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Preliminary Evaluation of Native *Bacillus thuringiensis* Isolate and Microbial Formulations against Pod Borer, *Helicoverpa armigera* in Pigeonpea

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The noctuid pod borer, *Helicoverpa armigera* Hubner is a key lepidopteran pest of pigeonpea in Punjab. Bioefficacy of a native *Bacillus thuringiensis* (*Bt*) isolate along with some microbial formulations of biopesticides was evaluated in a preliminary study against the pod borer, *H. armigera* in pigeonpea. Results revealed that among the various treatments *Beauveria bassiana* WP @ 1.5 kg/ha recorded significantly lowest larval population (3.50/plant) and was found to be statistically at par with the native *Bt* isolate @ 1.5 kg/ha, *B. bassiana* WP @ 1 kg/ha and *Bt*-1 @ 1.5 kg/ha. However, significantly lowest per cent pod damage (25.57%) was observed in *Bt*-1 @ 1.5 kg/ha followed by *B. bassiana* SP @ 300 mg/l and native *Bt* isolate @ 1.5 kg/ha. The grain yield obtained in the treatment local *Bt* isolate @ 1.5 kg/ha was also statistically at par to the best treatment. Thus, in the present studies it was found that amongst all the biopesticides evaluated, *Bt*-1 @ 1.5 kg/ha, *B. bassiana* WP @ 1.5 kg/ha and local *Bt* isolate @ 1.5 kg/ha were found to be superior in reducing the inflorescence damage due to pod borer larvae as against the untreated control in pigeonpea. The *Bt* re-isolated from dead *H. armigera* larvae (showing signs of *Bt* toxicity) collected from the field, when subjected to antibiotic spectra, showed antibiogram similar to that of the native *Bt* isolate sprayed.

Key words: *Helicoverpa armigera*, Pigeonpea, *Bacillus thuringiensis, Beauveria bassiana*, Microbial Biopesticides, Pod Damage.

Pigeonpea *Cajanus cajan* (L.) Millsp is an important crop in semi-arid tropical and subtropical farming systems, providing high quality vegetable protein, animal feed, and firewood. Pigeonpea is attacked by over 200 species of insects[1]. Few pests, however, can be devastating in epidemic situations. Insect pests feeding on flowers, pods, and seeds are the most important biotic constraint affecting pigeonpea yields. Among the insects feeding on reproductive parts, gram pod borer *Helicoverpa armigera* Hubner (Noctuidae: Lepidoptera) is an important insect pest inflicting heavy damage to pigeonpea in the vegetative and reproductive phase in south India[2]. Losses due to this pest in pigeonpea have been estimated at US$317 million in the semi-arid tropics[3].

Traditional control of economically important insect pests has relied for decades on a large family of chemical insecticides. However, their broad activity spectrum and the accumulation of persistent residues have increased the demand for environmental friendly alternatives. Since *H. armigera* has developed high levels of resistance to insecticides, it has become difficult to control this pest on pigeonpea and several other crops with conventional insecticides[4,5]. Farmers often resort to heavy use of insecticides to control this pest, and therefore, there is a need to develop

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alternative methods to minimize the extent of losses. Currently, attempts are also being focused on the use of safe chemicals like microbial pesticides to reduce the toxic effects of chemicals on the non-target organisms and prevent the environmental pollution. In recent years microbial insecticides have become a viable alternative to control lepidopteran pests. Biopesticides such as *Bacillus thuringiensis* (*Bt*), *Beauveria bassiana*, etc. can provide an alternative and environment friendly option to control these insect pests[6]. The ascomycete fungus *B. bassiana* is a pathogen of hundreds of insect species and is commercially produced as an environment friendly mycoinsecticide. *Beauveria* is one of the best-known genera of entomopathogenic fungi and worldwide numerous registered mycoinsecticide formulations based on *B. bassiana* (*Bb*) are used for control of insect pests[7]. Thus, attempts were made in the present investigation to carry out preliminary investigations to evaluate native *B. thuringiensis* isolate along with other microbial based formulations against pigeonpea pod borer.

MATERIALS AND METHODS

The present study was carried out at Pulses Research Farm and Microbiology Laboratory, Punjab Agricultural University, Ludhiana (Punjab).

Growth of native *Bt* culture

The local *B. thuringiensis* (*Bt*) isolate was grown in Luria broth at 28°C and 200 rpm until sporulation. The spore crystal pellet was extracted by centrifugation at 12,000 rpm for 20 min. the pellet was acetone-dried and stored at room temperature. The required concentration was prepared by suspending the pellet in water along with carboxy methyl cellulose CMC (0.1%) and Triton-X (0.01%).

Field experiment

The recommended pigeonpea variety, PAU 881 was grown by following the recommended agronomic practices. The crop was raised in 4.0 × 5.0 m plots with plant to plant spacing of 20 cm and row to row spacing of 50 cm. The experiment was laid out in randomized block design (RBD) with eight treatments (including untreated control) and each treatment was replicated thrice. The biopesticides evaluated in the present study included one locally isolated *Bt* strain evaluated at two different dosages, i.e. 0.75 and 1.5 kg/ha, two dosages of a mycoinsecticide *B. bassiana* SC formulation @ 250 and 300 mg/l/ha, *B. bassiana* W.P. @ 1.0 and 1.5 kg/ha, *Bt*-1 @ 1.5 kg/ha and an untreated control. *B. bassiana* SC formulation, *B. bassiana* WP formulation and *Bt*-1 were supplied by Directorate of Oilseeds Research (DOR), Hyderabad. Foliar spray of biopesticide treatments was given after 50% flowering coinciding with the pest incidence. The spray mixture of each treatment was prepared by mixing the required quantity of the formulations in water to make it equivalent to 250 l/ha. The spray mixtures were freshly prepared for each treatment. The population of *H. armigera* was recorded on five randomly selected plants in each plot before spray and subsequent observations were recorded after 72 hrs of spraying on same plants. Observations were recorded on number of healthy, damaged and total pods per plant at the time of harvest on ten randomly selected plants in each treatment and finally the per cent pod damage was computed. Per cent pod damage was computed as per the formula:

$$\text{Per cent pod infestation} = \frac{\text{Number of damaged pods}}{\text{Total number of pods}} \times 100$$

The data obtained were subjected to square root and arc sine transformations and then statistically analyzed. Yield of the pods harvested was also recorded plot-wise and then converted to kg/ha. The data was subjected to statistical analysis to compare the differences between treatments.

Re-isolation of native *Bt* isolate and intrinsic antibiotic resistance spectra

The dead *H. armigera* larvae collected from the field were re-isolated by crushing in sterile distilled water and plating on Luria Agar plates. An antibiotic resistance spectrum of this re-isolated native *Bt* isolate was studied for comparing it to the sprayed *Bt* isolate. Filter paper discs containing standard concentration of antibiotics *viz.* ampicillin, chloramphenicol, tetracycline, streptomycin, gentamycin, kanamycin, carbencillin etc. were used. Bacterial cultures were grown in their respective broth for 24 h and aliquot of 0.1 ml culture was spread on to the petri plates containing media. Then, antibiotic discs of different concentrations were placed on the lawns of bacteria and incubated at 28°C for 72 h. The plates were observed for zone of inhibition around antibiotic disc.

RESULTS AND DISCUSSION

The results presented in Table 1 showed that there were no significant differences in the *H. armigera* larval population before spray, thus indicating the uniform distribution of the pest in all the treatments. However, the number of *H. armigera* larvae 72 hrs after spray ranged from 3.50-5.16 and 5.87 per plant in different treatments and untreated control, respectively. Among the various treatments *B. bassiana* WP @ 1.5 kg/ha recorded significantly lowest larval population (3.50/plant) and was found to be statistically at par with local *Bt* isolate @ 1.5 kg/ha, *B. bassiana* WP @ 1 kg/ha and *Bt*-1 @ 1.5 kg/ha. As far as pod damage was concerned, significantly lowest per cent pod damage (25.57%) was observed in *Bt*-1 @ 1.5 kg/ha. However, it was statistically at par with *B. bassiana* SP @ 300 mg/l and local *Bt* isolate @ 1.5 kg/ha, indicating the relative efficacy of the native *Bt* strain against the pod borer. The untreated check recorded 5.87 larvae per plant and 39.45% pod damage due to pod borer. The grain yield ranged from 718-857 kg/ha in different treatments as compared to 592 kg/ha in untreated control. The treatment *Bt*-1 @ 1.5 kg/ha gave significantly highest grain yield (857 kg/ha). The grain yield obtained in the treatment native *Bt* isolate @ 1.5 kg/ha was also statistically at par to the best treatment. Thus, in the present studies it was found that amongst all the biopesticides evaluated, *Bt*-1 @ 1.5 kg/ha, *B. bassiana* WP @ 1.5 kg/ha and local *Bt* isolate @ 1.5 kg/ha were found to be superior in reducing the inflorescence damage due to pod borer larvae as against the untreated control in pigeonpea.

B. thuringiensis is an important entomopathogenic organism in protection against defoliating pests in Lepidoptera[8,9]. The present findings are in conformity to the previous studies reporting that the biopesticides like *B. thuringiensis* @ 1.5 kg/ha or *B. bassiana* @ 300 mg/lt were effective for the management of pod borer complex in pigeonpea[10]. *Bt* provided good protection and registered significantly lesser incidence of pod borer larvae and higher yield over control[11]. *B. thuringiensis* var. *kurstaki* based product (Spic-Bio Reg.) @ 2.5 l/ha was the best treatment, recording lesser *H. armigera* larval population (0.7/plant) in pigeonpea[12]. The pod

Table 1. Efficacy of different microbial biopesticides against *H. armigera* in pigeonpea

S. No.	Treatment	Dose/conc.	*H. armigera* larvae/plant		% Pod damage**	Yield (kg/ha)
			Before spray	72 hrs after spray		
1.	*Beauvaria bassiana* SC formulation	250 mg/lit	6.34	4.85 (2.45)	30.55 (33.45)	726
2.	*Beauvaria bassiana* SC formulation	300 mg/lit	6.44	4.66 (2.35)	26.26 (30.72)	776
3.	*Beauvaria bassiana* WP formulation	1.0 kg/ha	6.31	3.66 (2.15)	29.68 (32.96)	733
4.	*Beauvaria bassiana* WP formulation	1.5 kg/ha	6.48	3.50 (2.12)	27.45 (31.58)	825
5.	*Bacillus thuringiensis* (*Bt* -1)	1.5 kg/ha	6.45	3.66 (2.14)	25.57 (30.23)	857
6.	*Bacillus thuringiensis* (Native strain)	0.75 kg/ha	6.52	5.16 (2.50)	32.30 (34.50)	718
7.	*Bacillus thuringiensis* (Native strain)	1.5 kg/ha	6.39	3.66 (2.15)	26.56 (30.97)	792
8.	Untreated control	-	6.50	5.87 (2.62)	39.45 (38.43)	592
	C.D. 5%	NS	(0.27)	(3.11)	69	

NS= Non-significant
*Figures in parentheses are the transformed square root values
**Figures in parentheses are the transformed arc sine values

damage inflicted by *H. armigera* was recorded to be minimum with the application of NSKE followed by *Bt* at an interval of 20 days from the pod initiation stage onwards[13]. Similarly, combination of *B. thuringiensis* (Dipel) and deltamethrin (0.004% or 0.002%) was most effective in reducing the damage due to pod borers in pigeonpea with highest net profit[14]. The *Bt* formulations caused up to 58.72% mortality of third instar larvae after one day of application[15]. Similarly, the biopesticide *B. thuringiensis* was moderately effective against pigeonpea pod borer[16]. However, *B. bassiana* preparation was observed to be less effective (51.25% efficacy) against *H. armigera* in pigeonpea[17]. Besides, *B. thuringiensis* and *B. bassiana* were effective against spotted pod borer, *Maruca testulalis* in pigeonpea[18].

Fig. 1. *H. armigera* larvae showing toxicity due to native *Bt* isolate

Fig. 2. Re-isolated local *Bt* isolate from dead larvae

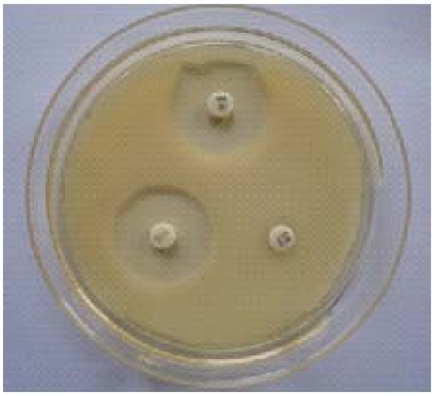

Fig. 3. Antibiotic sensitivity of re-isolated local *Bt* isolate

The *Bt* re-isolated from dead *H. armigera* larvae (showing signs of *Bt* toxicity) from the field, when subjected to antibiotic spectra, showed antibiogram similar to that of the native *Bt* isolate sprayed (Figs. 1 & 2). It showed sensitivity to gentamycin, chloramphenicol, streptomycin, tetracycline and kanamycin, whereas it was resistant to ampicillin, carbencillin, amoxicillin and penicillin (Fig. 3). This further confirmed larval mortality due to native *Bt* isolate sprayed.

Novel strains or toxins may assist in pest management programs by attempting to avoid or minimise the appearance of resistance to *B. thuringiensis* in field target insect populations. Because such native isolates may represent new genetic resources that can be used to develop new technologies, the outcomes of such studies may result in the development of new microbial insecticides against pest species in integrated pest management system. Thus, from the present findings it was concluded that the biopesticides like *B. thuringiensis*, particularly the locally isolated strains, could prove to be effective for the management of pod borer complex along with an increased yield and also to avoid development of resistance in pigeonpea ecosystem. This study indicates that the tested native *Bt* isolate may have the potential for the development of environmentally safe bioinsecticides against the pod borer, *H. armigera* in pigeonpea. These results form a basis for further investigation of the local *Bt* isolate such as determination of the Cry proteins therein and how temperature would affect its toxicity. It is also recommended that the toxicity of such native isolates be investigated against other local pests in order to determine their target range which may, ultimately have an important role in future biological control programs.

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REFERENCES

1. Reed, W., Lateef, S.S. Pigeonpea: Pest Management. In: *The Pigeonpea* (Nene YL, Hall SD, Sheila VK, ed). Wallingford, UK: CAB International, 1990; pp 349-374.
2. Shanower, T.G., Romeis, J., Minja, E.M. Insect pests of pigeonpea and their management. *Ann Rev Entomol*, 1999; **44**: 77-96.
3. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). The medium term plan. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India; 1992.
4. Kranthi, K.R., Jadhav, D.R., Kranthi, S., Wanjari, R.R., Ali, S.S., Russell, D.A. Insecticide resistance in five major insect pests of cotton in India. *Crop Prot.*, 2002; **21**:449-460.
5. Sharma, H.C. (ed). *Heliothis/Helicoverpa Management: Emerging Trends and Strategies for Future Research*. New Delhi, India: Oxford and IBH Publishers, 2005; pp 469.
6. Jeyarani, S., Karuppuchamy, P. Investigations on the enhancing efficacy of granulovirus on nucleopolyhedrovirus of *Helicoverpa armigera* (Hubner). *J. Biop.*, 2010; **3**(1): 172-176.
7. Xiao, G., Ying, S.H., Zheng, P., Wang, Z.L., Zhang, S., Xie, X.Q., Shang, Y., St. Leger R.J., Zhao, G.P., Wang, C., Feng, M.G. Genomic perspectives on the evolution of fungal entomopathogenicity in *Beauveria bassiana*. *Sci. Rep.* 2012; **2**: 483. doi: 10.1038/srep00483.
8. Frankenhuyzen, V.K.: The challenge of *Bacillus thuringiensis*. In: *Bacillus thuringiensis, an Environmental Biopesticide: Theory and Practice* (Entwistle PF, Cory JS, Bailey MJ, Higgs S, ed). New York: Wiley, 1993; pp 1-35.
9. Frankenhuyzen, V.K.: The development of *Bacillus thuringiensis* for control of spruce budworm, *Choristoneura fumiferana*, in Canada. In: *Bacillus thuringiensis Biotechnology and Environmental Benefits* (Feng JY, ed). Taipei, Taiwan: Hua Shiang Yuan, 1995; pp 405-423.
10. Sreekanth, M., Seshamahalakshmi, M. Studies on relative toxicity of biopesticides to *Helicoverpa armigera* (Hubner) and *Maruca vitrata* (Geyer) on pigeonpea (*Cajanus cajan* L.). *J. Biop.* 2012; **5**(2): 191-195.
11. Mohapatra, S.D., Srivastava, C.P. Bioefficacy of chemical and biorational insecticides against incidence of legume pod borer, *Maruca vitrata* (Geyer) in short duration pigeonpea. *Indian J. Plant Prot.*, 2002; **30**(1): 22-25.
12. Thilagam, P., Kennedy, J.S. Evaluation of *Bacillus thuringiensis* (Spic-Bio Reg.) against pod borer complex of pigeon pea. *J. Ecotoxicol. Environ. Monitor.*, 2007; **17**(3): 275-280.
13. Bhushan, S., Nath, P. Effect of certain insecticides against pod borer complex infesting pigeonpea grown under different cropping pattern. *J. Exp Zool.*, 2006; **9**(2): 305-309.
14. Reddy, C.N., Singh, Y., Singh, P.D. Bioefficacy of insecticides, biopesticides and their combinations against pod borers in pigeonpea. *Indian J. Entomol.*, 2001; **63**(2): 137-143.
15. Prabhakara, M.S., Srinivasa, N. Field persistence of *Bacillus thuringiensis* formulations against pigeonpea pod borer. *Indian J. Pulses Res.*, 1998; **11**(1): 63-67.
16. Sunitha, V., Lakshmi, K.V., Rao, G.V.R. Laboratory evaluation of certain insecticides against pigeonpea pod borer, *Maruca vitrata*. *J. Food Leg.*, 2008; **21**(2): 137-139.
17. Nahar, P., Yadav, P., Kulye, M., Hadapad, A., Hassani, M., Tuor, U., Keller, S., Chandele, A. G., Thomas, B., Deshpande, M.V. Evaluation of indigenous fungal isolates, *Metarrhizium anisopliae* M34412, *Beauveria bassiana* B3301 and *Nomuraea rileyi* N812 for the control of *Helicoverpa armigera* (Hubner) in pigeonpea field. *J. Biol. Control*, 2004; **18**(1): 1-7.
18. Manjula, K. Padmavathamma, K. Effect of microbial insecticides on the control of *Maruca testulalis* and on the predators of redgram pest complex. *Entomon*, 1996; **21** (3/4): 269-271.