http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 3 (8): 1296-1298, 2000

Field Efficacy of CAMB *Bacillus thuringiensis* Biopesticide to Control *Helicoverpa armigera* (Hübner) and *Earias vitella* (Fabricius) in Okra Crop

Shahid Karim, Ahmad Usman Zafar, Idrees Ahmad Nasir and Sheikh Riazuddin
National Centre of Excellence in Molecular Biology, University of the Punjab, Lahore-53700, Pakistan

Abstract: CAMB *Bacillus thuringiensis* formulation and two other commercial Bt formulations (Agree and Larvo Bt) were tested on Okra fields to control two lepidopteran pests, *Helicoverpa armigera* and *Earias vitella*. CAMB Bt formulation was tested from 250-g/h dose to 1500 g/h dose to see the efficacy against target insect pests. Commercial Bt formulations, Agree and Larvo Bt were used as standard with one dose of 1000 g/h. All microbial insecticides successfully controlled *H. armigera* and *E. vitella* larvae in okra field. The efficacy of locally developed Bt formulation was promising in comparison to Agree and Larvo BT.

Key words: *Bacillus thuringiensis*, *Helicoverpa armigera*, *Earias vitella*, Biopesticide, okra

Introduction

Helicoverpa armigera (Hübner) and *Earias vitella* (Fabricius) are the major lepidopteran insect pests of economically important crops in sub-continent. In Pakistan, *H. armigera* is a dominant pest of several legume crops including chickpea, vegetables and cotton (Karim, 2000). *E. vitella* is also a major pest on cotton and vegetables and can cause serious losses in cotton like *H. armigera* (Makhdoom *et al.,* 1997). Its high pest status arises from the preference of foraging larvae for plant structures rich in nitrogen (Fitt, 1989) such as flower, fruits and panicles. The control of *H. armigera* and *E. vitella* on different crops is usually carried out with the use of conventional chemical insecticides (Karim, 2000). However, in 1998 farmers in cotton growing areas were unable to control populations of *H. armigera* with insecticides. Ahmad *et al*. (1995, 1997), has confirmed high levels of resistance to chemical pesticides as a major cause of control failures. The development of insecticide coupled with an increasing awareness of possible detrimental effects of intensive insecticide use has stimulated interest in the development of environmentally safe and benign control options, which reduce pesticide inputs. There is an increased interest in development of alternative strategies for management of *H. armigera* and *E. vitella*. One of these alternatives is the use of microbial biopesticide, *Bacillus thuringiensis*, particularly in view of availability of improved strains of Bt for use as sprays and recent development of crop varieties expressing a Bt toxin (Karim *et al.,* 1999c; Karim and Riazuddin, 1997). The present work is the continuation of our work reported earlier (Karim *et al.,* 1999a; Zafar *et al.,* 2000). The bio-efficacy of CAMB Bt formulation and two commercial formulations of *Bacillus thuringiensis* against *H. armigera* and *E. vitella* in Okra fields was determined.

Materials and Methods

Formulation preparation: For the large scale production of CAMB Bt biopesticide formulation, the CAMB Bt isolate 3-023 (Karim *et al.,* 1999b; Zafar *et al*., 1999) was grown in a 14 liter "Microferm fermentor" New Brunswick, USA, model MF-114 using CSL-salts medium for 48 hrs. The cells were centrifuged at 7 K for 15 minutes in Beckman centrifuge. CAMB Bt formulation was prepared in fine powdered form. CAMB Bt formulation (4% active ingredient) was used for present studies. Agree and Larvo Bt was generously provided by Novartis and Zagro NPC (Pvt.) limited, Pakistan for the present studies.

Field studies: The tests were conducted on National Centre of Excellence in Molecular Biology, university of the Punjab Farm in Lahore during 1999. The field was planted in "Parbahari Kranti" okra cultivar imported from India in first week of April, 1999 and maintained using standard agronomic practices with furrow irrigation as needed. Plots were 9 rows 20 meters in length with 0.3 meter between rows and surrounded by a 1-meter strip of follow ground. Scouting for *Helicoverpa* and *Earias* sp. was initiated in the last week of April, before plants reached the flowering stage. Applications were initiated when thresholds were attained based on scouting (Table 1). The experimental design was a randomized complete block with seven treatments, two controls replicated four times. The treatments were as follows: (1) *Bacillus thuringiensis* Agree of Novartis, 50 WP (1000 g/ha), (2) LARVO BT of Troy Biosciences Inc. USA, 26.4% F. L. (1000 g/ha), (3) CAMB Bt, 4% active ingredient (250 g/ha), (4) CAMB Bt, 4% active ingredient (500 g/ha), (5) CAMB Bt, 4% active ingredient (750 g/ha), (6) CAMB Bt, 4% active ingredient (1000 g/h), (7) CAMB Bt, 4% active ingredient (1500 g/ha), (8) Untreated Control and (9) Base ingredients of CAMB formulation as Control. Treatments were applied on May 17 and May 25. Insect counts were taken 7 days after application. All applications were made using hand sprayer with single nozzle per row. A total of 12 liters were used per treatment. Larval

Table 1: An outline about the insect control agents, their application doses and Insect population density in the experimental plots before treatments

Plot number	Number of larvae (15/5/99)	Number of larvae (17/5/99)
1	120	127
2	106	166
3	136	153
4	123	170
5	142	196
6	120	191
7	120	185
8	131	239
9	107	237

and egg counts were made before each treatment and after application by a whole plant search, randomly selected plants within each replicate. Plants were monitored for *Helicoverpa* and *Earias* sp (Table 2).

Statistical analysis: Percent efficacy was calculated according to Henderson and Tilton (1955) formula. Data were analyzed by using analysis of variance (ANOVA) with means separated by LSD ($p<0.05$) when appropriate.

Results and Discussion

The increasing importance of Bt based crop protection systems, largely as a result of greater environmental awareness and food safety concerns, plus the failure of conventional chemicals due to an increasing number of insecticide-resistant species, has provided major niche for the development of Bt (Dent, 1993). Bt is most effective as a control agent when used in crop protection based on the table monitoring of pest population and the rational application of economy injury levels (Teakle, 1994). In present study, decisions on when to treat pest infestations were based on field scouting, rather than fixed thresholds (Table 1). Timing of Bt spray applications is a crucial factor (Ghidiu and Zehnder, 1993) because of differential toxicity against various life stages (Ferro and Lyon, 1991), feeding time preferences of certain insect larvae like *H. armigera* (Karim *et al.,* 1999a), UV sensitivity of Insecticidal Crystal Proteins (Pozsgay *et al.*, 1987) and other multiple environmental factors (Leong *et al.,* 1980). It is evident from Table 3 that all Bt formulations were toxic to the larvae of *Helicoverpa armigera* and *Earias vitella*, but CAMB Bt formulation proved to be the most effective followed by the commercial grade formulations at all the concentrations used in present study. Significant difference was found among different formulations for their effect on Okra ($F_{cal}1.00>F_{tab}0.500$). LSD showed specifically that there is significant difference among Larvo Bt and Agree (36.36>22.57) similarly among Larvo Bt and CEMB Bt (27.28>22.57) (Table 2). The promising performance of local CAMB Bt preparation over the other commercial formulations has already been reported (Karim *et al.,* 1998; Zafar *et al.,* 2000).

Table 1: An outline of experimental plots and dose applications

CAMB Bt 750 g/h	CAMB Bt 250 g/h	Agree Bt 1000 g/h	CAMB Bt 500 g/h	Plot 1
LARVO Bt 1000 g/h	CAMB Bt 500 g/h	LARVO Bt 1000 g/h	CHECK	Plot 2
CAMB Bt 1000 g/h	CAMB Bt 1000 g/h	CAMB Bt 500 g/h	CAMB Bt 1000 g/h	Plot 3
CAMB Bt 1500 g/h	LARVO Bt 1000 g/h	CAMB Bt 250 g/h	CHECK	Plot 4
CAMB Bt 1500 g/h	CAMB Bt 750 g/h	CAM Bt 1500 g/h	CHECK(Base)	Plot 5
CHECK (Base)	CAMB Bt 750 g/h	CAMB Bt 500 g/h	CHECK	Plot 6
CAMB Bt 250 g/h	CAMB Bt 750 g/h	CAMB Bt 1000 g/h	CAMB Bt 1500 g/h	Plot 7
CHECK (Base)	CAMB Bt 250 g/h	AGREE Bt 1000 g/h	AGREE Bt 1000 g/h	Plot 8
CHECK (Base)	AGREE Bt 1000 g/h	LARVO Bt 1000 g/h	CHECK	Plot 9

Table 2: Efficacy of Bt formulations in okra.

Insect control agents	Pre-treatment (Larvae)	7DAA (1) (% Mortality)	7DAA (2) (% Mortality)
Check	227	0	0
Check	210	0	0
Agree (1000 g/h)	208	72.72	100
Larvo Bt (1000 g/h)	155	36.36	100
Camb Bt (250 g/h)	216	66.67	100
Camb Bt (500 g/h)	158	45.45	100
Camb Bt (750 g/h)	169	66.67	100
CAMB Bt (1000 g/h)	160	63.64	100
Camb Bt (1500 g/h)	161	39.39	100

LDS= 33.2 at 5% level of confidence

Table 3: Camparision among different formulations

SOV	SS	D.F.	MSS	F Ratio	Prob.
7DAA (1&2)	2700.033	1	2700.033	15.079	0.0604
Formulations	358.116	2	179.058	1.000	0.5000
Error	358.116	3	179.058		
Total	3416.265	5			

They have found it more toxic than Agree towards *H. armigera* and one possible reason for this high potency may be the same micro-habitat of both local isolate and insect pest (Karamanlidou *et al.,* 1991; Kaelin *et al.* 1994). It is evident from present studies that Bt can be useful insect control agent to target insect pests like polyphagous *H. armigera* and *E. vitella* on its alternate host like Okra. Eradication or suppression of both pests on alternate hosts would eventually minimize the risk of outbreaks on cotton crop. Therefore, Bt could be promoted to target *H. armigera* and *E. vitella* on alternate host plant like Okra. In Pakistan, both insect pests build up their populations in Okra fields adjacent to cotton and later turn to cotton fields in high population. At this stage, it is impossible to control both pests at a very critical fruiting stage of cotton. Thus, among all tested formulations, CAMB Bt proved to be the most toxic and exhibited no detrimental effects on the predators in the field. It may, therefore, be suitably incorporated in the integrated pest management of these pests without disrupting the agro-ecosystem and the quality of the environment.

Acknowledgments

We thank Dr. M. A. Qadeer for his critical comments, Mr. Syed Intikhab Alam for his help in statistical analysis, Mr. Muhammad Munir, Mr. Abdul Karim, Mr. Sajjad Haider, Mr. Muhammad Kashif, and Mr. Waheed Anwar for their technical assistance. Ministry of Science and Technology, Pakistan financially supported research work.

References

Ahmad, M., I.M. Arif and Z. Ahmad, 1995. Monitoring insecticide resistance of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Pakistan. J. Econ. Entomol., 88: 771-776.

Ahmad, M., M.I. Arif and M.R. Attique, 1997. Pyrethroid resistance of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Pakistan. Bull. Entomol. Res., 87: 343-347.

Dent, D.R., 1993. The use of *Bacillus thuringiensis* as an Insecticide. In: Exploitation of Microorganisms, Jones, G. (Ed.). Chapman and Hall, London, pp: 19-44.

Ferro, D.N. and S.M. Lyon, 1991. Colorado potato beetle (Coleoptera: Chrysomelidae) larval mortality: Operative effects of *Bacillus thuringiensis* subsp. san diego. J. Econ. Entomol., 84: 806-809.

Fitt, G.P., 1989. The ecology of *Heliothis* species in relation to agroecosystems. Annu. Rev. Entomol., 34: 17-53.

Ghidiu, G.M. and G.W. Zehnder, 1993. Timing of the initial spray application of *Bacillus thuringiensis* for control of the Colorado potato beetle (Coleoptera: Chrysomelidae) in potatoes. Biol. Control, 3: 348-352.

Henderson, C.F. and E.W. Tilton, 1955. Tests with acaricides against the brown wheat mite. J. Econ. Entomol., 48: 157-161.

Kaelin, P., P. Morel and F. Gadani, 1994. Isolation of *Bacillus thuringiensis* from stored tobacco and *Lasioderma serricorne* (F.). Applied Environ. Microbiol., 60: 19-25.

Karamanlidou, G., A.F. Lambropoulos, S.I. Koliais, T. Manousis, D. Ellar and C. Kastritsis, 1991. Toxicity of *Bacillus thuringiensis* to laboratory populations of the olive fruit fly (*Dacus oleae*). Applied Environ. Microbiol., 57: 2277-2282.

Karim, S. and S. Riazuddin, 1997. *Bacillus thuringiensis*-endotoxins: Molecular mechanism of action and pest management. Proc. Pak. Acad. Sci., 34: 135-156.

Karim, S., M. Murtaza, and S. Riazuddin, 1998. Field applications of biopesticides for control of *Helicoverpa armigera* (Hubner)(Lepidoptera: Noctuidae). Proceedings of the 18th Pakistan Congress of Zoology, August 14, 1988, Zoology Society of Lahore.

Karim, S., 2000. Management of *Helicoverpa armigera*: A review and prospectus for Pakistan. Pak. J. Biol. Sci., 3: 1213-1222.

Karim, S., M. Murtaza and S. Riazuddin, 1999a. Field evaluation of bacillus thuringiensis, insect growth regulators, chemical pesticide against *Helicoverpa armigera* (Huber) (Lepidoptera: Noctuidae) and their compatibility for integrated pest management. Pak. J. Biol. Sci., 2: 320-326.

Karim, S., R. Makhdoom and S. Riazuddin, 1999b. Specificity and pesticidal activity of Pakistani isolates of *Bacillus thuringiensis* towards *Pectinophora gossypiella*. Pak. Entomol., Vol. 21.

Karim, S., A.U. Zafar and S. Riazuddin, 1999c. Use of *Bacillus thuringiensis* biopesticides for crop protection. Science Vision (Submitted).

Leong, K.L.H., R.J. Cano and A.M. Kubinski, 1980. Factors affecting *Bacillus thuringiensis* total field persistence. Environ. Entomol., 9: 593-599.

Makhdoom, R., S. Karim and S. Riazuddin, 1997. Pathogenicity of locally isolated *Bacillus thuringiensis* δ-endotoxins against *Earias vitella* and *Helicoverpa armigera*. Pak. Entomol., 19: 1-4.

Pozsgay, M., P. Fast, H. Kaplan and P.R. Carey, 1987. The effect of sunlight on the protein crystals from *Bacillus thuringiensis* var. *kurstaki* HD1 and NRD12: A Raman spectroscopic study. J. Invertebr. Pathol., 50: 246-253.

Teakle, R.E., 1994. Present use of and problems with, *Bacillus thuringiensis* in Australia. Agric. Ecosyst. Environ., 49: 39-44.

Zafar, A.U., M.A. Qadeer and S. Riazuddin, 1999. Process development of Bt. based biopesticide. Pakistan Patent Application No. 1033/99.

Zafar, A.U., S. Karim, I.A. Nasir and S. Riazuddin, 2000. Shelf life and field evaluation of CAMB *Bacillus thuringiensis* biopesticide against *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) on tomato. Pak. J. Biol. Sci., 3: 804-807.