Pakistan J. Zool., vol. 44(2), pp. 463-468, 2012.

Comparison of Antibiosis of Spotted Bollworm, *Earias vittella* (Fab.), on Two Bt– and One Non Bt– Cotton Varieties

Sohail Ahmed,[1]* Muhmmad Kashif Iqbal,[1]* Muhammad Shahid[2] and Rashad Rasool Khan[1]
[1]*Department of Agri. Entomology, University of Agriculture, Faisalabad, 38040, Pakistan*
[2]*Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, 38040, Pakistan*

Abstract.- In order to determine antibiosis of spotted bollworm (SBW), *Earias vittella* (Fab.) (Arctiidae: Lepidoptera) on two Bt (FH 113 and FH 114) and one non Bt (FH 2015) varieties, infestation, life cycle and enzymes activity of the bollworm were compared on these varieties. Results revealed that the seasonal infestation of squares was the highest (9.5%) on FH 2015 followed by FH 113 (6.2%) and FH 114 (4.9%). FH 2015 (3.2-17%) had statistically high flower infestation than FH 113 (1.5-1.9%) and FH 114 (0.6-2.0%). The seasonal percent infestation of bolls was significantly highest (6.8%) on FH 2015 than FH 113 (2.1%) and FH 114 (2.4%). The biology of SBW on these varieties showed that egg incubation and larval durations were longer on Bt varieties as compared to non Bt variety. The activities of non specific esterases were high in larvae reared on Bt varieties whereas total proteases were low on these varieties. The physiological adaptation of SBW on Bt cotton varieties is discussed.

Keywords: Spotted bollworm, *Earias vittella*, esterases, proteases, Bt cotton varieties

INTRODUCTION

The cotton varieties (*Gossypium hirsutum* L.) differ significantly among themselves with regards to the infestation of sucking and chewing types of insect pests (Godoy Avila *et al*., 2000; Abro *et al*., 2004; Dhillon and Sharma, 2009; Arshad and Suhail, 2010; Govindan *et al*., 2010). This difference is much pronounced where varieties have been compared with Bt cotton varieties. A number of studies have shown that Bt cotton recorded less infestation of bollworms, particularly of *Helicoverpa armigera* (Hub.), *Earias* spp. and *Pectinophora gossypiella* (Saund.) (Manjunatha *et al*., 2009). The criterion of distinguishing varieties has been comparison of infestation level and very few studies revealed difference in other biological parameters such as life history characteristics (Wu *et al*., 2009; Guo *et al*., 2010; Somashekara *et al*., 2011).

It has been stated that there is a temporal and spatial variation in CrylAc protein levels in Bt cotton (Luttrel and Mink, 1999). Although Bt toxin in transgenic cotton has adverse effects on larval survival and development of *H. armigera* (Bambawale *et al*., 2004), toxin level was different in various plants parts. This toxin level decreased as the crop matured and was very low or undetectable in squares (Kranthi *et al*., 2005) and bolls (Greenplate *et al*., 2000).

Since the toxin "Cry protein" in Bt cotton grown in the country being highly variable as was also evident in a survey conducted by Pakistan Agricultural Research Centre (PARC) in the year 2007 where 10 and 19 percent samples from 126 locations in 21 districts were devoid of the toxin or were below detectable limits (PARC, 2008). This variability in toxin may result in better survival and development of target pests, escaping direct or high doses of the toxin (Adamczyk and Gore, 2004). The insects that are in constant contact with Bt and its toxins most likely have a heightened innate cellular defense, and consequently undergo physiological adaptations (may be enzyme based) that result in resistance to the insecticidal action of Bt. By increasing fitness on the Bt varieties, resistance to insecticides in these insect pests on the introduced Bt cotton offers significant promise of studying their life sustaining parameters on Bt varieties. Sayyed *et al*. (2003) have also indicated this possibility and stated that insecticidal Bt toxins produced by transgenic crops can have nutritionally favorable effects that can increase the fitness of the resistant insects eating such crops.

Spotted bollworm (SBW), *Earias* spp. (Arctiidae: Lepidoptera) larvae stay away from

* Corresponding author: saha786_pk@yahoo.com
0030-9923/2012/0002-0463 $ 8.00/0

leaves and bore into the top tender growing shoots and tunnels down the stem, resulting drying of the attacked shoots and at the time of boll formation, larvae enter the bolls feed inside and then move to other bolls, provide a good model to investigate antibiosis. Since there is dearth of information on the biology of the spotted bollworm on the transgenic Bt cotton, the present study was aimed at recording the infestation of fruiting bodies in the field on one hand and the life cycle of SBW on these varieties in the laboratory on the other hand. Finally, proteases and esterases in the midgut of the bollworm were studied to determine physiological adaptation of SBW on Bt genotypes.

MATERIALS AND METHODS

The study was conducted to determine the comparative infestation of cotton bollworms *viz.*, spotted bollworm (*Earias vittella*) against two transgenic Bt (FH-113, FH-114) (characteristics given below) and one conventional cultivar (FH-2015), which were chosen from a pool of varieties grown in the experimental area of Directorate of Cotton, Ayub Agricultural Research Institute, Faisalabad, Pakistan. These varieties were laid out in randomized complete block design (RCBD), with 3 replications during 2010 crop season. Plot size of each experimental unit was 18.3 meter x 9.1 meter having row to row and plant to plant distance of 0.75 m and 0.30 m respectively. The data on the percentage infestation of SBW were recorded on the basis of the healthy and damaged fruiting bodies i.e., squares, flowers and bolls which were counted from five randomly selected plants on weekly basis from July to October, 2010. Percentage of damaged fruiting bodies was then calculated.

Genotypes	Parentage	Distinctive Features
FH 113	FH-113 FH-925×NuCot-N33B	Tall growing Bt variety possesses moderate tolerance to CLCuV. It has strong stem, prolonged flowering duration and require low inputs.
FH 114	FH-925×NuCot-N33B	A compact input intensive Bt variety with high tolerance to CLCuV and heat stress. Best suited to high plant population in late sowing.

Source: Mahmood *et al*., 2010 (personal communication)

Studies on biology

For investigating the biology of spotted bollworm on three varieties, observations on fecundity, larval and pupal duration, survival, adult emergence and longevity of male and female were made.

The larval population was collected from respective cotton variety and was reared in the laboratory on flowers of Bt and Non Bt varieties. The adults thus emerged from these larval populations were transferred into an iron cage 30 cm- long, 30-cm wide and 30cm high, wrapped around with nylon mesh. Flowers of each variety along with five mating adults were put into cages and the adults were allowed to lay eggs on these flowers. Total number of eggs laid by mating pairs were counted and averaged as eggs per female. Twenty five newly hatched larvae were transferred to flowers of respective variety on moistened filter paper in Petri dishes (9 cm diameter). The duration of larvae was thus counted and pupae formed were also noted. These pupae were put in iron cage and adult emergence (%) and longevity of male and female (in days) were recorded. All these studies were carried out in three replications under the laboratory conditions during month of July to October when ranges of temperature and relative humidity were 30±0.9 to 34±1.4°C and 54±9 to 72±8%, respectively.

Enzymes assay

Total proteases and non specific esterases were determined by methods of Drapeau (1974) and Shields *et al*. (1983), respectively. Protein content in enzyme supernatant was measured by method of Bradford (1976). To get supernatant, larvae of SBW were dissected in insect saline (NaCl 7.6g, KCl 3.79g and $CaCl_2$ 21.9g in 100 ml. distilled water). Guts were removed and remained in insect saline until homogenization of mid gut of four larvae replicated three times. The homogenization was carried out in phosphate buffer in a tube by electric driven homogenizer at 1500 rpm for 10 sec. the homogenate was centrifuged at 4000 rpm for 15 minutes. The supernatant thus obtained was then used for enzyme assays. Casein and α-naphthyl acetate were substrates for proteases and esterases, respectively.

Finally all data were subjected to Analysis of Variance (ANOVA) and treatment means were separated by Duncan Multiple Range (DMR) Test at 5% level of probability.

RESULTS

Infestation of fruiting bodies on three cotton varieties

The percent infestation of SBW on squares of three cotton varieties is given in Table I. The significantly high infestation of squares was found in FH 2015 in month of July, August and September than FH 113 and FH 114. The difference in infestation became non significant in October and November. The seasonal infestation of squares was the highest (9.5%) on FH 2015 followed by FH 113 (6.2%) and FH 114 (4.9%); latter two had no statistical difference between them. The infestation of flowers on three varieties had non significant difference in month of July, however, later in season statistical difference among varieties was evident. FH 2015 (3.2-17%) had statistically high flower infestation than FH 113 (1.5-1.9%) and FH 114 (0.6-2.0%) (Table II). The bolls' infestation on three varieties had significant difference among them throughout the season. The seasonal percent infestation of bolls was significantly highest (6.8%) on FH 2015 than FH 113 (2.1%) and FH 114 (2.4%); latter two were statistically at par between one another (Table III).

Table I.- Percent infestation (Mean±SE) of SBW on squares of three cotton varieties.

Months	Percent infestation			F value	p value
	FH 2015	FH 113	FH 114		
July	4.3±0.3a	1.6±0.4b	1.4±0.4b	17.1	0.00
August	10.0±1.0a	5.5±0.6b	4.9±0.5b	14.4	0.00
September	15.9±0.8a	8.6±0.6b	7.0±0.5b	54.8	0.00
October	5.6±0.8ns	6.6±0.5	4.6±0.6	2.2	0.1
November	4.6±0.6ns	4.7±0.6	3.6±0.2	10.0	0.1
Seasonal mean	9.5±0.8a	6.2±0.4b	4.9±0.3b	17.0	0.00

Means in a row having same letters are not significantly different at P<0.05.

Life cycle studies of SBW on three varieties

Regarding biology of SBW on three varieties, the egg incubation period and larval duration had statistical difference among varieties. Both egg incubation and larval durations were longer on Bt varieties as compared to non Bt variety (FH 2015). Incubation period on FH 2015, FH 113 and FH 114 was 3.0, 4.5 and 4.9 days, respectively. The larval duration on FH 2015, FH 113 and FH 114 was 14.16, 18.75 and 19.00 days, respectively. Incubation and larval duration were statistically at par on Bt varieties. The pupal duration, longevity of male and female adult and emergence of adults from pupae were statistically similar on three varieties (Table IV).

Table II.- Percent infestation (Mean±SE) of SBW on flowers of three cotton varieties.

Months	Percent infestation			F value	p value
	FH 2015	FH 113	FH 114		
July	1.9±0.3ns	1.8±0.3	1.7±0.2	0.16	0.8
August	10.1±0.7a	1.8±0.3b	2.0±0.3b	94.9	0.00
September	17.0±0.7a	1.8±0.2b	1.6±0.2b	336.7	0.00
October	7.7±0.5a	1.5±0.3b	1.3±0.3b	159.7	0.00
November	3.2±0.8a	1.9±0.5b	0.6±0.2b	6.25	0.03
Seasonal mean	10.2±0.8a	1.6±0.1b	1.5±0.1b	100	0.00

Means in a row having same letters are not significantly different at P<0.05.

Table III.- Percent infestation (Mean±SE) of SBW on bolls of three cotton varieties.

Months	Percent infestation			F value	p value
	FH 2015	FH 113	FH 114		
July	3.2±0.3a	0.7±0.2b	0.6±0.2b	42.2	0.00
August	5.7±0.3a	2.0±0.3b	2.3±0.3b	63.0	0.00
September	10.8±0.6a	4.0±0.5b	4.0±0.3b	56.4	0.00
October	6.6±0.7a	1.5±0.6b	2.4±0.3b	23.4	0.00
November	2.9±0.3a	0.3±0.1b	0.8±0.2b	39.9	0.00
Seasonal mean	6.8±0.5a	2.1±0.3b	2.4±0.2b	56.9	0.00

Means in a row having same letters are not significantly different at P<0.05.

Enzyme activities

The activities of non specific esterases and total proteases in midgut of larvae reared on non Bt and Bt varieties are shown in Fig.1. The esterases were significantly high in larvae reared on flowers of Bt varieties than non Bt variety (F 5.12, p 0.03) whereas proteases were significantly lower in the larvae on Bt varieties (F 5.54, p 0.02). In both cases, Bt varieties had non significant difference between one another for esterases and proteases.

Table IV.- Comparison of life cycle parameters (Mean±SE) of spotted boll worm on three cotton varieties.

Varieties	Fecundity	Durations (days)			Longevity (days)		Adult emergence (%)
		Incubation	Larval	Pupal	Male	Female	
FH 2015	239.0±25ns	3.0±0.3b	14.1±1.4b	7.2±0.7ns	14.3±1.6ns	15.5±1.6ns	90.3±1.6ns
FH 113	213.5±27	4.5±0.3a	18.7±1.0a	8.6±0.8	15.9±1.7	17.0±1.8	90.8±1.5
FH 114	182.0±15	4.9±0.4a	19.0±1.1a	9.2±1.0	16.4±1.8	17.5±1.9	89.0±0.3
F value	1.5	6.1	5.0	1.3	0.4	0.3	0.2
p value	0.2	0.00	0.01	0.3	0.7	0.7	0.8

Means in a column having same letters are not significantly different at P<0.05. ns, non significant.

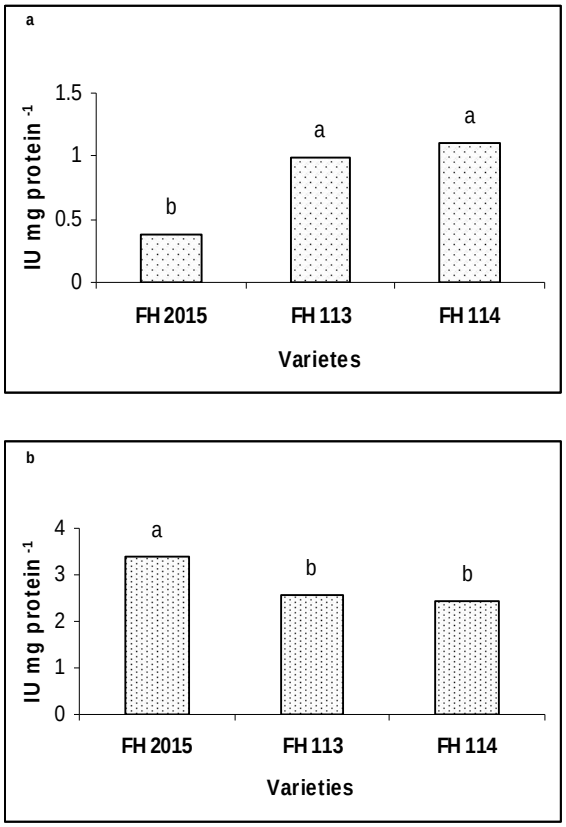

Fig. 1. Non specific esterases (a) and total proteases (b) activities in midgut of larvae reared on non Bt (FH 2015) and Bt (FH 113 and FH 114) cotton varieties. IU, international units. Means as bars with same letters are not significantly different at P<0.05.

DISCUSSION

The results of present studies are in line with earlier published literature which revealed that infestation of bollworms (*Earias* spp., *Pectinophora gossypiella* and *Helicoverpa armigera*) is reduced on Bt cotton varieties when compared with counterpart non Bt variety (Godoy Avila *et al*., 2000; Abro *et al*., 2004; Dhillon and Sharma, 2009; Govindan *et al*., 2010). In addition to direct toxicity of Bt toxin, change in behaviour is also accounted for this reduction. Food utilization efficiency of *E. vittella* on the squares of Bt cotton genotypes was greatly reduced than on non Bt genotypes (Somashekara *et al*., 2011). Bt cotton varieties may exhibit either resistance to toxin or non availability of the toxin at certain stage of crop growth. Regarding resistance to Bt toxin, the continued efficacy of Bt crops after 9 years refutes the worst scenarios predicting pest resistance to Bt crops in as little as 3 years (Tabashnik *et al*., 2005) but its occurrence cannot be ruled out. The evidence of non availability of Bt toxin at certain growth stage of the crop is increasing (PARC, 2008).

Apart from delay in larval development, Bt and non Bt varieties did not differ statistically in other characteristics such as fecundity, pupal period, longevity of male and female and adult emergence from pupae. There was also no difference in survival rate of larvae between Bt and non Bt varieties (data not shown). Liu *et al*. (2005) has also reported the delay in larval development of *H. armigera* on Bt genotype. The present data is supported by argument / expectation that Bt varieties may nutritionally favour bollworms (Sayyed *et al*., 2003). This is held true by elsewhere reported studies in that continuous selection pressure by Bt toxin may change detoxification enzymes in *Spodoptera exigua* that allows them to

better survive on Bt cotton in subsequent generations (Wu *et al*., 2008; Guo *et al*., 2010). According to present data on enzyme activities, the increase in esterases in midgut of *E. vittella* on Bt variety may allow incest to complete its development successfully on Bt varieties. The low activity of total proteases may have resulted for binding of some proteases for inactivating protein toxin ingested as a consequence of feeding (Terra *et al*., 1996).

The current results showed that despite low infestation level and slow rate of larval development of SBW, survivors with high detoxification enzyme may render the insect at advantage to resist insecticides used for its control program on Bt varieties.

REFERENCES

ABRO, G.H., SYED, T.S., MUNIO, G.M. AND KHUHRO, M.A., 2004. Performance of transgenic Bt cotton against insect pest infestation. *Biotechnology*, **3**: 75-81.

ADAMCZYK, J.J. AND GORE, J., 2004. Development of bollworms, *Helicoverpa zea*, on two commercial Bollgard(R) cultivars that differ in overall Cry1Ac levels. *J. Insect Sci*., **4**: 1-5.

ARSHAD, M. AND SUHAIL, A., 2010. Studying the sucking insect pests community in transgenic Bt cotton. *Int. J. Agri. Biol*., **12** (5): 764-768.

BRADFORD, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.,* **72***:* 248-254.

BAMBAWALE, O.M., SINGH, A., SHARMA, O.P., BHOSLE, B.B., LAVEKAR, R.C., DHANDAPANI, A., KANWAR, V., TANWAR, R.K., RATHOD, K.S., PATANGE, N.R. AND PAWAR, V.M., 2004. Performance of Bt cotton (MECH-162) under Integrated Pest Management in farmers' participatory field trial in Nanded district, Central India. *Curr. Sci*., **86**: 1628–1633.

DHILLON, M.K. AND SHARMA, H.C., 2009. Impact of *Bt*-engineered cotton on target and non-target arthropods, toxin flow through different trophic levels and seed cotton yield. *Karnataka J. agric. Sci.*, **22:** 462–466.

DRAPEAU, G., 1974. Protease from *Staphylococcus aureus*. In: *Methods in enzymology* (ed. L. Lorand), 45B, Academic Press, NY. p. 469,

GODOY AVILA, S., PALOMO GIL, A. AND GARCÍA HERNÁNDEZ, J.L., 2000. Evaluation of transgenic cotton (*Gossypium hirsutum* L.) varieties resistant to pink bollworm (*Pectinophora gossypiella* S.) I. Yield. *ITEA Producción Vegetal*, **96**: 157-164.

GOVINDAN, K., GUNASEKARAN, K., KUTTALAM, S. AND AISWARIYA, K.K., 2010. Laboratory evaluation of transgenic Bt cotton and non Bt cotton plant parts against third instar larvae of *Spodoptera litura* (Fab.) (Noctuidae: Lepidoptera). *J. Biopestic*., **3**: 432-436.

GREENPLATE, J., PENN, S.R., MULLINS, J.W. AND OPPENHUIZEN, M., 2000. Seasonal Cry1Ac levels in DP50B: the "Bollgard basis" for Bollgard II. *Proc. Beltwide Cotton Conf., San Antonio, USA*, 4-8 January, **2**: 1039–1040.

GUO, J.-Y., WU, G. AND WAN, F.-H., 2010. Activities of digestive and detoxification enzymes in multiple generations of beet armyworm, *Spodoptera exigua* (Hub.) in response o transgenic Bt Cotton. *J. Pestic. Sci*., **83**: 453-460.

KRANTHI, K.R., NAIDU, S., DHAWAD, C.S., TATWAWADI, A., MATE, K., PATIL, E., BHAROSE, A.A., BEHERE, G.T., WADASKAR, R.M. AND KRANTHI, S., 2005. Temporal and intra-plant variability of Cry1Ac expression in Bt-cotton and its influence on the survival of the cotton bollworm, *Helicoverpa armigera* (Hubner) (Noctuidae : Lepidoptera). *Curr. Sci.*, 89: 291–298.

LIU, X.D., ZHAI, B.P., ZHANG, X.X. AND MIN, J., 2005. Impact of transgenic cotton plants on a non target pest *Aphis gossypii* Glover. *Ecol. Ent*., **30**: 307-3015.

LUTTRELL, R.G. AND MINK, J.S., 1999. Damage to cotton fruiting structures by the fall armyworm, *Spodoptera frugiperda* (J.E.Smith) (Lepidoptera: Noctuidae). *J. Cott. Sci*., **3**: 35–44.

MAHMOOD, A., FAROOQ, J. AND NOOR-UL-ISLAM, 2010. *Introgression of desirable characters for growing cotton in Punjab*, Pakistan.

MANJUNATHA, R., MANJUNATHA, M., NAIK, M.I., SHIVANNA, B.K. AND HOSAMANI, V., 2009. Comparative performance of Bt and non-Bt cotton against bollworm complex. *Karnataka J. agric. Sci*., **22** (3-Spl. Issue): 646-647.

PARC (PAKISTAN AGRICULTURAL RESEARCH COUNCIL), 2008. *Status of cotton harboring Bt gene in Pakistan.* Instt. Agri-Biotechnol. Genetic Resources, National Agricultural Research Centre, Pakistan Agricultural Research Council, Islamabad.

SAYYED, A.H., CERDA, H. AND WRIGHT, D.J., 2003. Could *Bt* transgenic crops have nutritionally favourable effects on resistant insects? *Ecol. Lett*., **6**: 167-169.

SHIELDS C.R., ORTON T.J. AND STUBER, C.W., 1983. An outline of general resource needs and procedures for the electrophoretic separation of active enzymes from plant tissue. In: *Isozymes in plant genetics and breeding* (eds. S.D. Tanksley and T.J. Orton), Elsevier Sci. Publish., B.V., Amsterdam. pp. 443-512.

SOMASHEKARA, R., UDIKERI, S.S., PATIL, S.B. AND

BASAVANAGOUD, K., 2011. Food consumption indices for spotted bollworm *Earias vittella* (Fab.) on transgenic cottons expressing one or more Bt genes. *Karnataka J. agric. Sci.*, **24**: 140-142.

TABASHNIK, B.E., DENNELY, T.J. AND CARRIERE, Y., 2005. Delayed resistance to transgenic cotton in pink bollworm. *Proc. natl. Acad. Sci.*, **102**: 15389-15393.

TERRA, W.R., FERREIRA, C., JORDÃO, B.P. AND DILLON, R.J., 1996. Digestive enzymes. In: *Biology of the insect midgut*, Chapman and Hall, London. pp. 153–194.

WU, G., HARRIS, M.K., GUO, J.-Y. AND WAN, F.-H., 2009. Response of multiple generations of beet armyworm *Spodopetra exigua* (Hub.) feeding on transgenic Bt Cotton. *J. appl. Ent.*, **133**: 90-100.

(Received 30 July 2011, revised 14 August 2011)