (0.00%).

At 4<sup>th</sup> day among the treatment T<sub>6</sub> (Profenophos 50% EC @1000ml/ha) 100.00 % and T<sub>5</sub> (Neem oil Azadirechtin 0.03% @5000ml/ha) 90.00 % show the superior mortality over the other treatment, which is on par with each other. Among the doses of B. bassiana T<sub>4</sub> (B. bassiana @5000g/ha) 25.00% show the maximum mortality followed by T<sub>3</sub> (B. bassiana @3000g/ha) 17.50% which is on par with  $T_2$  (B. bassiana @2500g/ha) 15.00%. Lower percent mortality recorded in T<sub>1</sub> (B. bassiana @2000g/ha) 10.00 % but superior than control. At 5<sup>th</sup> day the treatment T<sub>6</sub> (Profenophos 50% EC @1000ml/ha) 100.00 % and  $T_5$  (Neem oil Azadirechtin 0.03%@5000ml/ha) 90.00 % show the superior mortality over the other treatment, which is on par with each other. Among the doses of B. bassiana T<sub>4</sub> bassiana @5000g/ha) 30.00% show the maximum mortality which is not on par with  $T_3$  (B. bassiana @3000g/ha) 17.50%, T<sub>2</sub> (B. bassiana @2500g/ha) 15.00% and  $T_1$  (B. bassiana @2000g/ha) 12.50 % but superior than control. At  $6^{th}$ day among the treatment T<sub>6</sub> (Profenophos 50% EC @1000ml/ha) 100.00 % and  $T_5$  (Neem oil Azadirechtin 0.03% @5000ml/ha) 100.00 % show the superior mortality over the other treatment, which is on par with each other. Among the doses of B. bassiana T<sub>4</sub> (B. bassiana @5000g/ha) 55.00% show the maximum mortality which is not on par with T<sub>3</sub> (B. bassiana @3000g/ha) 30.00% and  $T_2$  (B. bassiana @2500g/ha) 22.50%. Lower percent mortality recorded in T<sub>1</sub> (B. bassiana @2000g/ha) 12.50 % but superior than control  $T_7$  (0.00 %).

At 7<sup>th</sup> day among the treatment T<sub>6</sub> (Profenophos 50% EC @1000ml/ha) 100.00 % and  $T_5$  (Neem oil Azadirechtin 0.03% @5000ml/ha) 100.00 % show the superior mortality over the other treatment, which is on par with each other. Among the doses of B. bassiana T<sub>4</sub> (B. bassiana @5000g/ha) 67.50% show the maximum mortality followed by T<sub>3</sub> (B. bassiana @3000g/ha) 40.00% which is on par with  $T_2$  (B. bassiana @2500g/ha) 32.50%. Lower percent mortality recorded in T<sub>1</sub> (B. bassiana @2000g/ha) 12.50 % but superior than control  $T_7$  (0.00 %). At  $8^{th}$ day among the treatment T<sub>6</sub> (Profenophos 50% EC @1000ml/ha) 100.00 % and  $T_5$  (Neem oil Azadirechtin 0.03% @5000ml/ha) 100.00 % show the superior mortality over the other treatment, which is on par with each other. Among the doses of B. bassiana T<sub>4</sub> (B. bassiana @5000g/ha) 72.50% show the maximum mortality followed by  $T_3$  (B. bassiana @3000g/ha) 45.00% which is on par with  $T_2$  (B. bassiana @2500g/ha) 32.50%. Lower percent mortality recorded in  $T_1$  (B. bassiana @2000g/ha) 12.50% but superior than control  $T_7$  (0.00%).

Earlier similar work was done by Prasad, et al. (1990) recorded five entomopathogenic fungi for their infectivity to 2<sup>nd</sup> -instar larvae of Heliothis armigera [Helicoverpa armigera] by spraying them with conidial suspensions. Among them, B. bassiana (Bapatla isolate) was found to be the most virulent, recording the lowest LC<sub>50</sub> of  $2.17 \times 10^5$  conidia/ml. Tefera et al. (2003) also reported the effects of exposure methods, conidial concentrations, and temperature on mortality, mycosis and sporulation in second instar Chilo partellus cadavers infected by B. bassiana in laboratory. Larvae directly sprayed with conidia, exposed to conidia-treated leaves and dipped into conidial suspension resulted in high mortality (98–100%). The longest  $LT_{50}$  (3.5 days) and days to mortality (2.6 days) were observed in the treatedleaves exposure method. The shortest  $LT_{50}$  (1 day) and time for mortality (1 day) were recorded for the dipping method.. Exposure of larvae to treated-leaves resulted in high sporulation.

Prasad *et al.* (2010) also worked to control the pest population of *H. armigera* (Hubner) with an ecosafe entomopathogen *B. bassiana* (Balsamo). Four different concentrations (0.1, 0.125, 0.2 and 0.25 ml  $\times$  10<sup>8</sup> spores/ml) were sprayed topically against most damaging 4<sup>th</sup> instar larvae of *H. armigera* (Hubner) and a dose dependent mortality was observed that went up to 76.7 percent with highest dose of 0.25 ml  $\times$  10<sup>8</sup> spores/ml. Mortality started after two to three days of treatment. Senthamizhlselvan, *et al.* (2010)

collected fungal isolates from Karaikal and tested against seven insect pests using different inoculation methods *viz.*, spraying, crawling and dipping. Dipping method was found to be highly effective (82.50 per cent) to coccinellids followed by spraying and crawling methods (48.75 and 13.75 per cent). Larval mortality of spotted pod borer in pulses was more with spraying method (85.00 per cent). Among the three methods, spraying method was considered to be superior.

Similarly, Kumar and Chowdhry (2004) also reported that 18 B. bassiana isolates were found pathogenic to *H. armigera*. The larval mortality ranged from 40.0 to 90.0%. The maximum larval mortality was recorded in isolate HBB-2 (90.0%). followed by DBB-1 (87.5%) and HBB-1 (75.0%). The mean LC50 values for B. bassiana HBB-2 and M. anisopliae HMA-2 against the second instar larvae of *H. armigera* were  $0.955 \times 10^3$  and  $1.243 \times 10^3$ spores/ml, respectively. Hatting, (2012) bioassayed three entomopathogenic fungi, B. (Balsamo-Crivelli) Vuillemin, N. rileyi (Farlow) Samson and *Isaria fumosorosea* Wize in laboratory employing topical versus per os inoculation techniques. At a dose of  $3.75 \times 10^5$  conidia per larva, N. rileyi out-performed both I. fumosorosea and B. bassiana, causing a mean of 87±1.4% mortality. Notably, no difference was detected between the two inoculations techniques employed with any of the three fungal species assayed. The incubation periods for topical applications ranged from 4 days (PPRI 7201 and 8072) to 7 days (PPRI 7758) while per os treatments responded on Day 4 (PPRI 8072) and Day 5 (PPRI 7758 and 7201) post-inoculation.

Table 1. "In vitro bio-efficacy of Beauveria bassiana against H. armigera Hubner on Chickpea year 2015-16

T. N.	Treatments	Doses kg/ha	Percent mortality of H. armigera up to 8 days							
			1 day	2 day	3 day	4 day	5 day	6 day	7 day	8 day
$T_1$	B. bassiana 1.15%	@2000g/ha	0.00°	0.00 <sup>d</sup>	0.00 d	10.00 °	10.00°	15.00 d	15.00 <sup>d</sup>	15.00
	WP $(1x10^8 \text{ cfu/g})$		(0.544)	(0.544)	(0.544)	(18.439)	(18.439)	(22.500)	(22.500)	(22.500)
$T_2$	B. bassiana 1.15%	@2500g/ha	0.00°	$0.00^{d}$	$0.00^{d}$	15.00 °	15.00°	20.00 <sup>d</sup>	30.00 <sup>cd</sup>	32.50
	WP $(1x10^8 \text{ cfu/g})$		(0.544)	(0.544)	(0.544)	(22.500)	(22.500)	(26.565)	(33.055)	(34.557)
$T_3$	B.bassiana 1.15%	@3000g/ha	0.00°	$0.00^{d}$	5.00 <sup>d</sup>	15.00 °	15.00°	30.00°	40.00°	45.00
	WP $(1x10^8 \text{ cfu/g})$		(0.544)	(0.544)	(9.481)	(20.051)	(20.051)	(32.898)	(39.164)	(42.057)
$T_4$	B. bassiana 1.15%	@5000g/ha	0.00°	5.00°	15.00 °	25.00 °	30.00 <sup>b</sup>	60.00 b	75.00 b	75.00
	WP $(1x10^8 \text{ cfu/g})$		(0.544)	(9.481)	(22.500)	(29.889)	(32.832)	(50.895)	(63.453)	(63.453)
$T_5$	Neem oil	@5000ml/ha	60.00 <sup>b</sup>	72.50 b	85.00 b	90.00 <sup>в</sup>	100.00 a	100.00 a	100.00 a	100.00
	Azadirechtin 0.03% EC		(50.835)	(58.608)	(69.948)	(76.447)	(89.455)	(89.455)	(89.455)	(89.455)
$T_6$	Profenophos 50% EC	@1000ml/ha	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a	100.00
			(89.455)	(89.455)	(89.455)	(89.455)	(89.455)	(89.455)	(89.455)	(89.455)
T <sub>7</sub>	Control	-	$0.00^{c}$	$0.00^{d}$	$0.00^{d}$	$0.00^{d}$	$0.00^{d}$	$0.00^{e}$	0.00 e	$0.00^{e}$
			(0.544)	(0.544)	(0.544)	(0.544)	(0.544)	(0.544)	(0.544)	(0.544)
	CD at (0.05%)	·	2.677	6.663	9.594	11.556	9.082	6.136	10.847	11.449
	SE(m)		0.909	2.266	3.263	3.927	3.088	2.086	3.687	3.892
	C.V.		8.908	19.865	23.668	21.361	15.819	9.351	15.284	15.933

<sup>\*</sup>Figures in parentheses are arc sine transformed values.

**Table 2.** "In vitro bio-efficacy of B. Bassiana against H. armigera Hubner on Chickpea year 2016-17

T.	Treatments	Doses kg/ha	Percent mortality of H. armigera up to 8 days							
N.			1 day	2 day	3 day	4 day	5 day	6 day	7 day	8 day
$T_1$	B. bassiana 1.15%	@2000g/ha	0.00°	0.00	0.00	10.00	12.50	12.50	12.50	12.50
	WP $(1x10^8 \text{ cfu/g})$		(0.544)	(0.544)	(0.544)	(15.990)	(20.464)	(20.467)	(20.464)	(20.464)
$T_2$	B.bassiana 1.15%	@2500g/ha	0.00°	0.00	5.00	15.00	15.00	22.50	32.50	32.50
	WP $(1x10^8 \text{ cfu/g})$		(0.544)	(0.544)	(9.481)	(22.500)	(22.500)	(28.227)	(34.557)	(34.557)
T <sub>3</sub>	B. bassiana 1.15%	@3000g/ha	0.00°	0.00	5.00	17.50	17.50	30.00	40.00	45.00
	WP $(1x10^8 \text{ cfu/g})$		(0.544)	(0.544)	(7.043)	(24.535)	(24.535)	(32.898)	(39.104)	(42.057)
$T_4$	B. bassiana 1.15%	@5000g/ha	0.00°	5.00	15.00	25.00	30.00	55.00	67.50	72.50

	WP $(1x10^8 \text{ cfu/g})$		(0.544)	(9.481)	(19.680)	(29.736)	(32.832)	(47.947)	(58.998)	(61.941)
T <sub>5</sub>	Neem oil Azadirechtin 0.03% EC	@5000ml/ha	57.50 <sup>b</sup> (49.383)	77.50 (65.117)	90.00 (76.447)	100.00 (89.455)	100.00 (89.455)	100.00 (89.455)	100.00 (89.455)	100.00 (89.455)
T <sub>6</sub>	Profenophos 50% EC	@1000ml/ha	100.00 a (89.455)	100.00 (89.455)	100.00 (89.455)	100.00 (89.455)	100.00 (89.455)	100.00 (89.455)	100.00 (89.455)	100.00 (89.455)
T <sub>7</sub>	Control	-	0.00° (0.544)	0.00° (0.544)	0.00 <sup>d</sup> (0.544)	0.00 <sup>d</sup> (0.544)	0.00 f (0.544)	0.00 ° (0.544)	0.00 e (0.544)	0.00 ° (0.544)
	CD at (0.05%)		3.133	11.071	14.726	7.854	6.355	6.530	12.939	11.848
	SE(m) CV		1.065 10.523	3.765 31.714	5.004 34.478	2.669 13.723	2.160 10.801	2.220 10.058	4.397 18.500	4.028 16.668

<sup>\*</sup>Figures in parentheses are arc sine transformed values.

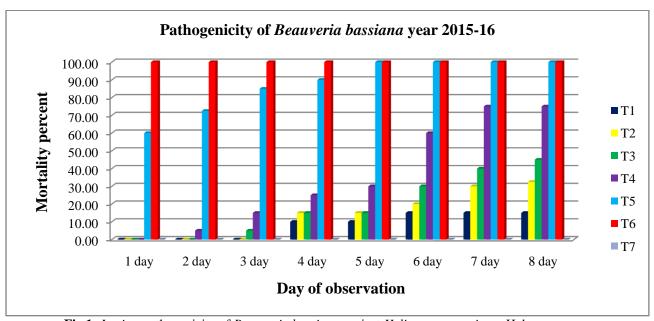


Fig 1: In vitro pathogenicity of Beauveria bassiana against Helicoverpa armigera Hubner

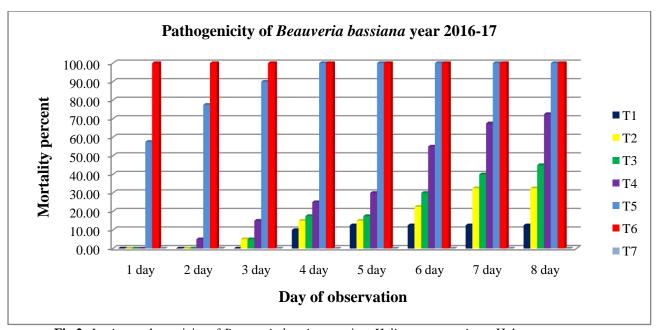


Fig 2: In vitro pathogenicity of Beauveria bassiana against Helicoverpa armigera Hubner

## **CONCLUSION**

In both the year among the doses of *B. bassiana*  $T_4$  (*B. bassiana* @5000g/ha) show the maximum mortality followed by  $T_3$  (*B. bassiana* @3000g/ha),  $T_2$  (*B. bassiana* @2500g/ha). Lower percent

mortality recorded in T<sub>1</sub> (*B. bassiana* @2000g/ha) but superior than control T<sub>7</sub>. In this experiment we can conclude that *B. bassiana* can useful biopesticide in integrated insect pest management for eco-friendly management of insect pests.

## REFERENCES

- Ahmed, K., Khalique, F. and Malik, B. A. (1998). Modified artificial diet for mass rearing of chickpea pod borer, *Helicoverpa armigera* (Hub.). *Pakistan Journal of Biological Sciences*, **1**(3):183-187.
- **Annonymous** (2016). India Stat, 2016, Ministry of Agriculture and Farmers Welfare, Govt. of India. pp.1-2.
- Elizabeth, R., H., Brown, P. M. J., Rothery, P., Ware, R. L. and Majerus, M. E. N. (2008). Interaction between the fungal pathogen *Beauveria bassiana* and three species of coccinellid: *Harmonia axyridis*, *Coccinella septempunctata* and *Adalia bipunctata*. *Biocontrol*, **53**: 265-276.
- **Fields, P. G.** (1998). Diatomaceous earth: advantages and limitations. Proceedings of the 7th International Working Conference on Stored product Protection, Beijing China, **1**: 781-784.
- **Hatting, J. L.** (2012). Comparison of three entomopathogenic fungi against the bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), employing topical vs per os inoculation techniques. African Entomology, **20** (1):91-100.
- **Hong, W.** (2003). Molecular biology of the entomopathogenic fungus *Beauveria bassiana*: Insectcuticle Degrading enzymes and development of a new selection marker for fungal transformation. PhD. Thesis, University of Heidelberg, Germany, pp. 147.
- Kontsedalov, S., Abu-Moch, F., Lebedev, G., Czosnek, H., Horowitz, R., Ghanim, M. (2012). *Helicoverpa armigera* biotype dynamics and resistance to insecticides in Israel during the years 2008-2010. *J Integrative Agric.*, **11**:312-320.
- **Krishnareddy, B. and Hanur, S. V.** (2015). Enhanced synthetic diet for rearing of *Helicoverpa armigera* under laboratory conditions. *Journal of Entomological and Zoology studies*, **3**(1):165-167.
- **Kumar, V. and Chowdhry, P. N.** (2004). Virulence of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* against tomato fruit borer, *Helicoverpa armigera*. *Indian Phytopathology*, **57**(2): 208-212.
- **Langle, T.** (2006). *Beauveria bassiana* (Bals.Criv.) Vuill.: A biocontrol agent with more than 100 years of history of safe use. REBECA, pp. 37.
- Li, Y. W. and J. H. Yang (1988). Prospects for the use of entomogenous fungus against forests pests, In: Study and application of entomogenous fungus in China, (Y. W. Li, Z. Z. Li, Z. Q. Liang, J. W. Wu, Z. K. Wu and Q. F. Xu ed.). Periodical Press, Beijing, pp. 10-14.
- **Lingappa, S., Saxena, H. and Devi Vimala, P. S.** (2005). Role of biocontrol agents in management of Helicoverpa armigera (Hubner). In: Recent advances

- in Helicoverpa armigera Management (Hem Saxena, A. B. Rai, R. Ahmad and Sanjeev Gupta eds.). Indian Society of Pulses Research and development, IIPR, Kanpur, pp 159-184.
- Mccaffery A. R., Head, D. J., Jianguo, T., Dubbeldam, A. A., Subramaniam, V. R. and Callaghan, A. (1998). Nerve insensitivity resistance to pyrethroids in Heliothine lepidoptera. *Pestic Sci*, **51**: 315-320.
- Namasivayam, S. K. R., Aarthi, R. and Anbazhahan, P. (2015). Studies on factors influencing the viability of entomopathogenic fungi *Metarhizium anisopliae* in soil adapting culture dependent method. *J. Biopest.*, **8**(1):23-27.
- Nauen R, and Bretschneider T. (2002). New modes of action of insecticides. *Pesticide outlook*, **13**:241-245
- Neoliya, N. K., Singh, D. and Sangwan, R. S. (2007). Azadirachtin-based insecticides induce alternation in *Helicoverpa armigera* Hub. head polypeptides. *J. Current Science*, **92** (1):94-99.
- Prakash, M. R., Ram, U. and Tariq, A. (2007). Evaluation of chickpea (*Cicer arietinum* L.) germplasm for the resistance to gram pod borer, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae). *J. entomol. Res.*, 31: 215-218.
- **Prasad, A., Syed, N. and Purohit, S.** (2010). *Beauveria bassiana* (Balsamo) vuillemin: a successful biopesticide against key pest *Helicoverpa armigera* (Hubner). *Internat. J. of Pharma and Bio Sciences*, **1** (2):30.
- **Prasad, V. D., Jayaraj, S. and Rabindra, R. J.** (1990). Susceptibility of gram caterpillar, *Heliothis armigera* Hbn. (Noctuidae: Lepidoptera) to certain entomogenous fungi. *J. of Biological Control*, **4**(1): 44-47.
- Senthamizhlselvan, P., Alice J., Sujeetha, R. P. and Jeyalakshmi, C. (2010). Efficacy of the native entomopathogenic fungal isolates with different inoculation methods. *J. of Biopesticides*, **3**(3): 550 552.
- Senthamizhlselvan, P., Alice J., Sujeetha, R. P. and Jeyalakshmi, C. (2010). Efficacy of the native entomopathogenic fungal isolates with different inoculation methods. *J. of Biopesticides*, **3**(3): 550 552.
- Sigsgaard, L., Greenstone, M. H. and Duffield, S. J. (2002). Egg cannibalism in *Helicoverpa armigra* on sorghum and *pigeonpea*. *Biological control*, 47: 151-165
- **Tefera, T. and Pringle, K. L.** (2003). Effect of exposure method to *Beauveria bassiana* and conidia concentration on mortality, mycosis, and sporulation in cadavers of *Chilo partellus* (Lepidoptera: Pyralidae). *Journal of Invertebrate Pathology*, **84**:90–95.