J. Biol. Control, 21(2): 217-221, 2007

Susceptibility of different instars of *Earias vittella* (Fabricius) to *Bacillus thuringiensis* insecticidal proteins

P. S. SHANMUGAM[1], R. BALAGURUNATHAN[1], N. SATHIAH[1] and N. G. V. RAO[2]

[1]Department of Agric. Entomology, Tamil Nadu Agricultural University
Coimbatore – 641003, Tamil Nadu, India

[2]Department of Biotechnology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth
Akola – 444104, Maharashtra, India

E-mail: psshanmugamk@yahoo.co.in

ABSTRACT: The susceptibility of different instars of *Earias vittella* (Fabricius) to *Bacillus thuringiensis* (*Bt*) insecticidal proteins (ICP) was studied. The LC_{50} of Cry1Aa, Cry1Ab, Cry1Ac, Cry1C and Cry1E were 108.54, 97.04, 66.62, 1240.83 and 1335.60 ng/cm^2, respectively to *E. vittella* neonates. The order of toxicity of different ICPs to second and third instar was Cry1Ac > Cry1Ab > Cry1Aa. Overlap 95 per cent fiducial limits at LC_{50} of Cry1Aa and Cry1Ab showed similar toxicity of these toxins to second and third instars of *E. vittella*. The possibility of these toxins and toxicity parameters in resistance management is discussed.

KEY WORDS: *Bacillus thuringiensis*, *Earias vittella*, insecticidal proteins, median lethal concentration, toxicity

INTRODUCTION

Bacillus thuringiensis Berliner (*Bt*) based biopesticides are now being increasingly used in India in many crops as one of the alternatives to chemical insecticides. Bt produces crystalline inclusions during sporulation composed of one or several proteins known as insecticidal crystal (Cry) proteins (ICPs) or δ-endotoxins. These toxins kill the insects by binding to and creating pores in the midgut membranes (Schnepf *et al.*, 1998). The Cry proteins are classified into four main groups, of which Cry1 and Cry2 proteins are mainly active against caterpillars (Crickmore *et al.*, 1998). Bt toxins are non-toxic to most non-target organisms including arthropod natural enemies (Entwistle *et al.*, 1993).

Transgenic cotton, engineered to continuously express δ-endotoxin from the Bt gene, holds great promise for controlling the bollworm complex (Barwale *et al.*, 2004). The transgenic cotton expressing Bt protein(s) could reduce the impact of chemical insecticides and create ecologically sound environment without reducing crop production as a part of an IPM strategy (Lutterell and Herog, 1994).

Determining the baseline susceptibility of key pest species to different Bt toxins is essential for monitoring the changes in their susceptibility over a period of time. Several common pest species have been selected for resistance to *B. thuringiensis* in the laboratory indicating that biological pesticides could suffer the same fate as

chemical pesticides (Tabashnik *et al.*, 1990; Tabashnik, 1994). Hence studies on the susceptibility of key pests such as *Earias vittella* to the Bt ICPs will be helpful in devising appropriate resistance management strategies for Bt transgenics.

MATERIAL AND METHODS

Insects

Earias vittella larvae were collected from bhendi and cotton fields in Coimbatore and Dharmapuri districts of Tamil Nadu. The field collected populations were reared on semi-synthetic diet (Gupta *et al.*, 1999). They were reared in the laboratory for four generations to develop diet-adapted laboratory population for bioassays.

Bt proteins

The Bt proteins Cry1Aa, Cry1Ab, Cry1Ac, Cry1E and Cry1C were obtained from Dr. Donald Dean, Ohio State University, USA. The multiplication and isolation of proteins from *E. coli* strains were carried out in the Insect Biotechnology Laboratory, Department of Entomology, Dr. PDKV, Akola, Maharashtra.

Multiplication and Isolation of Bt protein from *E. coli* cultures

E. coli culture was inoculated in 50 ml Luria broth (LB) medium with 50µg / ml ampicillin and incubated at 37°C, 150 RPM overnight. From the seed culture, 1% was inoculated to a fresh 400 ml LB medium with ampicillin and incubated at 37°C, 150 rpm for 8 h. After the incubation period, the LB medium should become thicker in consistency along with opacity, which is ready for protein isolation.

The procedure adopted for purification of protein from the incubated medium is given below:

1. To the medium 400µl isopropyl thio glycol (IPTG) was added and incubated for 2h. Then centrifugation was carried out at 5000 RPM at 4°C for 10 min.
2. The pellet was resuspended in 20 ml of lysis buffer (50 mM Tris pH 8.0, 50 mM EDTA and 18% sucrose), to this 1 mg / ml lysozyme was added and incubated for 2 h with slow shaking. Then centrifugation was carried out a 500 RPM, 4°C for 10 min.
3. Then the pellet was resuspended in crystal wash I (0.5 M NaCl, 2% Triton-X) along with protease inhibitor trypsin.
4. The sample was sonicated for 5 min (2 pulse). To this 10 ml crystal wash I was added and centrifuged at 500 RPM, 4°C for10 min.
5. The pellet was washed three times with crystal wash I and crystal wash II (0.5 M NaCl), and finally with sterilized distilled water. Each time the supernatant was discarded.
6. The obtained pellet was resuspended in 10 ml stabilizing buffer and incubated at 37°C for 3-4 h.
7. Then the sample was centrifuged at 10000 RPM, 4°C for 10 min. The supernatant was treated as crystal protein. The toxins were stored at –20°C for bioassays.

Bioassays of ICPs against *E. vittella*

Bioassays of ICPs were conducted by surface diet contamination (Liao *et al.*, 2002) using cryo vials. The concentrations of Cry1Aa, Cry1Ab, and Cry1Ac used in the bioassays ranged from 50 - 300, 50 - 400 and 50 - 175ng / cm^2, respectively. Similarly a control was also used. The experiments were conducted when the insect stock culture was in F_4 - F_6 generation. All the components of the semi-synthetic diet, except formalin were used for making the bioassay diet. Approximately 1 ml of the diet was dispensed into cryo vials (without touching the sides of vial) and allowed to settle. After the diet solidified the Cry protein was layered on the diet surface @ 10µl per vial using a sterile glass rod. The vials were allowed to dry for 30 min and to each vial a larva was released using a soft hairbrush. Each treatment was replicated four times with 10 larvae as an experimental unit. The larval mortality was recorded every 24 h, consecutively for seven days. Moribund larvae were also considered dead

when the observations were terminated. The experiments were carried out at 27±2°C, 70±5 per cent RH and natural day and light periods. The mortality data were subjected to probit analysis (Finney, 1971) to calculate median lethal concentration (LC_{50}), LC_{95} and fiducial limits. The probit analyses were carried out using the statistical package for social sciences (SPSS) version 10.0 SPSS Inc., USA.

RESULTS AND DISCUSSION

The neonates were the most susceptible to Bt ICPs than other instars studied. Among the ICPs, Cry1Ac was the most toxic against all instars of *E. vittella*. The LC_{50} and LC_{95} of Cry1Ac against *E. vittella* neonates were 66.62 and 343.05ng / cm^2, respectively (Tables 1 to 3).

The order of toxicity of different ICPs to *E. vittella* neonates was Cry1Ac > Cry1Ab > Cry1Aa > Cry1C > Cry1E (66.62 > 97.04 > 108.54 > 1240.83 > 1335.60ng / cm^2) (Table 1). As the Cry1C and Cry1E were relatively less toxic to *E. vittella* neonates, they were not evaluated against the late instars. The overlapping fiducial limits at LC_{50} for Cry1Aa and Cry1Ab reveal that they were almost similar in toxicity to *E. vittella* neonates. The order of toxicity against second instar was Cry1Ac > Cry1Aa > Cry1Ab (107.96 > 161.96 > 192.99 ng/cm^2) (Table 2), whereas the order of toxicity against third instar was Cry1Ac > Cry1Ab > Cry1Aa (169.96 > 197.11 > 213.27ng / cm^2) (Table 3). Even though variation occurred in the toxicity of Cry1Aa and Cry1Ab, the overlapping fiducial limits at LC_{50} indicated no significant difference in their toxicity to *E. vittella* (Table 2 and 3).

The less toxic nature of Cry1C and Cry1E has been recorded earlier (Liao *et al.*, 2002; Chakrabarti *et al.*, 1998).

Susceptibility to bioinsecticides typically declines as the larvae develop (Hornby and Gardner, 1987). The late instars of *Cadra cautella*, *Plodia interpunctella*, and *Helicoverpa zea* have been reported to be less susceptible to *B. thuringiensis* than their early instars (Ali and Young, 1996).

The more toxic nature of Cry1Ac to lepidopterans was already recorded by Kranthi *et al.* (1999a and 2000) and Jalali *et al.* (2004). Mahapatro *et al.* (2001) recorded the LC_{50} of Cry1Aa, Cry1Ab and Cry1Ac to *E. vittella* third instar was 224.5, 300.5 and 141.2 ng/cm^2, respectively. Kranthi *et al.* (1999b) reported 4-6 fold variation in the susceptibility of *E. vittella* to Cry1Aa, Cry1Ab and Cry1Ac.

In the present study, even though variation occurred in the toxicity of Cry1Aa and Cry1Ab, the overlapping fiducial limits at LC_{50} indicated there was no significant difference in their toxicity to

Table 1. Relative toxicity of *B. thuringiensis* insecticidal crystal proteins against *Earias vittella* neonates

Toxin	LC_{50} (ng / cm^2)	95% fiducial limits		LC_{95} (ng / cm^2)	95% fiducial limits		'b' (±SE)	Chi square $(\chi^2)^{\#}$ (n-2)
		Lower	Upper		Lower	Upper		
Cry1Aa	108.54	98.39	119.08	353.35	284.36	490.08	3.20 ± 0.35	3.57
Cry1Ab	97.04	87.02	107.35	321.95	232.54	476.29	3.15 ± 0.39	3.98
Cry1Ac	66.62	52.13	86.56	343.05	198.31	434.89	3.10 ± 0.34	3.38
Cry1C	1240.83	1153.73	1331.95	2858.90	2384.69	3868.07	4.53 ± 0.60	2.09
Cry1E	1335.60	1334.37	1531.24	3124.37	2643.53	4051.09	4.45 ± 0.52	2.62

#In each case, the χ^2 value from the goodness of fit test was less than tabular value (P = 0.05), indicating that the data fit the probit model

Table 2. Relative toxicity of *B. thuringiensis* insecticidal crystal proteins against second instar *Earias vittella* larvae

Toxin	LC_{50} (ng/cm²)	95% fiducial limits		LC_{95} (ng/cm²)	95% fiducial limits		'b' (±SE)	Chi square $(\chi^2)^{\#}$ (n-2)
		Lower	Upper		Lower	Upper		
Cry1Aa	161.96	151.79	172.26	354.56	307.06	440.15	4.83 ± 0.52	2.88
Cry1Ab	192.99	171.46	216.94	380.88	303.08	677.99	4.57 ± 0.54	2.52
Cry1Ac	107.96	97.68	118.60	358.48	287.17	502.00	3.15 ± 0.35	4.42

In each case, the χ^2 value from the goodness of fit test was less than tabular value (P = 0.05), indicating that the data fit the probit model

Table 3. Relative toxicity of *B. thuringiensis* insecticidal crystal proteins against third instar *Earias vittella* larvae

Toxin	LC_{50} (ng/cm²)	95% fiducial limits		LC_{95} (ng/cm²)	95% fiducial limits		'b' (±SE)	Chi square $(\chi^2)^{\#}$ (n-2)
		Lower	Upper		Lower	Upper		
Cry1Aa	213.27	192.46	234.66	725.37	578.05	1025.95	3.09 ± 0.35	3.28
Cry1Ab	197.11	177.70	216.05	628.01	514.66	844.99	3.26 ± 0.35	3.24
Cry1Ac	169.96	159.08	181.64	390.86	331.51	504.17	4.54 ± 0.52	2.90

In each case, the χ^2 value from the goodness of fit test was less than tabular value (P = 0.05), indicating that the data fit the probit model

E. vittella (Table 2 and 3). These results corroborate that of Mahapatro *et al.* (2001). In the present study the toxicity of Cry toxins to different instars of *E. vittella* was studied and the findings could be useful to maintain toxicity levels in transgenic cotton that will manage all the instars.

REFERENCES

Ali, A. and Young, S. Y. 1996. Activity of *Bacillus thuringiensis* Berliner against different ages and stages of *Helicoverpa zea* (Lepidoptera : Noctuidae) on cotton. *Journal of Entomological Science*, **31**: 1-8.

Barwale, R. B., Godwal, V. R., Usha, Z. and Zehr, B. 2004. Prospects for Bt cotton technology in India. *AgbioForum*, **7**: 23-26.

Chakrabarti, S. K., Mandaokar, A., Kumar, P. A. and Sharma, R. P. 1998. Efficacy of lepidopteran-specific delta-endotoxins of *Bacillus thuringiensis* against *Helicoverpa armigera*. *Journal of Invertebrate Pathology*, **72**: 336-337.

Crickmore, N., Niegler, D. R., Fietelson, J., Schnepf, E., Van Rie, J., Lereclus, D., Baum J. and Dean, D. H. 1998. Revision of the nomenclature for *Bacillus thuringiensis* pesticidal crystal proteins. *Microbiology and Molecular Biology Reviews*, **62**: 807-813.

Entwistle, P. F., Cory, J. S., Bailey, M. J. and Higgs, S. 1993. *Bacillus thuringiensis*, an environmental biopesticide. John Wiley and Sons. Inc., New York, N.Y., USA.

Finney, D. J. 1971. *Probit Analysis*. 3rd edition. Cambridge University Press, Cambridge.

Gupta, G. P., Mahapatro, G. K. and Chandra, A. 1999. Partial larval rearing of *Earias vittella* (F.) on artificial diet. *Insect Science and its application*, **18**: 371-375.

Hoftey, H. and Whiteley, H. P. 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiology Review*, **53**: 242-255.

Hornby, J. A. and Gardner, W. A. 1987. Dosage / mortality response of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and other noctuid larvae to beta-exo toxin of *Bacillus thuringiensis*. *Journal of Economic Entomology*, **80**: 925-929.

Jalali, S. K., Mohan, K. S., Singh, S. P., Manjunath, T. M. and Lalitha, Y. 2004. Baseline susceptibility of the old-world bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera, Noctuidae) populations from India to *Bacillus thuringiensis* Cry1Ac insecticidal protein. *Crop Protection*, **23**: 53-59.

Kranthi, K. R., Kranthi, S., Ali, S. and Banerjee, S. K. 2000. Resistance to Cry1Ac δ-endotoxin of *Bacillus thuringiensis* in a laboratory selected strain of *Helicoverpa armigera* (Hübner). *Current Science*, **78**: 1001-1004.

Kranthi, S., Kranthi, K. R. and Lavhe, N. V. 1999a. Baseline toxicity of Cry1A toxins to the spotted bollworm *Earias vittella* F. *Crop Protection*, **18**: 551-555.

Kranthi, S., Kranthi, K. R. and Lavhe, N. V. 1999b. Geographical variation in susceptibility of *Earias vittella* to Cry toxins. *Crop Protection*, **18**: 537-555.

Liao, C., Heckel, D. G. and Akhurst, R. 2002. Toxicity of *Bacillus thuringiensis* insecticidal proteins for *Helicoverpa armigera* and *Helicoverpa punctigera* (Lepidoptera: Noctuidae), major pests of cotton. *Journal of Invertebrate Pathology*, **80**: 55-63.

Lutterell, R. G. and Herzog, G. A. 1994. Potential effect of transgenic cotton expressing Bt on cotton IPM programme. *Proceedings of Beltwide Cotton Conference*, **5**: 806-809.

Mahapatro, G. K., Gupta, G. P. and Ananda Kumar, P. 2001. Bioassay of insecticidal *Bacillus thuringiensis* Cry1A delta-endotoxins against the spotted bollworm *Earias vittella* Fabricius. *Insect Science and its Application*, **21**: 225-228.

Schnepf, E., Crickmore, N., Van Rie, J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D. R. and Dean, D. H. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiology and Molecular Biology Reviews*, **62**: 755-806.

Tabashnik, B. E. 1994. Evolution of resistance to *Bacillus thuringiensis*. *Annual Review of Entomology*, **39**: 47-79.

Tabashnik, B. E., Cushing, N. L., Finson, N. and Johnson, M. W. 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). *Journal of Economic Entomology*, **83**: 1671-1676.

Wu, K., Guo, Y. and Nan, L. V. 1999. Geographic variation in susceptibility of *Helicoverpa armigera* to *Bacillus thuringiensis* insecticidal protein in China. *Journal of Economic Entomology*, **92**: 273-278.

(Received: 16.06.2006; Revised: 11.04.2007; Accepted: 21.04.2007)