Field Performance for Genetically Modified Egyptian Cotton Varieties (*Bt* Cotton) Expressing an Insecticidal- Proteins Cry 1Ac and Cry 2Ab Against Cotton Bollworms

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Abstract: The present study is the first attempt in Egypt which devoted to evaluate the field performance of some Egyptian cotton varieties *Gossypium barbadense* L. (Giza 80, Giza 90 and Giza 89) which genetically modified by *Bacillus thuringiensis* (*Bt*) genes against bollworms, pink bollworm *Pectinophora gossypiella* and spiny bollworm *Earias insulana*. However, no variety of *Bt* cotton has yet been approved for commercial planting in Egypt. This work was conducted on three Egyptian cotton varieties (Giza 80, 90 and 89) in which were Genetically Modified (GM) by transfer tow genes (Cry 1Ac and Cry 2Ab) from *Bacillus thuringiensis* (*Bt*) to the American cotton *Gossypium hirsutum* by the gene particle gun, then transfer those tow genes to the three Egyptian cotton varieties by crossing between the American cotton and the Egyptian cotton varieties. Cotton bollworms *P. gossypiella* and *E. insulana* belong to the order Lepidoptera and therefore are sensitive to *Bt* Cry I Ac and Cry II Ab proteins, which are specific to them. The maximum percent of bollworms infestation during 2011 cotton season for Giza 80 were 2.0 and 22.0 % by *P. gossypiella* for *Bt* cotton and non *Bt*, respectively, and for *E. insulana* the maximum percent reached 1.0 and 90.0 % for *Bt* cotton and non *Bt*, respectively. For Giza 90, the maximum percent were 1.0 and 21.0 % by *P. gossypiella* for *Bt* cotton and non *Bt* , respectively and for *E. insulana* the maximum percent reached 2.0 and 82.0 % for *Bt* cotton and non *Bt*, respectively. For Giza 89, the maximum percent were 1.0 and 14.0 % by *P. gossypiella* for *Bt* cotton and non *Bt*, respectively, and for *E. insulana* the maximum percent reached 1.0 and 32.0 % for *Bt* cotton and non *Bt*, respectively. On the other hand, the artificial infestation for the three cotton varieties by the *P. gossypiella* and *E. insulana* neonate larvae at laboratory cleared that the infestation % was 0.0 % for Giza 80, 90 and 89 *Bt* progenies compared to 76 – 100 % for Giza 80, 90 and 89 non *Bt* varieties. These attempts were elucidate to rationalize the using of insecticides via IPM program on cotton crop in Egypt.
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Key words: Genetically modified cotton; Egyptian *Bt* cotton; Insecticidal proteins; Cry1Ac; Cry 2Ab; Bollworms infestation; *Pectinophora gossypiella* and *Earias insulana*.

1. Introduction

Cotton is one of the major fiber crops of global significance. It is cultivated in tropical and subtropical regions of more than eighty countries of world occupying nearly 33 million hectare with an annual production of 19 to 20 million tones of bales. China, U.S.A., India, Pakistan, Uzbekistan, Australia, Brazil, Greece, Argentina and Egypt are major cotton producing countries. These countries contribute nearly 85% of the global cotton production (**Mayee *et al.*, 2001**).

The cotton crop has numerous invertebrate pests and more than one thousand species are found on cotton but only ten or a dozen of them are significant potential pests **(Deguine *et al.*, 2008).** Several million pounds are paid every year for controlling of cotton pests, the control of *P. gossypiella* and *E. insulana* based mainly on foliage treatments with chemical synthetic insecticides. The widespread and intensive use of different synthetic insecticides for controlling these pests caused increasing environmental problems including insect resistance, excessive persistence of residues, human health hazards and harmful effect on non-target organisms (**Dahi, 2012**).

The transgenic plants are the new approach around the world for controlling the insect pests. The first transgenic plant was developed in 1983 in tobacco (**Fraley *et al.*, 1983**) in U.S.A. In cotton, the first transgenic plant was developed in 1987 in U.S.A. by Monsanto (**Benedict and Altman, 2001)**.

Cotton is currently the third most important transgenic crop in terms of surface area (after soybean and maize) **(James, 2004)**.The transgenic cotton is of two types' viz. (1) Bollgard (*Bt* cotton) and (2) Roundup ready cotton (RR cotton). The former confers resistance to bollworms and the latter is resistant to herbicides. However, *Bt* transgenic cotton has spread to several countries (such as USA, China and India etc). Development and commercialization of cotton varieties expressing insecticidal proteins (Cry toxins) from *Bacillus thuringiensis* (*Bt* cotton) has offered an alternative to traditional synthetic insecticides for control of important agricultural lepidopterous pests.

B. thuringiensis (*Bt*) Berliner is a ubiquitous, spore-forming soil bacterium that produces crystalline inclusions containing entomocidal proteins, also referred as *Bt* toxins, or δ-endotoxins, during the sporulation process. Preparations containing spores and protein crystal of *Bt* have been used as microbial pesticides since the 1970s **(Navon, 2000)**. *Bt* strains produce a variety of crystal proteins each with its distinct host ranges **(Kumar *et al.*, 1996).** At least ten genes encoding different *Bt* toxins have been engineered into different crops plants: Cry 1 Aa, Cry 1Ab, Cry 1 Ac, Cry 1 Ba, Cry1Ca, Cry1H, Cry2 Aa, Cry3A, Cry6A and Cry9C **(Schuler *et al.*, 1998)** and most of the commercial transgenic cotton express Cry1Ac (**Luttrell *et al.*, 1999; Perlak *et al.*, 2001 and Dutton *et al.*, 2002 and Baur and Boethel, 2003).** The second generation of *Bt* cotton combines Cry1Ac with a second *B. thuringiensis* toxin (Cry2Ab) and provides growers with a product that offers a broader spectrum of pest control and reduced chances of insects developing *B. thuringiensis* resistance **(Ferre and Rie , 2002** and **Tabashnik *et al.*, 2002**). Also, a mixture of different toxins could be more effective than a single toxin **(Yunus *et al.*, 2011)**.

One of the most effective controlling measures against bollworms (*P. gossypiella* and *E. insulana*) and cotton leafworm *S. litorallis* is planting *Bt* transgenic cotton, expressing an insecticidal- protein derived from *Bacillus thuringiensis* subsp. Kuristaki (Berliner) **(Vojech, 2005 and Ladan *et al.*, 2011**). Registration of *Bt* cotton in USA in 1996 marked the beginning of a major change in pest management in Arizona cotton **(Timothy *et al.*, 2007)**. In order to control of lepidopteran pests, plants have been genetically engineered to express insecticidal proteins from *Bacillus Thuringiensis* (e.g. *Bt* cotton) are now planted in the around the world **(Wu, 2007 and Torres and Ruberson, 2008).** Transgenic cotton with the *Bacillus thuringeinsis* (*Bt*) Berliner gene or genes producing proteins toxic to the pink bollworm, *P. gossypiella* and other lepidopterous pests have been grown commercially in Arizona since 1996 without loss in control efficacy (**Tabashnik *et al.*, 2004; Tabashnik *et al.*, 2006).**

Bt cotton, to be released shortly in Egypt, primarily targets the bollworms *P. gossypiella*, *E. insulana* and *Heliocoverpa armegira*) and cotton leafworm *S. littoralis*. In Egypt **Dahi (2012),** reported that the Egyptian *Bt* cotton have a high resistant to the Egyptian cotton leafworm *S. littoralis*.

The present study was devoted to evaluate the field performance of some Egyptian cotton varieties (Giza 80, 90 and 89) which genetically modified by *Bacillus thuringiensis* (*Bt*) genes against *P. gossypiella* and *E. insulana*. These attempts were elucidate to rationalize the using of insecticides via IPM program on cotton crop in Egypt.

2. Material and Methods

I- Transgenic cotton:

This study was conducted on three Egyptian cotton varieties (Giza 80, Giza 90 and Giza 89) in which were Genetically Modified (GM)- during the co-ordinate project between Monsanto company and Egyptian Ministry of Agriculture, Agricultural Research Center (ARC) included Agricultural Genetic Engineering Research Institute (AGERI), Cotton Research Institute (CRI) and Plant Protection Research Institute (PPRI) – by transfer tow genes (Cry1Ac and Cry2Ab) from *Bacillus thuringiensis (Bt)* to the American cotton *Gossypium hirsutum* by the gene particle gun, then transfer those tow genes to the three Egyptian cotton varieties *Gossypium barbadense* by crossing between the American cotton and the Egyptian cotton varieties.

The transgenic Egyptian cotton varieties plants Bollgard II (MON15985 "*Bt* cotton") expressing Cry 1Ac and Cry 2Ab proteins and untransformed control cultivars (Giza 80, Giza 90 and Giza 89"non *Bt*") were cultivated during 2011 cotton season at Agricultural Research Center Research Stations. Giza 80 and Giza 90 at Sids Research Station (Beni Suef Governorate) and Giza 89 at Sakha Research Station (Kafr El Sheikh Governorate).

II-Experimental Design:

- Trials sites and Giza lines:
  - Sids/Beni-Suef: Giza 80 and Giza 90
  - Sakha/Kafr El Sheikh: Giza 89
- Experimental Design: Randomized Complete Block Design.
- Replications: 4 (every replication will have 2 meters alley separation).
- Plot size: 6 meters length x 10 rows.
- Plant Density: Adapted to the agronomic conditions of every site.
- Plot buffers: No plot buffers are needed.
- Trial buffers: Trial will be surrounded by 15 meters/15 rows of conventional Giza iso-line seed.
- Planting date: as commercial fields where the site is located, (May 10th at Sids/Beni-Suef and May 27th at Sakha/Kafr El Sheikh).

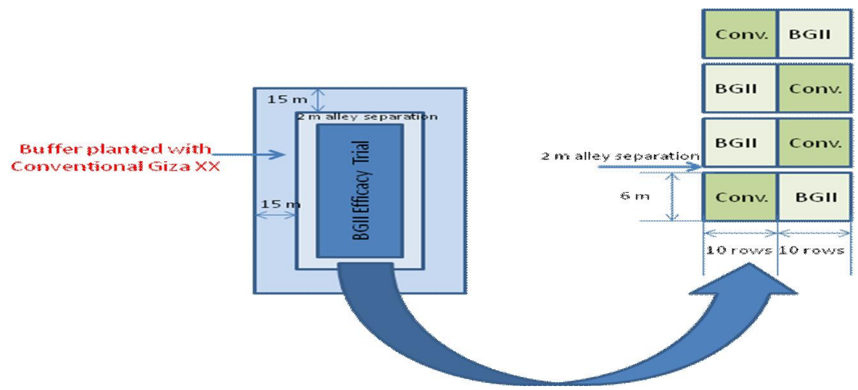

Fig. 1: The experimental design for MON 15985 in GIZA cotton varieties 80, 90, and 89 (*Gossypium barbadense*) against bollworms.

III-Population density of *P. gossypiella* and *E. insulana* larvae infesting *Bt* cotton and non *Bt*:

The population fluctuation of *P. gossypiella* and *E. insulana* larvae in cotton bolls was estimated during 2011 cotton growing season for Giza 80, 90 and 89(*Bt* cotton and non *Bt*). Samples of 100 bolls in four replicates (25 bolls / each) were picked up weekly at random for Giza 80, 90 and 89(*Bt* cotton and non *Bt*).The bolls were examined in the laboratory for the presence of *P. gossypiella* and *E. insulana* larvae. Counts included bolls from which the larvae had departed but had left characteristic traces. The average infestation percent in 100 bolls were calculated for the three cotton varieties *Bt* cotton and non *Bt*. Sampling started on 1st week of July for both Giza 80 and Giza 90 at Sids Station (Beni-Suef Governorate) and continued till last week of September. For Giza 89 at Sakha Station (Kafr El Sheikh Governorate) the sampling started on 2nd week of August and continued till the 3rd week of October.

IV-The Artificial infestation by *P. gossypiella* and *E. insulana* for *Bt* cotton and non *Bt* in laboratory.

At August 20th, Fifty bolls from each variety (Giza 80, 90 and 89) *Bt* cotton and non *Bt* were collected from field and transfer to laboratory to evaluate the artificial infestation % of *P. gossypiella* and *E. insulana* under 25 ± 1°C constant temperature. Source of water for every cotton boll was occurred by put some of wetted cotton fiber around the boll stem .Fifty newly hatched larvae in five replicates (10 per each) from both *P. gossypiella* and *E. insulana* were transferred to fifty bolls of Giza 80, 90 and 89 cotton varieties (*Bt* cotton and non *Bt*) . After two weeks the bolls were examined for the presence of *P. gossypiella* and *E. insulana* larvae. Counts included bolls from which the larvae had departed but had left characteristic traces. The average infestation percent in 50 bolls were calculated for the three cotton varieties *Bt* cotton and non *Bt*. The bolls of *Bt* cotton and non *Bt* when mixed in one cadge the *Bt* bolls marked by a point of white ink (corrector pen).

3. Results

I-Field performance

Giza 80

Data recorded in Table (1) revealed that the maximum of boll infestation percent reached 2.0 % at September 10th by *P. gossypiella* for Giza 80 *Bt* cotton, but it reached to 22.0 % at September 3th for Giza 80 non *Bt* . For *E. insulana* the maximum of boll infestation percent reached 1.0 % at September 3 th for Giza 80 *Bt* cotton and it reached to 90.0 % at September 10th for Giza 80 non *Bt*.

Giza 90

Data in Table (2) revealed that the maximum of boll infestation percent by *P. gossypiella* reached to 1.0 % at September 3th and 21.0 % at September 24th for both Giza 90 *Bt* cotton and non *Bt*, respectively. For *E. insulana* the maximum of boll infestation percent reached to 2.0 % at September 17th and 82.0 % at September 10th for both Giza 90 *Bt* cotton and non *Bt*, respectively.

Giza 89

Data in Table (3) indicated that the maximum of boll infestation percent by *P. gossypiella* reached to 1.0 % at October 12nd and 14.0 % at August 31st for both Giza 89 *Bt* cotton and non *Bt*, respectively. For *E. insulana* the maximum of boll infestation percent reached to 1.0 % at September 28th and 32.0 % at October 12nd for both Giza 89 *Bt* cotton and non *Bt*, respectively.

Table (1): Infestation % by *P. gossypiella* and *E. insulana* for Giza 80 (*Bt* cotton and non *Bt*) at Sids Station during 2011 cotton season.

Date of inspection	Infestation %			
	Bt cotton		Non *Bt*	
	P.	*E.*	*P.*	*E.*
2- July	0	0	4	0
9	0	0	5	0
16	0	0	4	0
23	0	0	3	2
30	0	0	2	6
6- Aug.	0	0	7	4
13	0	1	11	26
20	0	0	17	24
27	0	0	15	38
3- Sept.	0	1	22	43
10	2	0	20	90
17	0	0	21	82
24	0	0	20	78

P. = *Pectinophora gossypiella* *E.* = *Earias insulana*

Table (2): Infestation % by *P. gossypiella* and *E. insulana* for Giza 90(*Bt* cotton and non *Bt*) for at Sids Station during 2011 cotton season.

Date of inspection	Infestation %			
	Bt cotton		Non *Bt*	
	P.	*E.*	*P.*	*E.*
2- July	0	0	4	0
9	0	0	4	0
16	0	0	2	4
23	0	0	0	6
30	0	0	4	10
6- Aug.	0	0	6	12
13	0	0	8	25
20	0	0	10	22
27	0	2	10	32
3- Sept.	1	0	10	46
10	0	0	16	82
17	0	2	20	80
24	0	0	21	76

P. = *Pectinophora gossypiella* *E.* = *Earias insulana*

Table (3): Infestation % by *P. gossypiella* and *E. insulana* for Giza 89(*Bt* cotton and non *Bt*) at Sakha Station during 2011 cotton season.

Date of inspection	Infestation %			
	Bt cotton		Non *Bt*	
	P.	*E.*	*P.*	*E.*
10- Aug.	0	0	0	0
17	0	0	4	0
24	0	0	4	2
31	0	0	14	1
7- Sept.	0	1	10	8
14	0	0	12	8
21	0	0	11	12
28	0	1	10	28
5- Oct.	0	0	10	24
12	1	0	11	32
19	0	0	8	30

P. = *Pectinophora gossypiella* *E.* = *Earias insulana*

II-The Artificial infestation by *P. gossypiella* and *E. insulana* for *Bt* cotton and non *Bt* in laboratory.

Data in Table (4) indicate to the artificial infestation by *P. gossypiella* and *E. insulana* for Giza 80, 90 and 89 *Bt* cotton and non *Bt* in laboratory. The infestation % by *P. gossypiella* was (zero & 76.0), (zero & 80.0) and (zero & 76.0 %) for Giza 80, 90 and 89 (*Bt* cotton & non *Bt*), respectively. For *E. insulana*, the infestation % was (zero & 86.0), (zero & 90.0) and (zero & 90.0 %) for Giza 80, 90 and 89 (*Bt* cotton & non *Bt*), respectively.

Table (5) indicate that the artificial infestation % by *P. gossypiella* and *E. insulana* for the three Egyptian genetically modified cotton varieties (Giza 80, 90 and 89 *Bt* cotton and non *Bt*) when cotton bolls (*Bt* and non *Bt*) mixed for each variety in one cadge. The infestation % by *P. gossypiella* was (zero & 76.0), (zero & 76.0) and (zero & 80.0 %) for Giza 80, 90 and 89 (*Bt* cotton & non *Bt*), respectively. On the other hand, the infestation % by *E. insulana* was (zero & 90.0), (zero & 90.0) and (zero & 100.0 %) for Giza 80, 90 and 89 (*Bt* cotton & non *Bt*), respectively.

The data in Table (6) show that the infestation % by two bollworms (*P. gossypiella* and *E. insulana*) for Giza 80, 90 and 89 (*Bt* cotton and non *Bt*) when cotton bolls and two pests mixed in one cadge for each variety separately. The infestation % by two bollworms was (zero &100.0) for Giza 80, 90 and 89 (*Bt* cotton & non *Bt*), respectively.

Table (4): Artificial infestation % by *P. gossypiella* and *E. insulana* for Giza 80, 90 and 89 *Bt* cotton and non *Bt*.

Pests	Varieties		No. of bolls	No. of larvae	Infestation %
P. gossypiella	Giza 80	*Bt* cotton	50	50	0
		Non *Bt*	50	50	76
	Giza 90	*Bt* cotton	50	50	0
		Non *Bt*	50	50	80
	Giza 89	*Bt* cotton	50	50	0
		Non *Bt*	50	50	76
E. insulana	Giza 80	*Bt* cotton	50	50	0
		Non *Bt*	50	50	86
	Giza 90	*Bt* cotton	50	50	0
		Non *Bt*	50	50	90
	Giza 89	*Bt* cotton	50	50	0
		Non *Bt*	50	50	90

Table (5): Artificial infestation % by *P. gossypiella* and *E. insulana* for Giza 80, 90 and 89 *Bt* cotton and non *Bt* when cotton bolls (*Bt* and non *Bt*) mixed in one cadge.

Pests	Varieties	No. of		Infestation %	
		Bolls (*Bt* and non *Bt*)	Larvae	*Bt* cotton	Non *Bt*
P. gossypiella	Giza 80	100	100	0.0	76
	Giza 90	100	100	0.0	76
	Giza 89	100	100	0.0	80
E. insulana	Giza 80	100	100	0.0	90
	Giza 90	100	100	0.0	90
	Giza 89	100	100	0.0	100

Table (6): Infestation % by two bollworms for Giza 80, 90 and 89 (*Bt* cotton and non *Bt*) when cotton bolls and two pests mixed in one cadge.

Varieties	No. of		Infestation %	
	Bolls (*Bt* and non *Bt*)	Two pests larvae	*Bt* cotton	Non *Bt*
Giza 80	100	100	0.0	100
Giza 90	100	100	0.0	100
Giza 89	100	100	0.0	100

4. Discussion

Transgenic cotton with genes expression of the crystalline insecticidal protein of *B. thuringiensis* can be considered as an effective contributor in pest management program of cotton fields in Egypt (**Dahi, 2012**). In addition, the bollworms *P. gossypiella* and *E. insulana* are the most important pests in cotton fields. Among the alternatives for controlling these pests, the use of *Bt* transgenic plants has gained attention due to its efficiency, low cost of the pest management programs and no impact on natural enemies (**Schuler *et al.*, 1999, 2002** and **2003** and **Romeis *et al.*, 2004** and **2006).** Previously, the impact of *Bt* cotton expressing insecticidal proteins from *B. thuringiensis* on the growth and survival of Noctuidae (Lepidoptera) larvae was studied by **Stewart *et al.*, (2001) and Fabrick *et al.*, (2009)** Moreover, laboratory and field evaluations of *Bt* transgenic soybean for control of lepidopteran pests was confirmed **(Macrae *et al.,* 2005)**.

According to the susceptibility of the above-described species to different lepidopteran-specific *B. thuringiensis* toxins, Cry1Ac cotton was selected as the best choice for commercial release. The second generation of *Bt* cotton combines Cry1Ac with a second *B. thuringiensis* toxin (Cry2Ab) and provides growers with a product that offers a broader spectrum of pest control and reduced chances of insects developing *B. thuringiensis* resistance (**Ferré and Rie, 2002**; **Tabashnik *et al.,* 2002** and **María *et al.,* 2006**).

The effectiveness of the control of bollworm by *Bt* cotton cultivation has resulted in a decrease in the amount of insecticides used on *Bt* cotton compared to conventional cotton. This has led to a control of lepidopterous pests by *Bt* cotton due to the reduction in broad-spectrum of used insecticide and consequently to not transformation of a minor pest to a main one.

All of these studies of other references here in, were emphasized the effectiveness of the *Bt* transgenic plants for control of lepidopteran pests. The present study is the first attempt in Egypt to evaluate the effect of *Bt* cotton against the bollworms *P. gossypiella* and *E. insulana*. Our findings confirm that the transgenic cotton containing a Cry 1Ac and Cry 2Ab genes have significantly more efficacy against *P. gossypiella* and *E. insulana* than the conventional cotton, for all things measured. The effect of Egyptian *Bt* cotton varieties on growth, development and metamorphosis of bollworms was similar to those were reported for the most studied pest species from the family Noctuidae by **Stewart *et al.,* (2001), Macrae *et al.,* (2005) and Sivasupramaniam *et al.,* (2008).**In the literature assays in which larvae were fed fresh plant tissue expressing both Cry 1Ac and Cry 2Ab were more toxic to bollworms, *Heliciverpa zea* (Boddie), fall armyworms, *S. frugiperda* (J.E. Smith), and beet armyworms, *S. exigua* (Hubner), than single-toxin cultivars expressing only Cry 1Ac (**Stewart *et al.,* 2001). Yunus *et al.* (2011)** reported that a mixture of different toxins could be more effective than a single toxin.

Our results are agreement with **Timothy *et al.,* 2004**; they reported that the field evaluations of efficacy of *Bt* cotton were conducted by the Arizona Cotton Research and Protection Council in adjacent pairs of *Bt* and non-*Bt* fields at 40 Arizona locations. Statewide, large pink bollworm larvae were found in an average of 21.7% (range 0 to 100%) of non-*Bt* bolls sampled from borders of refuge fields. Bolls from adjacent *Bt* cotton (Bollgard'™) fields yielded an average of 0.340% (range 0.0 to 4.69%) bolls infested with large larvae of *P. gossypiella*.

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