Incidence of Insect Predators and Parasitoids on Transgenic Bt Cotton in Comparison to Non-Bt Cotton Varieties

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Abstract.-Transgenic Bt (*Bacillus thuringiensis*) cotton (*Gossypium hirsutum* L.) genetically modified from and expressing delta-endotoxin protein has been increasingly preferred by Pakistani farmers due to high production potential and targeted control of bollworms. A field study was conducted to evaluate the impact of Bt cotton on insect predators and parasitoids population in comparison to conventional (non Bt) cotton varieties. The available non-Bt cotton varieties/line (AARI-FH- 942, NIBGE-NN3, NIAB-112) and Bt cotton varieties/line (AARI-FH-114, NIBGE-IR-3701, 4B-Tarzan-1) were sown during 2012-13 cotton season. Population of insect predators and parasitoids were observed from 1st week of July to 2nd week of November. The results revealed that the abundance of insect predators; *Chrysoperla carnea, Coccinella septempunctata, Geocoris* spp., *Menochilus sexmaculata*, and parasitoids: *Trichogramma* spp., *Apanteles* spp., did not differ significantly on non- Bt and transgenic Bt cotton have no adverse impact on population dynamics and distribution of insect predators and parasitoids under field conditions.

Key words: Insect predators, parasitoids, transgenic Bt cotton.

INTRODUCTION

Biological control has considered a reliable and long term solution of the insect pest problems due to self-perpetuating nature and environment friendly tactic (Bale et al., 2008). However, increasingly intensive farming strongly influences the population dynamics of insect predators and parasitoids and the activity by these natural enemies. The restricted use of pesticides and landscape biodiversity management help to conserve the biocontrol agents in agro-ecosystems and favors the development of sustainable agriculture. The adoption of transgenic Bt cotton genetically modified through recombinant DNA from Bacillus (Bt) is increasing thuringiensis since its commercialization in 1996 and according to the report of ISAA (International Service for the Acquisition of Agr-Biotech Application), Pakistan planted 2.85 million hectares of Bt cotton in 2014 cotton season (James, 2014). Bt cotton (Gossypium hirsutum L.) is cultivated extensively and preferred

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by farmers due to higher production potential, less dependence on insecticides and targeted control of specific lepidopterous pests (Arshad and Suhail, 2011; Arshad *et al.*, 2015). Cotton hosts the rich diversity of natural enemies (insect predators and parasitoids) that attack on different life stages of insect pests (egg, larval, pupal and adult stages) and provide natural balance.

Predators are essential biological control agents (Sathe and Bhosle, 2001; Sattar et al., 2007). Predators such as Chrysoperla carnea, Coccinella septempunctata, Geocoris spp., Menochilus sexmaculatus, and parasitoids Trichogramma spp and Apanteles spp. suppress the population of various cotton insect pests especially Helicoverpa armigera, Spodoptera exigua, Aphis spp. and Bemisia tabaci. Tricogramma spp. is reported an important egg parasitoid of lepidopterous pests (Ahmad et al., 1998). Apanteles spp. is a larval parasitoid and parasitizes larvae of lepidopteran pests (S. exigua, and H. armigera). Coccinellid predators (C. septempunctata and M. sexmaculata) are the important natural enemies of aphid and keep the aphid population below the economic threshold level (Wells et al., 2001). Geocorus spp. and C. carnea are voracious feeders of cotton soft bodied insect pests (Mari et al., 2007) and thus reduce the

need of insecticides application.

Field studies have been conducted to evaluate the impact of transgenic Bt cotton on the non-target arthropods which are natural enemies of important insect pests (Torres and Ruberson, 2006; Sisterson et al., 2007). Similarly, valuable laboratory studies reported negative impact of transgenic Bt cotton on predators and parasitoids (Hillbeck et al., 1999; Ponsard et al., 2002). However, in field studies typical minor effects of transgenic Bt cotton on nontarget arthropods have been reported (Al-Deeb and Wilde, 2003; Naranjo, 2005). Studies on the cultivation of Bt cotton may (Hillbeck et al., 1999; Ponsard et al., 2002) or may not (Moar et al., 2002, Torres and Ruberson, 2006) affect the predators and parasitoids within cotton field. The present study was aimed at comparing the abundance and population dynamics of insect predators and parasitoids under field conditions of transgenic Bt cotton and conventional non-Bt varieties.

MATERIALS AND METHODS

The field experiment was laid out in a randomized complete block design (RCBD), and conducted at Plant Protection Division Research area, Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. The experiment consisted of two treatments *i.e.*, sets of non-Bt and Bt cotton varieties each with three replications. In a set of three non-Bt cotton varieties/line viz., AARI-FH-942, NIBGE-NN3, NIAB-112 and three transgenic Bt cotton varieties/line viz., AARI-FH-114, NIBGE-IR-3701, 4B-Tarzan-1 were sown during the 1st week of April, 2012. Data collection regarding the abundance of population insect predators and parasitoids was initiated during 1st week of July and then taken fortnightly until 2nd week of November, resulting in 11 sampling collection dates. Sampling was done early in the morning because most of insects become active when temperatures is about 25-30°C (Garcia et al., 1982). Bag collection method was used for the sampling of canopy and foliage dwelling beneficial insects. A polythene plastic sheet bag (dimension: width 75 cm x length 75 cm) was used to collect beneficial insects by covering the plant, then shaking and arresting dropped insects in bag. Five samples were taken randomly per plot, so that a total of 15 samples per variety. The insect specimens were killed in cyanide jars and preserved for sorting, counting and identification with the help of available literature (Nagarkatti and Nagaraja, 1997; Whitfield *et al.*, 2001) and comparison with reference insect collection present in National Insect Museum, NARC, Islamabad, Pakistan.

All data on population of insect predators and parasitoids in both fields were analyzed using the analysis of variance (ANOVA). Tucky's honestly significance difference (HSD) test was used to compare the means.

RESULTS AND DISCUSSION

The parasitoids recorded were *Apanteles* spp. and *Trichogramma* spp. Insect predators studied were *Chrysoperla carnea* (Stephens) *Geocoris* spp. *Coccinella septempunctata* (Linnaeus), *Menochilus sexmaculata* (Fabricius). Average mean seasonal population observed was for *Trichogramma* spp. (2.25) followed by *C. carnea* (2.21), *C. septempunctata* (1.83), *Geocoris* spp. (1.48), *M. sexmaculata* (0. 90 and *Apanteles* spp. (0.28) (Fig.1).



Fig. 1. Average means of predators and parasitoids during the study periods.

Geocoris spp. observed first on from June 16, reached highest peak on September 02 (3.44/15) bags) and then declined onward till November 16 (0.28/15) bags) (Fig. 2). Seasonal mean population was recorded as 1.48/15 bags (Fig. 1). Similar to our findings, Khuhro *et al.* (2002) reported the

population of Geocoris spp. in cotton and higher population in 4th week of July, mean population 0.30/15 sweeps from June to September. Hafeez et al. (2006) recorded maximum population of Geocoris spp. 7.2/25 plants in cotton belt from Vehari. Solangi et al. (2008) found Geocoris punctipes (1.28/plant) from June to September in cotton fields. We found non-significant difference of *Geocoris* spp. population on both types of cotton (non-Bt and Bt) during all assessment dates (Table I). The seasonal mean population did not differ significantly (P = 0.73) although slightly higher numbers in Bt varieties (1.51) with comparison to non-Bt varieties (1.46) (Fig. 3). Our results did not differ significantly of Geocoris spp. population level in both types of fields (non-Bt and Bt). Similar to our findings, Moar et al. (2002) reported greater number of natural enemies in their evaluation in transgenic cotton then non-transgenic cotton for insects (parasitic wasps, Nabis spp., Orius sp., green lacewings, Geocoris spp. and spiders). Torres and Ruberson (2006) observed no lethal or sub-lethal effects of transgenic Bt cotton on development and reproduction of Geocoris punctipes through feeding on prey. However, in contrast to our findings. Ponsard et al. (2002) observed the adverse impact of Bt toxin on development and biology of insect predators under laboratory conditions by consumption of Btintoxicated host. Head et al. (2005) reported nonsignificantly negative impacts of Bt toxin on population of insect predators (Geocoris spp., Orius spp., spiders and lady beetles) in comparison with non-Bt cotton treated with insecticides.

Population of *C. carnea* was observed from 1^{st} week of August which gradually increased and peaked on September 16 (4.11/15 bags) and then these numbers declined till 1^{st} week of November (0.94/15 bags) (Fig. 2). The seasonal mean population observed was 2.21/15 bags (Fig. 1). Khuhro *et al.* (2002) found higher numbers of *C. carnea* in 4th week of June and mean population 0.23/15 sweeps from June to September in cotton crop. Solangi *et al.* (2008) reported *Chrysoperla* spp. in cotton crop from June to September (2.07/plant). A non-significant difference of *C. carnea* population was observed in non-Bt varieties with respect to Bt varieties during all sampling dates

(Table I). The seasonal mean population difference recorded non-significantly (P = 0.38) relatively greater numbers in non-Bt varieties (2.29) then Bt varieties (2.13) (Fig.3). Our results showed that although relatively higher population in Bt varieties but statistically non-significant difference. C. carnea do not preferred either non-Bt or Bt varieties. Their population was almost equally distributed in both types of fields (non-Bt and Bt). Similar to our studies, Dutton et al. (2002), under laboratory condition reported no adverse impact of Bt toxin on biology of C. carnea through Bt intoxicated prey (aphids) reared on transgenic maize. Contrary to our results, Sharma et al. (2007) reported higher population of predators (chrysopids, coccinellids and spiders) in transgenic Bt cotton.



Fig. 2. Fifteen days interval population of predators and parasitoids on overall Bt and non-Bt cotton varities.



Fig. 3. Average mean of predators and parasitoids on non Bt and Bt cotton during the study periods.

The coccinellid beetles, *M. sexmaculata* and *C. septempunctata* were observed from September

						Date	Dates of observations	ations				
Insects	Treatments	16/06/2012	2/7/2012	16/07/2012	2/8/2012	16/8/2012	2/9/2012	16/09/2012	2/10/2012	16/10/2012	2/11/2012	16/11/2012
Chrysoperla	Bt varieties Non-Bt	i i			0.56 ± 0.29 1.11±0.29	1.67 ± 0.38 1.78 ± 0.22	3.33 ± 0.51 2.67 ± 0.38	3.56 ± 0.48 4.67 ± 0.96	3.11 ± 0.29 2.78 ± 0.48	1.78 ± 0.22 2.00 ± 0.38	0.89 ± 0.11 1.00 ± 0.33	
nauten	varieties											
	P-Value	ı	ī	ı	0.44	0.82	0.07	0.46	0.66	0.74	0.68	ı
	Tukey HSD	·	ı			·	ī		ļ		ı	I
Geocoris spp.	Bt varieties Non-Bt	0.44 ± 0.22 0.22 ± 0.22	0.78 ± 0.29 1.00 ± 0.33	1.56 ± 0.11 1.11 ± 0.22	1.11 ± 0.11 1.67 ± 0.38	2.89±0.11 2.56±0.29	3.56±0.78 3.33±0.69	2.67±0.51 1.78±0.11	1.67 ± 0.69 1.67 ± 0.33	1.22 ± 0.29 1.78 ± 0.48	0.44 ± 0.29 0.67 ± 0.19	0.22 ± 0.11 0.33 ± 0.19
	varieties P-Value	0.66	0.42	0.26	0.29	0.42	0.53	0.20	1.00	0.12	0.42	0.42
	Tukey HSD		ī	ī	·		ī				·	
Menochilus	Bt varieties	,	ĩ	,	1	1	ī	0.56 ± 0.22	0.33 ± 0.19	0.56±0.40	1.67±0.51	1.22 ± 0.29
sexmaculata	Non-Bt	ï						0.11 ± 0.11	1.11 ± 0.29	1.44 ± 0.11	1.22 ± 0.40	0.78 ± 0.40
	varieties P-Value	,		,	,	,		0.270	0.19	0.09	0.18	0.54
	Tukey HSD	'n	ī	Ţ	,	ı	ī				ī	,
Coccinella	Bt varieties	,	ī				ī	0.89 ± 0.40	1.78 ± 0.11	2.56±0.48	2.22±0.56	1.89±0.11
septempunctata	Non-Bt	T	ī			т	T	0.89 ± 0.11	1.44 ± 0.11	3.00 ± 0.00	2.22 ± 0.29	1.44 ± 0.11
1 1	varieties							001		0.45	1 00	0.10
	Tukey HSD							- 1.00		.+.0	- 1.00	01.0
Table II Po	pulation of in	Population of insect parasitoids per 15 bags on Bt and non-Bt cotton at NIAB – Faisalabad Pakistan during crop year 2012.	ids per 15 h	ags on Bt an	d non-Bt co		B – Faisala	bad Pakistaı	1 during cro			
Insect nests						Dates of observations	rvations					
L		010010	1 / 101 101 10	0101010								

Population of insect predators per 15 bass on Bt and non-Bt cotton at NIAB – Faisalabad Pakistan during crop year 2012. Table I.-

Insect pests					Da	Dates of observations	ions				
	Treatments 2/7/2012	2/7/2012	16/07/2012	2/8/2012	16/8/2012	2/9/2012	16/09/2012	2/10/2012	16/10/2012	2/11/2012	16/11/2012
-		110.110	00000000		010.000			000.000		000.110	110.110
Apanteles spp.		0.11±0.11	0.00±0.00	0.11±0.11	0.33±0.19	0.11±0.11	0.20±0.40	0.0±0.22	0.33±0.19	0.44 ± 0.29	0.11±0.11
	Non-Bt variatias	0.00 ± 0.00	0.11 ± 0.11	0.11 ± 0.11	0.33 ± 0.19	0.78 ± 0.22	0.67 ± 0.19	0.22 ± 0.11	0.33 ± 0.19	0.22 ± 0.22	0.22 ± 0.11
	P-Value	0.42	0.42	1.00	1.00	0.73	0.83	0.22	1.00	0.18	0.42
	Tukey HSD	ĩ	·	,		1		,	,	,	,
Trichogramma		0.33 ± 0.19	1.78 ± 0.29	2.44 ± 0.59	2.78 ± 0.59	3.22 ± 0.29	3.11 ± 0.48	3.00 ± 0.51	2.56 ± 0.48	1.89 ± 0.11	0.89 ± 0.40
spp.	Non-Bt	0.22 ± 0.11	2.00 ± 0.38	3.00 ± 0.51	2.67 ± 0.19	3.00 ± 0.19	3.89 ± 0.22	3.00 ± 0.38	2.56 ± 0.59	1.56 ± 0.29	1.11 ± 0.59
	varieties										
	P-Value	0.73	0.77	0.46	0.85	0.68	0.36	1.00	1.00	0.42	0.79
	Tukey HSD	ï	ı	,	,	,		ī	,	ı	,

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16 and their number gradually increased and peaked on November 02 (1.44/15 bags) and October 16 (2.78/15 bags) respectively and then decline during following dates (Fig. 2). The mean seasonal population of М. sexmaculata and С. septempunctata recorded were 0.90/15 bags and 1.83/15 bags, respectively (Fig. 1). In similar field Khuhro *et al.* conditions. (2002)reported *Coccinellids* from June to September with maximum population on 3rd week of July. Ashfaq et al. (2011) observed maximum population of C. septempunctata 1.42/leaf on August 10. We found non-significant difference of beetles' population (M. sexmaculata and C. septempunctata) throughout cotton season (Table I). A non-significantly higher population of *M. sexmaculata* in non-Bt varieties (0.93) then Bt varieties (0.87)while C. septempunctata was non-significantly (P = 0.5) higher in Bt varieties (1.87) with respect to non-Bt varieties (1.80) (Fig. 3). Our studies showed that Bt cotton have no negative impact on population and distribution of both beetles (M. sexmaculata and C. septempunctata). Similar to our studies, Head et al. (2005) observed equal distribution and abundance of population dynamics of coccinellid beetles in non-Bt and transgenic Bt cotton. Mellet and Schoeman (2007) reported that cultivation of transgenic Btcotton had no adverse impact on abundance of coccinellids spp. However, Naranjo (2005) reported typically minor effects of Bt toxin on beneficial insects (C. carnea, G. punctipes Coccinellids and Aphelinids parasitoids) compared to alternative use of insecticides.

Trichogramma spp. was first observed during 1st week of July and higher numbers were observed on September 16 (3.50/15 bags), then low population was recorded till November 16 (1.00/15 bags) (Fig. 2). The seasonal mean population was 2.25/15 bag (Fig. 1). In similar field conditions. Ahmad et al. (1998) reported Trichogramma spp. population from Aug to Oct. In our present study a non-significant difference was observed in Trichogramma spp. population of both field types (non-Bt and Bt cotton) during all observation dates (Table II). The seasonal mean population difference was non-significant (P = 0.59) in non-Bt (2.30) and Bt varieties (2.20) (Fig. 3). A similar trend of population was observed in both types of fields of cotton (non-Bt and Bt). Similarly Fernandes *et al.* (2007) reported no adverse impact of transgenic Bt maize on the population of ladybird beetles and *Trichogramma* wasps. Wu and Guo (2005) found higher population of parasitoids (*Trichogrammatids*, *Microplitis* and *Campoletis*) in non-Bt as compared to transgenic Bt cotton.

Apanteles spp. population was noted from 1st week of July and maximum number on September 16 (0.61/15bags) then numbers declined till November 16 (0.17/15 bags) (Fig.2). The seasonal mean population recorded were 0.28/15 bag (Fig. 1). Almost similar to our findings, Hafeez et al. (2006) reported maximum population of Apanteles spp. 0.6/25 plants from August to October during cotton season. We found non-significant difference of Apanteles spp. population throughout the cotton season on all observed dates (Table II). A nonsignificant difference of seasonal mean population (P = 0.68) of *Apanteles* spp. recorded slightly higher in non-Bt varieties (0.30) in comparison to Bt varieties (0.27) (Fig. 3). Our findings showed that population of Apanteles spp. did not differ significantly in non-Bt with respect to the Bt varieties. Almost a similar trend of Apanteles spp., was present throughout cotton season in both types of cotton fields (non-Bt and Bt). Similar to our findings, Schuler et al. (1999) showed no negative impact of Bt toxin on development and biology of parasitoids. Similarly, Dhillon and Sharma (2013) noted no adverse impact of Bt toxin on diversity of arthropods. Contrary to our results Pilcher et al. (2005) reported higher population of parasitoids in non-Bt as compared to transgenic Bt cotton. Men et al. (2003) reported decreased diversity of natural enemies (predators and parasitoids) in transgenic crops as compared to non-transgenic crops.

CONCLUSION

Our findings conclude an equal abundance of observed parasitoids and predators in both transgenic Bt and non-Bt cotton varieties throughout the cotton season. Therefore beneficial insect community is not affected by the cultivation of transgenic Bt cotton.

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(Received 10 February 2015, revised 4 April 2015)

						Date	es of observ	ations				
Insects	Treatments	16/06/2012	2/7/2012	16/07/2012	2/8/2012	16/8/2012	2/9/2012	16/09/2012	2/10/2012	16/10/2012	2/11/2012	16/11/2012
Chrysoperla	Bt varieties	-	-	-	0.56±0.29	1.67±0.38	3.33±0.51	3.56±0.48	3.11±0.29	1.78±0.22	0.89±0.11	-
carnea	Non-Bt varieties	-	-	-	1.11±0.29	1.78±0.22	2.67±0.38	4.67±0.96	2.78±0.48	2.00±0.38	1.00±0.33	-
	P-Value	-	-	-	0.44	0.82	0.07	0.46	0.66	0.74	0.68	-
	Tukey HSD	-	-	-	-	-	-	-	-	-	-	
Geocoris spp.	Bt varieties	0.44±0.22	0.78±0.29	1.56±0.11	1.11±0.11	2.89±0.11	3.56±0.78	2.67±0.51	1.67±0.69	1.22±0.29	0.44±0.29	0.22±0.11
	Non-Bt varieties	0.22±0.22	1.00±0.33	1.11±0.22	1.67±0.38	2.56±0.29	3.33±0.69	1.78±0.11	1.67±0.33	1.78 ± 0.48	0.67±0.19	0.33±0.19
	P-Value	0.66	0.42	0.26	0.29	0.42	0.53	0.20	1.00	0.12	0.42	0.42
	Tukey HSD	-	-	-	-	-	-	-	-	-	-	-
Menochilus	Bt varieties	-	-	-	-	-	-	0.56±0.22	0.33±0.19	0.56±0.40	1.67±0.51	1.22±0.29
sexmaculata	Non-Bt varieties	-	-	-	-	-	-	0.11±0.11	1.11±0.29	1.44 ± 0.11	1.22±0.40	0.78 ± 0.40
	P-Value	-	-	-	-	-	-	0.270	0.19	0.09	0.18	0.54
	Tukey HSD	-	-	-	-	-	-	-	-	-	-	-
Coccinella	Bt varieties	-	-	-	-	-	-	0.89±0.40	1.78±0.11	2.56±0.48	2.22±0.56	1.89±0.11
septempunctata	Non-Bt varieties	-	-	-	-	-	-	0.89 ± 0.11	1.44±0.11	3.00±0.00	2.22±0.29	1.44±0.11
	P-Value	-	-	-	-	-	-	1.00	0.22	0.45	1.00	0.18
	Tukey HSD	-	-	-	-	-	-	-	-	-	-	-

Table I. Population of insect predators per 15 bags on Bt and non-Bt cotton at NIAB – Faisalabad Pakistan during crop year 2012.

 Table II. Population of insect parasitoids per 15 bags on Bt and non-Bt cotton at NIAB – Faisalabad Pakistan during crop year 2012.

Insect pests					Da	tes of observat	ions				
	Treatments	2/7/2012	16/07/2012	2/8/2012	16/8/2012	2/9/2012	16/09/2012	2/10/2012	16/10/2012	2/11/2012	16/11/2012
Apanteles spp.	Bt varieties Non-Bt varieties P-Value	0.11±0.11 0.00±0.00 0.42	0.00±0.00 0.11±0.11 0.42	0.11±0.11 0.11±0.11 1.00	0.33±0.19 0.33±0.19 1.00	0.11±0.11 0.78±0.22 0.73	0.56±0.40 0.67±0.19 0.83	0.56±0.22 0.22±0.11 0.22	0.33±0.19 0.33±0.19 1.00	0.44±0.29 0.22±0.22 0.18	0.11±0.11 0.22±0.11 0.42
	Tukey HSD	-	-	-	-	-	-	-	-	-	-
<i>Trichogramma</i> spp.	Bt varieties Non-Bt varieties	0.33±0.19 0.22±0.11	1.78±0.29 2.00±0.38	2.44±0.59 3.00±0.51	2.78±0.59 2.67±0.19	3.22±0.29 3.00±0.19	3.11±0.48 3.89±0.22	3.00±0.51 3.00±0.38	2.56±0.48 2.56±0.59	1.89±0.11 1.56±0.29	0.89±0.40 1.11±0.59
	P-Value Tukey HSD	0.73	0.77 -	0.46 -	0.85 -	0.68 -	0.36 -	1.00	1.00 -	0.42	0.79 -