Assessment the Effects of Transgenic Egyptian *Bt* Cotton that Contain Two Genes Expressing Cry 1Ac and Cry 2Ab Delta-Endotoxin on the Abundance of the Non Target Organisms Community

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Abstract: The effect of *Bt* cotton, i.e. genetically modified cotton that contain genes Cry 1Ac and Cry 2Ab expressing delta-endotoxin, on non-target pest arthropods and non target beneficial arthropods populations was determined by field study at Giza 80 Egyptian cotton variety. Although *Bt*-cotton is lepidopteran specific, non-lepidopteran arthropod populations may be indirectly influenced by the endotoxin. Abundance of non target pest arthropods (Aphids, whiteflies, leafhopper green bugs, and spider mites) and non-target beneficial arthropods (green lacewing, ladybird *coccinella,* rove beetle*,* Orius *bugs* and true spider) were used as measures to determine possible effects on the populations under investigation. The present study is the first attempt in Egypt which devoted to assessment the effects of transgenic *Bt* cotton *Gossypium barbadense* L. (Giza 80), which was genetically modified (GM)- during the co-ordinate project between Monsanto company and Ministry of Agriculture, Agriculture Research Center (ARC) including Cotton Research Institute (CRI), Agricultural Genetic Engineering Research Institute (AGERI) and Plant Protection Research Institute (PPRI) by *Bacillus thuringiensis* (*Bt*) genes against cotton leafworm *Spodoptera littoralis* and bollworms, (pink bollworm *Pectinophora gossypiella* and spiny bollworm *Earias insulana*) on the function of the non target organisms community (pests and natural enemies). However, no variety of *Bt* cotton has yet been approved for commercial planting in Egypt. Tow genes (Cry 1Ac and Cry 2Ab) from *Bacillus thuringiensis* (*Bt*) were introduced to the American cotton *Gossypium hirsutum* by the particle bombardment, then crossed and back crossed with Egyptian cotton variety Giza 80 to transfer those two genes to the Egyptian cotton variety Giza 80.The Scouting was conducted on a weekly basis from 7th weeks of the plant emergence and continued until the cotton bolls started to open (10 weeks of data).The statistical analysis cleared that, no significant different between the abundance of non-target pest arthropods and non-target beneficial arthropods on *Bt* cotton and non *Bt*. These attempts were elucidate to evaluate the effect of *Bt* cotton on non target organisms in cotton fields to release the Egyptian *Bt* cotton as a new commercial product at large scale for rationalize the using of insecticides via IPM program on cotton crop in Egypt.

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Key words: *Bt* cotton, GM Crops, non-target pest arthropods, non target beneficial arthropods, Cry1Ac, Cry 2Ab, Delta-Endotoxin and seasonal abundance.

1. Introduction

In **1997**, 4 million hectares were planted with crops genetically engineered to produce toxins derived from the bacterium *Bacillus thuringiensis* (*Bt*) **James (1997).** By 2011, the global area planted to *Bt* crops covered over 66 million hectares, during this time, maize and cotton covered the majority of the world's agricultural landscape devoted to *Bt* crops **James (2011).**In cotton, the proteins expressed (Cry1Ac and Cry2Ab) confer protection from a broad array of lepidopteran herbivores, enabling the use of broad spectrum insecticides to be greatly reduced. *Bt* cotton (particularly varieties expressing Cry1Ac and Cry2Ab) has been registered for commercial use in USA, Argentina, Australia, China, Colombia, India, Mexico and South Africa. Some of the most exciting possibilities for such a product exist in tropical systems where substantial broad spectrum insecticides would otherwise be used. In areas of Asia, such as India and China, cotton crops may be sprayed more than ten times in a year in the absence of *Bt* cotton in an attempt to control severe lepidopteran pest outbreaks (**Wu and Guo, 2005**).

Crops genetically engineered to produce *Bacillus thuringiensis* (*Bt*) toxins are planted on millions of hectares worldwide (**James, 2011**). Considerable effort has been expended to determine the effects of *Bt* crops on non-target arthropods (**Zwahlen *et al.*, 2000; Dutton *et al.*, 2002; Al-Deeb and Wilde, 2003; Jasinski *et al.*, 2003; Men *et al.*, 2003; Sisterson *et al.*, 2004; Torres and Ruberson, 2006 and Sisterson *et al.*, 2007**). In particular, the number of insecticide sprays applied in Arizona was lower for *Bt* cotton than non *Bt* cotton (**Carpenter and Gianessi, 2001 and Cattaneo *et al.*, 2006**). Transgenic *Bacillus thuringiensis* (*Bt*) cotton did not exert any adverse effects on natural enemies and the activity of coccinellids and spiders was more or less uniform on *Bt* and non-*Bt* cotton fields in India **(Rao and Rao, 2008)**. Because *Bt* crops are often less harmful than insecticides to non-target arthropods (**Bhatti *et al.*, 2005; Dively, 2005; Naranjo, 2005; Cattaneo *et al.*, 2006** and **Aaron and William, 2012**), it reduced insecticide use in *Bt* crop fields could benefit some non-target species. If *Bt*- toxins produced by transgenic *Bt* cotton do not pose direct and indirect threats to beneficial insects, such as green lacewing, ladybird *coccinella,* rove beetle, Orius *bugs* and true spider or positively influence pest populations such as aphids, whiteflies, leafhopper green bugs, and spider mites by increasing their numbers, and if predator – prey interaction are not changed by *Bt* cotton cultivation, then it can play an important role in reducing the pest damage to this crop. Transgenic crops producing the insecticidal proteins of *Bacillus thuringeinsis* Berliner (*Bt*) have the potential to contribute to natural enemy conservation through both their selective activity and associated reductions in the broad-spectrum insecticides they replace **(Edge *et al*,. 2001; Shelton *et al.*, 2002 and Federici, 2003).**

The aims of this study are, therefore, to determine the effects of cultivation of *Bt* cotton on non-target pest arthropods (aphids, whiteflies, leafhopper green bugs, and spider mites) and non-target beneficial arthropods (green lacewing, ladybird *coccinella*, the rove beetle, Orius *bugs* and true Spider) populations. There are at least two different ways to evaluate the potential negative effects of *Bt* cotton on non target organisms (pests and natural enemies). A fundamental question one might ask is if *Bt* cotton has any negative effects on non target organisms (pests and natural enemies) complex. Alternatively, one might ask what is the relative impact of *Bt* cotton on non target organisms (pests and natural enemies) complex compared with that of conventionally grown cotton (treated with multiple applications of synthetic insecticides). Therefore this field study was initiated to determine exactly what effects *Bt* cotton would have on non target organisms (pests and natural enemies) complex.

2.Material and Methods

Experimental Design:

Trials sites and Giza lines:

Location: Sids Station /Beni-Suef Governorate.

Varity :Giza 80 Egyptian cotton variety.

Design: Randomized Complete Block Design (RCBD) with three entries:

1. MON 15985 (Giza 80)
2. Conventional Giza 80 sprayed with insecticide to control lepidopterous.
3. Conventional Giza 80 without spray to control lepidopterous.

- Replications of the entries: 4 (every replication will have 2 meters alley separation)
- Plot size: 6 meters length x 10 rows x 0.80 meter width.
- Plant Density: Adapted to the agronomic conditions of every site.
- Plot buffers: two empty rows between the plots will be implemented.
- Trial buffers: Trial will be surrounded by 15 meters/15 rows of conventional Giza iso-line seed.
- Planting date: as commercial fields (April 10th 2011).

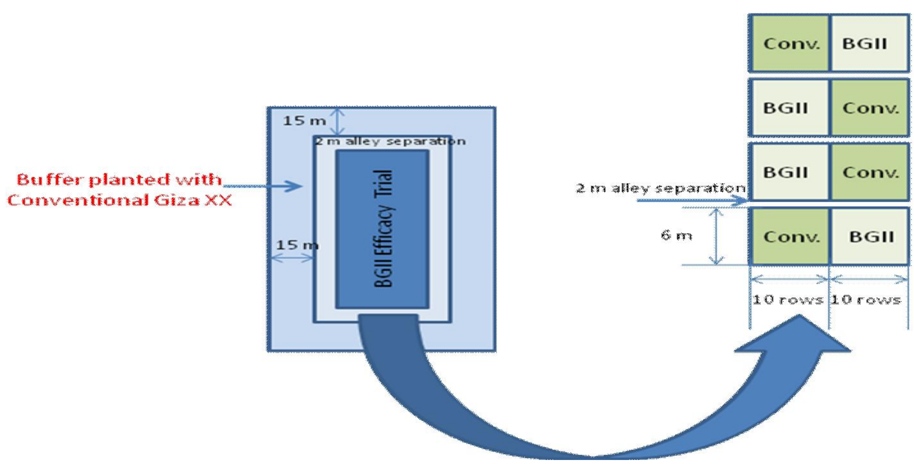

Fig. 1: The experimental design for MON 15985 (*Bt* cotton) in Giza 80 cotton variety (*Gossypium barbadense*) against cotton leafworm and bollworms.

Scouting:

During the season, scouting was conducted on a weekly basis from 7th weeks of the plant emergence and continued until the cotton bolls started to open (10 weeks of data) according to (**Mellet and Schoeman, 2007.** The number of non-target pest arthropods (aphids, whiteflies, leafhopper green bugs, and spider mites) and non-target beneficial arthropods (Green lacewing, ladybird coccinella; the rove beetle; Orius *bugs* and true Spider) were determined. No distinction was made between all species of non-target pest arthropods and non-target beneficial arthropods. Abundance of non-target pest arthropods and non-target beneficial arthropods were determined in two ways as follows:

1. Most arthropods were sampled using a standard sweep net (38 cm diameter) that was swung perpendicular to a single row in a figure-eight pattern for Giza 80 cotton variety (*Bt* cotton, non *Bt* and sprayed non *Bt*) at Sids Station / Beni-Suif Governorate from the 7th week of plant emergence and continued until the cotton bolls started open (10 weeks of data).Two sets of 25 sweeps were collected in each plot using a random starting point. The contents of the net were frozen and later sorted in the laboratory for account both the non target pest arthropods (adult and nymph for each leafhopper and green bugs) and the non target beneficial arthropods (green lacewing (adult and nymph), ladybird *coccinella (larvae and adult),* rove beetle (adult and nymph), Orius *bugs* (adult and nymph) and true spider (adult and spider lings)).
2. Sample consists of 25 cotton leaves randomly selected from each plot (4 replicates) for Giza 80 cotton variety (*Bt* cotton, non *Bt* and sprayed non *Bt*). The samples transfer to the laboratory for account the non –target pest arthropods (aphid, whiteflies, and spider mites) and non –target beneficial arthropods (true spider egg sack and egg stage for green lacewing).

Treatments with insecticides:

Insecticide name	Target insect	Date of application
Chlorpyrifos	Cotton leafworm	2nd week of June
Spinetoram	Bollworms	2nd week of July
Lambda-cyhalo thrin	Bollworms	4th week of July

Statistical analysis:

ANOVA was used to analyses parametric data.

3. Results

1. Abundance of non- target pest arthropods on *Bt* cotton, non *Bt* and sprayed non *Bt*.

Data in Table (1) indicate that, the abundance of non- target pest arthropods on Giza 80 (*Bt* cotton, non *Bt* and sprayed non *Bt*) at Sids Station Beni- Suif Governorate during 2011 cotton season. The general mean of aphids abundance was 82.6, 83.9 and 50.1individual aphid for *Bt* cotton, non *Bt* and sprayed non *Bt*, respectively. Statistical analysis, no significant differences between the aphid abundance on *Bt* cotton and non *Bt*, whereas, there are a significant differences between the aphid abundance on sprayed non *Bt* compare to *Bt* cotton and non *Bt*.

Leafhopper abundance, the general mean of leafhopper abundance was 96.2, 102.4 and 73.8 leafhopper (adult and nymph) for *Bt* cotton, non *Bt* and sprayed non *Bt*, respectively. Statistically, no significant differences between the leafhopper abundance on *Bt* cotton and non *Bt*, whereas, there are a significant differences between the leafhopper abundance on sprayed non *Bt* compare to *Bt* cotton and non *Bt*.

Whiteflies abundance, the general mean of whiteflies abundance was 39.2, 38.7 and 18.8 whitefly (adult and nymph) for *Bt* cotton, non *Bt* and sprayed non *Bt*, respectively. Statistically, no significant differences between the whiteflies abundance on *Bt* cotton and non *Bt*, whereas, there are a significant differences between the whiteflies abundance on sprayed non *Bt* compare to *Bt* cotton and non *Bt*.

Green bugs and spider mites abundance, the general mean of green bugs abundance was 7.3, 7.2 and 6.2 green bug (adult and nymph) and 15.2, 13.1 and 12.2 individual spider mites for *Bt* cotton, non *Bt* and sprayed non *Bt*, respectively. Statistically, no significant differences between all values for green bugs and spider mites abundance on *Bt* cotton, non *Bt* and sprayed non *Bt*.

2. Abundance of non- target beneficial arthropods on *Bt* cotton, non *Bt* and sprayed non *Bt*.

Table (2) show that the abundance of non-target beneficial arthropods on Giza 80 (*Bt* cotton, non *Bt* and sprayed non *Bt*) at Sids Station during 2011 cotton season. Abundance of green lacewing eggs, the general mean was 10.7, 10.0 and 8.7 egg for *Bt* cotton, non *Bt* and sprayed non *Bt*, respectively. No significant differences between all values for green lacewing eggs abundance on *Bt* cotton, non *Bt* and sprayed non *Bt*. The abundance of adult and larvae green lacewing was11.8, 13.2 and 9.1 (adult and larvae) on *Bt* cotton, non *Bt* and sprayed non *Bt*, respectively. Statistical analysis, no significant differences between (adult and larvae) green lacewing abundance on *Bt* cotton and non *Bt*, whereas, there are a significant differences between the green lacewing abundance on sprayed non *Bt* compare to *Bt* cotton and non *Bt*. The same table indicates to the abundance of *ladybird coccinella* adult and larvae, it was 22.5, 24.2 and 15.2 *ladybird* on *Bt* cotton, non *Bt* and sprayed non *Bt*, respectively. Statistically, no significant differences between the *ladybird* abundance on *Bt* cotton and non *Bt*, whereas, there are a significant differences between the *ladybird* abundance on sprayed non *Bt* compare to *Bt* cotton and non *Bt*.

The rove beetle abundance, the general mean of adult and nymph of rove beetle was 3.3, 3.9 and 2.0 rove beetle (adult and nymph) on *B*t cotton, non *Bt* and sprayed non *Bt*, respectively. Statistical analysis, no significant differences between the general mean of adult and nymph for rove beetle abundance on *Bt* cotton and non *Bt*, whereas, there are a significant differences between the rove beetle abundance on sprayed non *Bt* compare to *Bt* cotton and non *Bt*.

The same Table (2) clear that the Orius bugs abundance was 15.8, 19.0 and 12.3 Orius (adult and nymph) on *B*t cotton, non *Bt* and sprayed non *Bt*, respectively. Statistically, no significant differences between the Orius bugs abundance on *Bt* cotton and non *Bt* & between *Bt* cotton and sprayed non *Bt,* whereas, a significant different between non *Bt* and sprayed non *Bt*.

Abundance of true spider (egg sack), it was 3.5, 4.0 and 3.2 egg sack on *B*t cotton, non *Bt* and sprayed non *Bt*, respectively. Statistically, no significant differences between all values for *Bt* cotton, non *Bt* and sprayed non *Bt* .The abundance of true spider (adult and spider lings), it was 5.3, 5.1 and 4.3 true spider (adult and spider lings) on *B*t cotton, non *Bt* and sprayed non *Bt*, respectively. Statistically, no significant differences between all values for *Bt* cotton, non *Bt* and sprayed non *Bt*.

4. Discussion:

Collectively, the non-target studies performed to date demonstrate that *Bt* crops do not have any unexpected toxic effects on natural enemy species, as would be predicted from knowledge of the mode of action and specificity of *Bt* proteins. Because of this specificity, *Bt* crops effectively preserve local populations of various economically important biological control organisms that can be adversely impacted, at least transiently, by broad-spectrum chemical insecticides. The only indirect effects on non-target organisms that have been observed with *Bt* crops are local reductions in numbers of certain specialist parasitoids whose hosts are the primary targets of *Bt* crops. Such trophic effects will be associated with any effective pest control technology, whether it be transgenic, chemical, or cultural, as well as with natural fluctuations in host populations **(Graham, 2005)**.

The lack of effect of transgenic cotton on aphid, chrysopid and coccinellid abundance is, however, further supported by the literature. **Lumbierres *et al.* (2004)** found that the aphid *Rhopalosiphum padi* L. was not influenced by *Bt* when fed on transgenic maize for several generations, **Dutton *et al.* (2002)** reported that the intrinsic rate of natural increase of *R. padi* was not influenced when fed on *Bt*-maize in a laboratory study and the green lacewing *Chrysoperla carnea* was not negatively influenced when fed on these transgenic maize raised aphids. **Romeis *et al.* (2004)** exposed *C. carnea* to Cryl Ab toxin concentrations at a factor 10,000 higher than what would have been ingested when feeding on *Bt*-reared lepidopteran larvae and found no direct toxicity towards the lacewings. **Pilcher *et al*., 1997** reported that transgenic corn pollen had no significant effect on *Chrysoperla carnea* and *Coleomegilla maculata* movement in corn. No detrimental effects on chrysopid and coccinellid populations in the transgenic corn were observed over a study period of two years in the field.

There were no adverse effects on non-target arthropods in Bollgard cotton (*Bt* cotton) fields compared with conventionally grown cotton. When conventionally grown cotton requires synthetic insecticide treatments for tobacco budworm or cotton bollworm control, Bollgard cotton fields often have significantly more non-target arthropods than conventionally grown cotton fields **(Moar *et al.*, 2002)**. Transgenic *Bt* cotton did not exert any adverse effects on natural enemies and the activity of coccinellids and spiders was more or less uniform on *Bt* and non-*Bt* cotton fields in India **(Rao and Rao, 2008).** In USA, **Naranjo, 2005,** reported, no effects of *Bt* cotton on nature enemy function in Arizona cotton and further showed that minor reductions in density of several predator taxa in *Bt* cotton observed in a companion study may have little ecological manning relative to natural enemy impact on key pests in the system. Also, these studies showed essentially no significant different between the abundance of non-target pest arthropods and non-target beneficial arthropods on *Bt* cotton and non *Bt*. In any case, these attempts were elucidate to evaluate the effect of *Bt* cotton on non target organisms in Egyptian cotton fields to release the Egyptian *Bt* cotton as a new commercial product at large scale for rationalize the using of insecticides via IPM program on cotton crop in Egypt.

Table (1): Abundance of non- target pest arthropods on Giza 80 (*Bt* cotton and non *Bt*) cotton variety during 2011 cotton season.

Non-target pest arthropods	Giza 80	Date of inspection										General mean	L.S.D 5%
		May	June					July					
English name		25	1	8	15	22	29	9	16	23	30		
Aphids	*Bt*	32.3	39	42	55.3	59.3	89	102	142.6	156	108	82.6 a	13.9
	Non *Bt*	30.6	42.3	39.3	55	63	85.3	125	138.6	149	111	83.9 a	
	Sprayed non *Bt*	63	38.6	39.3	59	58	84	36.6	33	39.3	50	50.1 b	
Leafhopper	*Bt*	40.6	56.3	99	82	47.3	54.6	83	152	175	172.3	96.2 a	8.7
	Non *Bt*	44	48	94.3	79.3	47	59.3	74	143.3	210.6	224	102.4 a	
	Sprayed non *Bt*	36	45	98	96.3	50	62	23.3	96	65.3	166	73.8 b	
Whiteflies	*Bt*	2.3	2	8	9	17.3	52.3	53	78	88.3	81.3	39.2 a	8.7
	Non *Bt*	0	3	6.6	11	23.3	42	54.4	74	81.3	91.3	38.7 a	
	Sprayed non *Bt*	1	4	4	9	19	36.3	34.6	33.3	27	19.3	18.8 b	
Green bugs	*Bt*	2	5.3	4	4.3	7	8.3	9	9.3	11	13	7.3 a	6.3
	Non *Bt*	3	4.3	3	8.3	9	8	5.6	11.3	9	10	7.2 a	
	Sprayed non *Bt*	1	4	5	6	8.3	10.3	7.3	6	6	8.3	6.2 a	
Spider mites	*Bt*	0	0	4.3	5	11.3	23	30	23	25.3	30.3	15.2 a	5.3
	Non *Bt*	0	0	0	3.3	7	21.6	20	22.3	24	33	13.1 a	
	Sprayed non *Bt*	1	0	0	4.3	5.3	27	22.6	20	15.3	26	12.2 a	

For general mean column, the values have the same letters vertically for separately pest are non-significant different.

Table (2): Abundance of non- target beneficial arthropods on Giza 80 (*Bt* cotton and non *Bt*) cotton variety during 2011 cotton.

Non-target beneficial arthropods		Giza 80	Date of inspection										General mean	L.S.D. 5%
			May	June					July					
English name	Stage		25	1	8	15	22	29	9	16	23	30		
Green lacewing	Egg	*Bt*	2	2.3	2	11.3	7	13.3	15	17	17.3	20	10.7 a	2.4
		Non *Bt*	0.6	3	4.3	7	6.3	15	12.3	10	22	19.3	10.0 a	
		Sprayed non *Bt*	0	2	2	4	9	13	17	11	15	14	8.7 a	
	Adult+ Larvae	*Bt*	2.3	3.6	4	9.3	10	15.6	18	15.3	17	23.3	11.8 a	3.3
		Non *Bt*	3.3	3	5.3	12.3	12.6	15.3	16	16	22	26	13.2 a	
		Sprayed non *Bt*	0.3	3.3	3	15.3	21.3	16.3	11	6.6	9	5.3	9.1 b	
Ladybird *coccinella*	Adult+ Larvae	*Bt*	4	6	11.3	10	9	16.6	26	41	40.3	61	22.5 a	7.6
		Non *Bt*	5.3	5	8.3	13.3	16.3	22	32	33.3	42.3	64.6	24.2 a	
		Sprayed non *Bt*	6	3.3	6.3	9	15	18	19.3	22.6	19.3	33	15.2 b	
Rove beetle	Adult+ Nymph	*Bt*	0	0	2	4.3	3	2.6	5	7	6.3	3	3.3 a	0.97
		Non *Bt*	0	0	0.6	5	3	3	7.3	5.6	6	8.3	3.9 a	
		Sprayed non *Bt*	0	0	0	4	4.6	3	5.6	2.3	0	0	2.0 b	
Orius ***bugs***	Adult+ Nymph	*Bt*	0	0	0	3	2	15	16	40	37	45	15.8 ab	2.47
		Non *Bt*	0	0	0	6	11.3	17	23.3	41	40.3	51.3	19.0 a	
		Sprayed non *Bt*	0	0	0	6	15	22.3	17.3	22.3	17.3	23	12.3 b	
True Spider	Egg Sack	*Bt*	0.6	2.3	3	4	3.6	3	7.3	4	3	4.6	3.5 a	0.61
		Non *Bt*	0	2.3	3.3	2	0.6	5	5.3	5	7.3	9	4.0 a	
		Sprayed non *Bt*	1	0	3.6	5	4	2.3	4	3	2	7.3	3.2 a	
	Adult+ Spider lings	*Bt*	0	1.6	0	7.3	10	8.3	4	6.6	5	10.6	5.3 a	0.83
		Non *Bt*	1	3.3	3	6.3	5	4.3	6.3	6	7	8.3	5.1 a	
		Sprayed non *Bt*	0	3.3	4.6	9	5.3	5.3	4	4.6	3.3	4	4.3 a	

For general mean column, the values have the same letters vertically for separately stage are non-significant different

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