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Comparative study on oviposition and larval preference of spotted bollworm, *Earias vittella* on Bt and non-Bt cotton

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Abstract

Oviposition and larval preference of spotted bollworm, *Earias vittella* (Fabricius) was assessed on four transgenic Bt cotton hybrids, viz. MRC 6304 Bt (*cry1Ac* gene), JKCH 1947 Bt (modified *cry1Ac* gene), NCEH 6R Bt (*cry1Ab*/*cry1Ac* fused gene) and MRC 7017 BG II (*cry1Ac* and *cry2Ab* genes) in comparison to the respective isogenic cotton. The results showed that Bt toxin did not deter oviposition preference of *E. vittella* moths as there was no significant difference in the number of eggs laid on squares/bolls of Bt and non-Bt cotton hybrids, across different crop growth stages. There was also no behavioral change in larval preference with respect to selecting non-Bt cotton in comparison to Bt cotton. Floral bodies from Bt and the respective isogenic cotton genotypes were equally preferred by both first and third instar larvae after 24 hrs indicating that initial selection was independent of susceptibility to Bt toxin. However, *E. vittella* larvae showed significant difference in preference for different cotton genotypes. Studies on the relative preference indicated that third instar larvae had greater preference for bolls (7.29-7.50%) than for the squares (5.0-5.21%) and reverse was true for the first instar larvae which showed greater preference for squares (7.08-7.29%) than for the bolls (5.21-5.42%), in a multiple-choice test. It may be concluded that oviposition and larval preference of *E. vittella* did not differ significantly between Bt and isogenic non-Bt cotton genotypes.

Key words

Bt cotton, Earias vittella, Larval preference, Oviposition, Spotted bollworm.

Introduction

Cotton (*Gossypium* spp.) is the most important commercial crop in India and plays a vital role in agricultural, industrial, social and monetary affairs of the country. India is the only country in the world where all the four cultivated species of cotton, viz. *Gossypium arboreum* L., *G. hirsutum* L., *G. herbaceaum* L. and *G. barbadense* L. along with intra- and inter- specific hybrids are cultivated along the diverse agro-climatic conditions, varying from 8-32° N latitude and 70-80° longitude on commercial scale spanning around 11.6 million hectares. Area wise, India ranks first in global scenario (about 35% of the world cotton area) but with regard to production, it rank next to China, the top producer (Anonymous 2014).

As many as 1326 species of insects have been recorded on cotton crop right from sowing to maturity in different cotton growing areas of the world (Hargreaves, 1948) and 162 species have been reported on the cotton crop in India, of which 24 species have attained pest status (Arora *et al*., 2011). Among these, the bollworm complex comprising of American bollworm [*Helicoverpa armigera* (Hübner)], spotted bollworm [*Earias vittella* (Fabricius)], spiny bollworm [*E. insulana* (Boisduval)] and pink bollworm [*Pectinophora gossypiella* (Saunders)] are the key pests in Punjab. Transgenic Bt cotton expressing genes from soil inhabiting spore forming bacterium, *Bacillus thuringiensis* Berliner (*Bt*) toxins for bollworm management was first approved for commercial cultivation by the Genetic Engineering Approval Committee (GEAC), Ministry of

Environment and Forests, Government of India in Central and South India in 2002 and in North India in 2005. So far, six transgene events, viz. MON 531 (*cry1Ac*), Event 1 (modified *cry1Ac*), GFM event (fusion *cry1Ab*/*cry1Ac*), BNLA-601 (*cry1Ac*), MLS-9124 (*cry1C* gene) and MON 15985 (*cry1Ac* and *cry2Ab*) have been approved by GEAC in India (Choudhary and Gaur, 2011).

As the expression of Cry toxin in Bt cotton varies among plant structures (Kranthi *et al*., 2005), the changes in intra-plant pest behaviour are a concern and have been investigated as a potential mechanism contributing to control failures and selection for resistance. Studies on oviposition behaviour of bollworms have demonstrated that ovipositing female moths clearly preferred the top five nodes of cotton plants for egg placement (Torres and Ruberson, 2006). This behaviour resulted in contact of neonate larvae with terminal plant tissues that have higher toxin expression. However, bollworm females laying eggs on plant parts with low toxin expression within the plant canopy increased the likelihood of survival for their offspring (Gore *et al*., 2002). It may allow initial larval development to an older stage more tolerant of Cry1Ac and can lead to control failures and increase resistance selection. Therefore, a behavioural change in bollworm egg placement may facilitate escape from exposure to the highest dosages of Cry1Ac toxin expression (Torres and Ruberson, 2006). The toxin expression differs in different tissues, and the insect pests can avoid the tissues that contain higher amount of toxins or select low toxin or toxin free tissues. Several lepidopteran pests including *H. armigera* (Zhang *et al*., 2004; Men *et al*., 2005; Rao and Rao, 2008), *Ostrinia nubilalis* (Hübner) (Huang *et al*., 2001), *H. zea* (Boddie) (Gore *et al*., 2002) and *Trichoplusia ni* (Hübner) (Li *et al*., 2007) have shown the ability to avoid food containing Bt toxins. However, no such preference studies have been reported in the literature in case of spotted bollworm, *E. vittella* to Bt cotton containing single or dual toxin genes. As development of insect resistance depends at least partly upon the behavioural responses of insects to toxins (Tabashnik, 1994), studies were conducted on the oviposition and larval preference of spotted bollworm, *E. vittella* on Bt and non-Bt cotton.

Materials and Methods

Selected cotton genotypes: Four Bt cotton genotypes from private sector, one each belonging to different events, viz. MRC 6304 Bt (*cry1Ac* gene), JKCH 1947 Bt (modified *cry1A*c gene), NCEH 6R Bt (*cry1Ab*/*cry1Ac* fused gene) and MRC 7017 BG II (*cry1Ac* and *cry2Ab* genes) along with the respective isogenic non-Bt genotypes were used for the present study. The selected Bt cotton along with their isogenic non-Bt genotypes were grown at row-to-row spacing of 67.5 cm and plant-to-plant spacing of 75 cm at the Cotton Farm, Department of Plant Breeding and Genetics, Punjab Agricultural University (PAU), Ludhiana. All the agronomic practices were applied as per PAU recommendations except that no plant protection measures were adopted against the bollworms throughout the cropping season. The squares from 1st internode and bolls from 2nd internode were used for the present studies. They were plucked and disinfected with 0.1% sodium hypochlorite for 30 seconds as and then washed with water. The excess water was reduced using layers of tissue papers and dried at room temperature.

Rearing of *Earias vittella*: Culture of *E. vittella* was maintained from the field collected larvae in plant growth chamber (Saveer Biotech Limited, India) at 27 ± 2^{0}C temperature and 70 ± 5 % relative humidity. Larvae were collected from cotton and okra grown fields and were reared in glass jars (10 x 15 cm) covered with muslin cloth on okra, *Abelmoschus esculentus* (L.) fruits, the most preferred natural food (Mehta, 1971). Food was changed daily till the onset of pupation. Pupae were separated (♂ and ♀) on the basis of well developed knob like structure at the antero-dorsal end of male cocoon (Gupta, 1978) and placed in jars having moist sponge at bottom covered with filter paper. Freshly emerged male and female moths were paired and released into glass jars (15 x 20 cm) lined with muslin cloth for oviposition. A cotton-swab dipped in 10 per cent honey solution was hung from top of muslin cloth covering the mouth of the jar which provided food for the adults. The female moth laid eggs on the lined muslin cloth which was removed daily and replaced with new one to facilitate further oviposition. Freshly emerged adults were used for oviposition preference and first as well as third instar larvae were used for feeding preference.

Studies were conducted to know the oviposition preference of adult females and settling preference of *E. vittella* larvae on Bt and non-Bt cotton genotypes at different crop ages, viz. 90, 120 and 150 days old crop, in a plant growth chamber at 27 ± 2^{0}C temperature and 70 ± 5 % relative humidity at Entomological Research Farm, Department of Entomology, PAU, Ludhiana.

Oviposition preference: The oviposition preference of adults was studied on squares and bolls in a free-choice test with following three combinations: only squares, only bolls and both squares and bolls together. All the three combinations were studied separately and replicated thrice. The samples (squares and bolls) were collected from raised crop and were kept randomly in wooden screen cage (18" x 18" x 26") at equidistant from each other in each of the combinations. In the first (test of squares) and second combination (test of bolls), two samples of each genotype, i.e., 16 squares and 16 bolls were used per replicate,

respectively. In the third combination (test of squares and bolls), 8 squares and 8 bolls of each genotype were kept per replication. Five pairs of freshly emerged adults of *E. vittella* from the insect culture were released in each replication. Cotton-swab dipped in 10 per cent honey solution was hung from top providing food for the moths. The number of eggs laid on floral bodies was counted after 48 hrs of release.

Larval preference: The studies on feeding preference were assessed with first and third instar larvae on squares and bolls in a free-choice test (Dhillon and Sharma, 2004) with following three combinations: (1) only squares, (2) only bolls and (3) both squares and bolls together. Samples (squares and bolls) were collected from raised crop. These were kept randomly along the periphery of plastic container (18" diameter x 6" depth) at equidistant from each other in each of the combinations. All the three combinations were studied separately and replicated thrice. In the first (test of squares) and second combination (test of bolls), two samples of each genotype, i.e. 16 squares and 16 bolls were used per replicate, respectively. In the third combination (test of squares and bolls), 8 squares and 8 bolls of each genotype were kept per replication. The container was covered with black cloth because of photopositive behaviour of larvae. Twenty larvae were released in the centre of each vessel in each replication and number of larvae settled on fruiting bodies of different genotypes, were recorded after 24 hrs of release. The data so collected were converted to per cent preference based on the total number of larvae released to evaluate differences between squares (1st combination), bolls (2nd combination) and squares plus bolls (3rd combination) of Bt and non-Bt cotton.

Statistical analysis : All the three combinations were analyzed separately in case of both oviposition and larval preference. Data were subjected to transformation prior to statistical analysis of variance (ANOVA; SAS Institute, 2005) using factorial complete randomized design. Square root transformation was used because the percentages ranged from 0 to 30 % (Gomez and Gomez, 1984). The significance of differences were tested by F-tests, while the significance of difference between treatment means were compared using least significant difference (LSD) test at 5 % probability level.

Results and Discussion

The mean number of eggs laid varied from 19.56 to 20.78 per female on squares of different Bt and non-Bt cotton genotypes with non-significant differences. The mean number of eggs laid on 90, 120 and 150 days old crop was 20.25, 20.63 and 19.67 per female, respectively, and the differences among the three crop stages were non-significant (Table 1). On bolls also, non-significant differences were observed with respect to egg laying on Bt as well as non-Bt genotypes in which the number of eggs laid varied from 19.56 to 21.22 per female. The mean number of eggs laid per female was 20.33, 20.67 and 19.75 on 90, 120 and 150 days old crop, with non-significant differences (Table 1). Interaction between days and genotypes was also found to be non-significant on both squares and bolls.

Results of relative oviposition preference of *E. vittella* when both squares and bolls of Bt and non-Bt cotton hybrids were offered together revealed non-significant

Table 1 : Number of eggs laid by *Earias vittella* adult female when only squares (1st combination) and only bolls (2nd combination) of Bt cotton and their isogenic non-Bt genotypes were offered under multiple choice test.

Genotypes	Average number of eggs laid / female after 48 hours							
	Squares (1st combination)				Bolls (2nd combination)			
	90 DAS	120 DAS	150 DAS	Pooled mean	90 DAS	120 DAS	150 DAS	Pooled mean
MRC 6301 Bt	20.00±0.58	20.33±2.03	19.67±1.45	20.00±1.35	20.00±1.15	20.33±0.88	19.33±1.20	19.89±1.07
MRC 6301 non-Bt	20.00±1.15	20.33±1.76	19.33±0.67	19.89±1.19	20.00±1.00	20.67±1.45	19.33±0.67	20.00±1.04
JKCH 1947 Bt	19.67±0.88	20.00±1.73	19.00±1.00	19.56±1.20	19.67±0.88	20.00±1.15	19.00±1.00	19.56±1.01
JKCH 1947 non-Bt	19.67±1.20	20.00±1.15	19.00±1.15	19.56±1.17	19.67±1.45	20.00±1.15	19.00±1.15	19.56±1.25
NCEH 6R Bt	20.33±1.76	20.67±1.45	20.00±1.53	20.33±1.58	20.33±1.20	20.67±1.45	19.67±1.76	20.22±1.47
NCEH 6R non-Bt	20.67±1.20	21.00±1.73	20.00±1.00	20.56±1.31	20.67±0.33	20.67±1.20	20.00±1.00	20.44±0.84
MRC 7017 BG II	20.67±1.86	21.33±1.20	20.33±1.20	20.78±1.42	21.33±1.20	21.33±1.20	20.67±0.88	21.11±1.09
MRC 7017 non-Bt	21.00±1.53	21.33±1.33	20.00±1.00	20.78±1.29	21.00±1.53	21.67±1.20	21.00±1.53	21.22±1.42
Mean	20.25±1.27	20.63±1.55	19.67±1.13	-	20.33±1.09	20.67±1.21	19.75±1.15	-
P value	Days	0.398					0.307	
	Genotypes	0.898					0.544	
	Interaction	1.000					1.000	

DAS – days after sowing; Data were transformed by $\sqrt{(n+1)}$ transformation before analysis and original values ± standard error are given

Table 2.: Mean oviposition preference (%) of *Earias vittella* adult female on Bt cotton and their isogenic non Bt genotypes when both squares and bolls were offered together under multiple choice test (3rd combination)

Treatments	Mean oviposition preference (%)		
	90 DAS	120 DAS	150 DAS
Genotypes			
MRC 6301 Bt	6.21±0.51	6.16±0.43	6.14±0.44
MRC 6301 non Bt	6.28±0.20	6.20±0.17	6.15±0.20
JKCH 1947 Bt	5.83±0.19	5.88±0.16	5.81±0.34
JKCH 1947 non Bt	5.88±0.33	5.91±0.31	5.91±0.47
NCEH 6R Bt	6.23±0.28	6.23±0.24	6.25±0.40
NCEH 6R non Bt	6.41±0.39	6.42±0.35	6.44±0.34
MRC 7017 BG II	6.58±0.31	6.60±0.33	6.69±0.45
MRC 7017 non Bt	6.60±0.35	6.61±0.38	6.64±0.39
p value	0. 252	0.171	0.316
Fruiting bodies			
Squares	6.42±0.39	6.39±0.35	6.40±0.34
Bolls	6.08±0.24	6.11±0.23	6.09±0.41
p value	0.057	0.090	0.137

DAS – days after sowing; Data were transformed by $\sqrt{\ }$ (n+1) transformation before analysis and original values ± standard error are given

differences at all the three crop growth stages, i.e. 90, 120 and 150 days old crop. Among the cotton hybrids, the egg laying varied from 5.83 to 6.60 %, 5.88 to 6.60 % and 5.81 to 6.69 % on 90, 120 and 150 days old crop, respectively (Table 2). It is evident from the present results that Bt toxin did not deter oviposition preference of *E. vittella* moths as there was no significant difference in the number of eggs laid on squares/bolls of Bt and non-Bt cotton hybrids, across different crop growth stages. No such studies with respect to egg laying by releasing adults of *E. vittella* on Bt in comparison to non-Bt cotton have been reported in literature. However, studies on oviposition preference of *H. armigera* have revealed no significant differences in egg laying on Bt and non-Bt cotton (Basavaraja *et al*., 2012) which is in agreement with present findings on *E. vittella*. Liu *et al*., (2002) also reported that the Bt toxin did not deter oviposition of pink bollworm as neither susceptible nor resistant females laid fewer eggs on Bt cotton bolls than on non-Bt cotton bolls in choice tests on caged cotton plants in greenhouse. It has been reported that the quantitative levels of Cry protein differed significantly among different commercial Bt hybrids, declined with age of the crop and also varied in plant parts (Adamczyk *et al*., 2001; Kranthi *et al*., 2005). As a resistance mechanism, female moths may change their behaviour and select plant parts with no and/or low toxin expression for egg laying. Such behavioural change in egg placement may allow them to escape high toxin expression tissues and increase the possibility of offspring survival, thus leading to control failures (Gore *et al*., 2002). However, the present studies showed that *E. vittella* female moths showed no preference in egg laying on non-Bt over Bt cotton genotypes. Tate *et al*. (2006) reported that the evolution of behavioural preference for cultivars without Bt toxin genes over cultivars carrying Bt toxin genes is not expected due to the inability of female moths to sense the expression of the Bt toxin protein in plant cells.

When only squares were offered to first instar larvae, MRC 6301 Bt as well as MRC 6301 non-Bt genotypes were the least preferred, whereas, JKCH 1947 along with JKCH 1947 non-Bt were the most preferred genotypes after 24 hours of observation at all the selected crop growth stages, i.e. 90, 120 and 150 days old crop. Based on pooled mean, significantly less number of larvae were on MRC 6301 Bt (7.78%) and it was on a par with MRC 6301 non-Bt (8.33%) followed by MRC 7017 BG II and MRC 7017 non-Bt (10.56 %). Significantly more larvae were recorded on JKCH 1947 Bt and its isogenic non-Bt and both were equally preferred (17.22%) ($F_{7,48}$=15.83; p<0.001). Similar trend was observed when only bolls were offered to first instar larvae ($F_{7,48}$ =13.82; p<0.001) (Table 3).

When squares and bolls of different Bt and non-Bt cotton hybrids were offered together to the first instar larvae in multiple-choice test, the results showed significant differences for fruiting structures as well as among cotton genotypes. However preference for all Bt hybrids in comparison with the respective isogenic non-Bt genotypes did not differ significantly on 90 day old crop, first instar larvae significantly had greater preference for squares (7.29%) than bolls (5.21 %) ($F_{1,32}$=9.82; p=0.004). Similar trend was also observed in 120 (7.29% on squares and 5.21% on bolls) ($F_{1,32}$=8.39; p=0.007) and 150 (7.08% on squares and 5.42% on bolls) ($F_{1,32}$=4.49; p=0.042) days of croping. Among cotton hybrids, first instar larvae had significantly lower preference for MRC 6301 (4.17%) and MRC 6301 non-Bt (4.17%) which was at par with MRC 7017 BG non-Bt (4.17%) and MRC 7017 BG II (5.00%). However, JKCH 1947 Bt (8.33%) and its isogenic non-Bt (10.00%) were significantly more preferred ($F_{7,32}$=4.22; p=0.002). Similar trend was also observed in 120 and 150 days of croping ($F_{7,32}$=2.96-4.27; p=0.002-0.016) (Table 4).

When only squares of 90, 120 and 150 days old crop were offered to third instar larvae, MRC 6301 Bt and its isogenic non-Bt were less preferred, while JKCH 1947 Bt and non-Bt were more preferred genotypes ($F_{7,16}$=5.07-9.01; p=0.000-0.003) Based on pooled mean ($F_{7,48}$ =19.91; p<0.001), significantly less number of larvae were recorded on MRC 6301 Bt (7.78%) and its isogenic non-Bt (8.33%), both of which were at par (Table 5). These were followed by MRC 7017 BG II which was equally preferred as its non-Bt counterpart (10.56%). The larval preference for NCEH 6R Bt (12.78%) was at par with NCEH 6R non-Bt (13.33%). Significantly more larvae were on squares of JKCH 1947 Bt

Table 3 : Mean preference (%) of first instar *Earias vittella* larvae when only squares (1st combination) and only bolls (2nd combination) of Bt cotton and their isogenic non-Bt genotypes were offered under multiple choice test.

Genotypes	Larval preference (%)							
	Squares (1st combination)				Bolls (2nd combination)			
	90 DAS	120 DAS	150 DAS	Pooled Mean	90 DAS	120 DAS	150 DAS	Pooled Mean
MRC 6301 Bt	8.33±1.67^{a}	6.67±1.67^{a}	8.33±1.67^{a}	7.78±1.67^{a}	10.00±0.00^{ab}	8.33±3.33^{a}	8.33±1.67^{a}	8.89±1.67^{a}
MRC 6301 non-Bt	8.33±1.67^{a}	8.33±1.67^{a}	8.33±1.67^{a}	8.33±1.67^{ab}	8.33±1.67^{a}	8.33±1.67^{a}	8.33±1.67^{a}	8.33±1.67^{a}
JKCH 1947 Bt	16.67±1.67^{d}	18.33±1.67^{c}	16.67±1.67^{bc}	17.22±1.67^{d}	18.33±1.67^{d}	16.67±1.67^{bc}	18.33±1.67^{d}	17.78±1.67^{c}
JKCH 1947 non-Bt	16.67±1.67^{d}	16.67±1.67^{c}	18.33±3.33^{c}	17.22±2.22^{d}	16.67±1.67^{cd}	18.33±1.67^{c}	16.67±1.67^{cd}	17.22±1.67^{c}
NCEH 6R Bt	13.33±1.67^{bcd}	15.00±0.00^{c}	13.33±1.67^{abc}	13.89±1.11^{c}	13.33±1.67^{bc}	13.33±1.67^{abc}	13.33±1.67^{bcd}	13.33±1.67^{b}
NCEH 6R non-Bt	15.00±0.00^{cd}	15.00±2.89^{bc}	13.33±1.67^{abc}	14.44±1.52^{cd}	13.33±1.67^{bc}	13.33±1.67^{abc}	13.33±1.67^{bcd}	13.33±1.67^{b}
MRC 7017 BG II	10.00±0.00^{ab}	10.00±0.00^{ab}	11.67±1.67^{ab}	10.56±0.56^{b}	10.00±0.00^{ab}	10.00±0.00^{a}	11.67±1.67^{abc}	10.56±0.56^{a}
MRC 7017 non-Bt	11.67±1.67^{abc}	10.00±0.00^{ab}	10.00±0.00^{a}	10.56±0.56^{b}	10.00±0.00^{ab}	11.67±1.67^{ab}	10.00±0.00^{ab}	10.56±0.56^{a}
P value	0.003	<0.001	0.010	<0.001	0.001	0.016	0.003	<0.001

DAS – days after sowing; Data were transformed by $\sqrt{(n+1)}$ transformation before analysis and original values ± standard error are given; Figures within a column followed by same superscript letters did not differ significantly (p>0.05, LSD test)

and its isogenic non-Bt, both of which were equally preferred (18.33%). Similar trend was observed when only bolls were offered to third instar larvae ($F_{7,48}$=18.09; p<0.001) (Table 5).

When squares and bolls of different Bt and non-Bt cotton hybrids were offered together to the third instar larvae, significant differences among cotton genotypes and between fruiting structures were observed. However, preference for all Bt hybrids in comparison with the respective isogenic non-Bt genotypes did not differ significantly. In contrast to first instar larvae, third instar larvae had significantly greater preference for bolls (7.50%) than squares (5.00%) on 90 day old crop ($F_{1,32}$=11.87; p=0.002). Similarly, significant differences were also observed for fruiting structures on 120 (7.50% on squares and 5.00% on bolls) ($F_{1,32}$=10.49; p=0.003) and 150 (7.29% and 5.21% on bolls) ($F_{1,32}$=9.82; p=0.004) days old crop. Among cotton hybrids, on 90 day old crop ($F_{7,32}$=3.12; p=0.013), third instar larvae had significantly lower preference for MRC 6301 Bt (4.17%) and its isogenic non-Bt (4.17%), both of which were at par with MRC 7017 non-Bt (5.00%), MRC 7017 BG II (5.00%) and NCEH 6R non-Bt (6.67%). These were followed by NCEH 6R Bt (7.50%) which was at par with NCEH 6R non-Bt, MRC 7017 BG II and MRC 7017 non-Bt. JKCH 1947 non-Bt (9.17%) and its isogenic Bt (8.34%) were significantly more preferred by *E. vittella* larvae. Similar trend was also observed on 120 ($F_{7,32}$=6.58; p<0.001) and 150 ($F_{7,32}$=4.22; p=0.002) days of crop for preference by third instar larvae (Table 6).

In the present study, no behavioural change in larval preference was observed with respect to selecting non-Bt cotton in comparison to Bt cotton as floral bodies from Bt and the respective isogenic non-Bt genotypes were equally preferred by both first and third instar larvae. However, *E. vittella* larvae showed significant differences in preference for different cotton genotypes. Variation in settling preference among the cotton genotypes might be due to differences in the morphological (pubescence and gossypol glands) and/or due to differential plant volatiles of the genotypes. In accordance to present findings, Liu *et al*. (2002) reported lack of discrimination between Bt and non-Bt cotton bolls by pink bollworm indicating that mining initiations were independent of susceptibility to Cry1Ac as the number of entrance holes per boll did not differ between Bt cotton and non-Bt cotton for susceptible and resistant neonates. However, studies on larval preference in other bollworm

Table 4: Mean preference (%) of first instar *Earias vittella* larvae on Bt and their isogenic non Bt genotypes when both squares and bolls were offered together under multiple choice test (3rd combination)

Treatments	Mean larval preference (%)		
	90 DAS	120 DAS	150 DAS
Genotypes			
MRC 6301 Bt	4.17±1.67^{a}	4.17±0.84^{a}	4.17±0.84^{ab}
MRC 6301 non Bt	4.17±0.84^{a}	4.17±0.84^{a}	5.00±1.67^{ab}
JKCH 1947 Bt	8.33±1.67^{c}	8.33±0.84^{c}	10.00±1.45^{c}
JKCH 1947 non Bt	10.00±0.00^{c}	9.17±0.84^{c}	9.17±0.84^{c}
NCEH 6R Bt	6.67±0.84^{abc}	7.50±1.67^{bc}	6.67±0.84^{bc}
NCEH 6R non Bt	7.50±1.67^{bc}	6.67±1.67^{abc}	7.50±1.67^{bc}
MRC 7017 BG II	5.00±1.67^{ab}	5.00±1.67^{ab}	4.17±0.84^{ab}
MRC 7017 non Bt	4.17±0.84^{a}	5.00±1.67^{ab}	3.33±1.67^{a}
p value	0.002	0.016	0.002
Fruiting bodies			
Squares	7.29±1.04^{b}	7.29±0.83^{b}	7.08±1.19^{b}
Bolls	5.21±1.25^{a}	5.21±1.67^{a}	5.42±1.30^{a}
p value	0.004	0.007	0.042

DAS – days after sowing; Data were transformed by $\sqrt{(n+1)}$ transformation before analysis and original values ± standard error are given

Table 5: Mean preference (%) of third instar *Earias vittella* larvae when only squares (1st combination) and only bolls (2nd combination) of Bt cotton and their isogenic non-Bt genotypes were offered under multiple choice test

Genotypes	Larval preference (%)							
	Squares (1st combination)				Bolls (2nd combination)			
	90 DAS	120 DAS	150 DAS	Pooled mean	90 DAS	120 DAS	150 DAS	Pooled mean
MRC 6301 Bt	8.33±1.67^{a}	6.67±1.67^{a}	8.33±1.67^{a}	7.78±1.67^{a}	8.33±1.67^{a}	8.33±0.00^{a}	8.33±1.67^{a}	8.33±1.11^{a}
MRC 6301 non-Bt	8.33±1.67^{a}	8.33±1.67^{ab}	8.33±1.67^{a}	8.33±1.67^{a}	8.33±1.67^{a}	10.00±1.67^{ab}	8.33±1.67^{a}	8.89±1.67^{a}
JKCH 1947 Bt	18.33±1.67^{a}	18.33±1.67^{c}	18.33±1.67^{d}	18.33±1.67^{d}	20.00±0.00^{c}	16.67±1.67^{c}	18.33±1.67^{c}	18.33±1.11^{c}
JKCH 1947 non-Bt	20.00±0.00^{a}	18.33±1.67^{c}	16.67±1.67^{cd}	18.33±0.1.11^{d}	18.33±1.67^{c}	16.67±1.67^{c}	18.33±1.67^{c}	17.78±1.67^{c}
NCEH 6R Bt	11.67±1.67^{ab}	13.33±1.67^{bc}	13.33±1.67^{bcd}	12.78±1.67^{bc}	13.33±1.67^{b}	13.33±1.67^{bc}	11.67±1.67^{ab}	12.78±1.67^{b}
NCEH 6R non-Bt	13.33±1.67^{b}	13.33±1.67^{bc}	13.33±1.67^{bcd}	13.33±1.67^{c}	11.67±1.67^{ab}	13.33±1.67^{bc}	13.33±1.67^{bc}	12.78±1.67^{b}
MRC 7017 BG II	10.00±0.00^{ab}	11.67±1.67^{b}	10.00±0.00^{ab}	10.56±0.56^{b}	10.00±0.00^{ab}	11.67±1.67^{ab}	10.00±0.00^{ab}	10.56±0.56^{b}
MRC 7017 non-Bt	10.00±0.00^{ab}	10.00±0.00^{ab}	11.67±1.67^{abc}	10.56±0.56^{b}	10.00±0.00^{ab}	10.00±0.00^{ab}	11.67±1.67^{ab}	10.56±0.56^{b}
P value	<0.001	0.001	0.003	<0.001	<0.001	0.006	0.002	<0.001

Table 6: Mean preference (%) of third instar *Earias vittella* larvae on Bt and their isogenic non Bt genotypes when both squares and bolls were offered together under multiple choice test (3rd combination)

Treatments	Mean larval preference (%)		
	90 DAS	120 DAS	150 DAS
Genotypes			
MRC 6301 Bt	4.17±0.84^{a}	4.17±0.84^{ab}	4.17±1.67^{a}
MRC 6301 non Bt	4.17±0.84^{a}	3.34±0.84^{a}	4.17±0.84^{a}
JKCH 1947 Bt	8.34±0.84^{c}	9.17±0.84^{cd}	8.33±1.67^{c}
JKCH 1947 non Bt	9.17±0.84^{c}	11.67±0.84^{d}	10.00±0.00^{c}
NCEH 6R Bt	7.50±1.67^{bc}	6.67±0.84^{bc}	6.67±0.84^{abc}
NCEH 6R non Bt	6.67±0.84^{abc}	7.50±1.67^{bcd}	7.50±1.67^{bc}
MRC 7017 BG II	5.00±1.67^{ab}	4.17±0.84^{ab}	5.00±1.67^{ab}
MRC 7017 non Bt	5.00±1.67^{ab}	3.34±2.28^{a}	4.17±0.84^{a}
p value	0.013	<0.001	0.002.
Fruiting bodies			
Squares	5.00±1.46^{a}	5.00±1.25^{a}	5.21±1.25^{a}
Bolls	7.50±0.83^{b}	7.50±0.99^{b}	7.29±1.04^{b}
p value	0.002	0.003	0.004

DAS – days after sowing; Data were transformed by $\sqrt{(n+1)}$ transformation before analysis and original values ± standard error are given; Figures within a column followed by same superscript letters did not differ significantly (p>0.05, LSD test)

species/lepidopteran pests showed behaviour change with respect to ability of avoiding or detecting food containing high amount of toxins or selecting low toxin or toxin free tissues. When *H. zea* larvae were placed on the terminal of Bt or non-Bt plants, Gore *et al*. (2002) found that more larvae moved off from the site of infestation (terminal) on Bt cotton plants as compared to those on non-Bt plants. Zhang *et al*. (2004) reported that *H. armigera* larvae showed the ability to detect the transgenic Bt and CpTi-Bt cotton and selectively feed more on the non-transgenic cotton, especially the neonates which had high capacity of avoiding transgenic cotton. Similarly, Li *et al*. (2007) found that *T. ni* larvae were able to detect Bt or non-Bt leaves. Rao and Rao (2008) also reported that third instar larvae of *H. armigera* warded off Bt plants immediately after making initial bite thus, indicating the ability of larvae to detect the toxic Bt proteins.

Data on relative feeding preference indicated that third instar larvae had greater preference for bolls than for squares, whereas, first instar larvae which showed greater preference for squares than for the bolls in a free-choice test which corroborates the findings of Dhillon and Sharma (2004) on conventional cotton. Differences in food preference between different larval instars might be attributed to their nutritional requirements, since the older larvae of Lepidoptera had increased appetite (Raubenheimer and Browne, 2000) and greater need for proteins. Vennila *et al*. (2005) on the basis of feeding preference coefficients also indicated maximum preference for squares by the first two larval instars of *E. vittella* followed by green bolls and flowers whereas, third as well as fourth instar preferred more of green bolls as compared to other fruiting parts.

The present study thus showed that Bt toxin did not deter oviposition preference as *E. vittella* female did not differentiate cotton genotypes as Bt and non-Bt in egg laying indicating no behavioural preference for non-Bt cotton cultivars without Bt toxin over cultivars carrying toxins from soil bacterium, *B. thuringiensis*. Both first and third instar larvae showed lack of discrimination between Bt and non-Bt fruiting structures indicating that initial selection was independent of susceptibility to Bt toxin. Thus, there was no shift in behaviour of *E. vittella* larvae in selecting non-Bt cotton in comparison to Bt cotton. Further, research in this area should focus on the possibility that this species may develop changes in future in response to selection pressures that larvae do preferentially feed on structures with lower toxins after more time with Bt cotton in the landscape.

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