

Efficacy of *Bt* Transgenic Egyptian Cotton Varieties expressing Cry 1Ac and Cry 2Ab Genes Against *Spodoptera littoralis* (Boisd.)

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Abstract: The present study is the first attempt in Egypt to evaluate the effect of *Bt* cotton against *Spodoptera littoralis* (Boisd.). This study was conducted on three Egyptian cotton varieties *Gossypium barbadense* L. (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 Cotton Research Institute (CRI), Agricultural Genetic Engineering Research Institute (AGERI) and Plant Protection Research Institute (PPRI) – 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. The GM Egyptian cotton varieties clearly indicate high resistant against the cotton leafworm *Spodoptera littoralis* (Boisd.) as follow: the mortality percent for larvae feed on Egyptian cotton varieties (non *Bt*) were 9.0, 5.7 and 4.3 % for Giza 80, Giza 90 and Giza 89, respectively. On the other hand, the larvae feed on GM Egyptian cotton varieties (*Bt* cotton) the mortality percent were 97.7, 97.7 and 99.0 % for Giza 80, Giza 90 and Giza 89, respectively. The fecundity for female moths which resulted from larvae fed on *Bt* cotton & non *Bt* was (257.5 & 726.3) for Giza 80, (440.0 & 585.3) for Giza 90 and (317.0 & 491.7 eggs / female) for Giza 89, respectively. Also the fertility percent for eggs resulted from female moths which resulted from larvae fed on *Bt* cotton & non *Bt* was (68.5 & 97.0), (78.7 & 92.0) and (71.0 & 94.0 %) for Giza 80, Giza 90 and Giza 89, respectively. Another biological aspects for *S. littoralis* stages (larval duration, pupal weight, pupal duration, emergence %, malformed adult %, male & female longevity and sex ratio were affected (as a latent effect) by Cry 1Ac and Cry 2Ab of *Bacillus thuringiensis* genes which transfer to three Egyptian cotton varieties.

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Key word: *Spodoptera littoralis*; Cry 1Ac; Cry 2Ab; Transgenic cotton; *Bt* cotton and Genetically Modified (GM).

1. Introduction

The Egyptian cotton, *Gossypium barbadense* L., is considered a major economic crop in Egypt; it represents the first cash crop for the national income. Thus, the governmental policy in Egypt is offering all facilities to encourage farmers to increase the production of cotton in order to meet the increasing requirements for local production and allow surplus for exportation.

The cotton leafworm *Spodoptera littoralis* (Boisd.) is the major cotton destructive phytophagous lepidopterous insect pest in Egypt, which cause great reduction in quantity and quality of the yield (Hosny *et al.*, 1986). Several million pounds are paid every year for controlling of cotton pests, the control of *S. littoralis* based mainly on foliage treatments with chemical synthetic insecticides. The widespread and intensive use of different synthetic insecticides for controlling this pest caused increasing environmental problems including insect resistance, excessive persistence of residues, human health hazards and harmful effect on non-target organisms.

Development and commercialization of corn and cotton varieties expressing insecticidal proteins (Cry toxins) from *Bacillus thuringiensis* (*Bt* corn and *Bt*

cotton) have offered an alternative to traditional synthetic insecticides for control of important agricultural pests *Bt* cotton and *Bt* corn have been adopted by farmers in 29 countries to control lepidopteran pests such as corn borers (mainly *Ostrinia nubilalis*) in corn and the budworm-bollworm complex (*Heliothis virescens*, *Helicoverpa* spp., *Pectinophora gossypiella*) in cotton.

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). The inactive protoxins are proteolytically digested in the insect midgut to form active toxins. Their toxicity is achieved by binding to the midgut cells of insects and causing osmotic lysis through pore formation in the midgut (Gill *et al.* 1992 and Khove, 1998).

Bacillus thuringiensis (Berliner) is a soil bacterium that produces a diversity of Cry proteins that are selectively toxic against a wide variety of insect pests **Crickmore et al. (1998)**. 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 ME 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 the cotton leafworm *S. littoralis* is planting *Bt* transgenic sugar beet, 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**).

Bt cotton, to be released shortly in Egypt, primarily targets the cotton leafworm *S. littoralis* and Bollworms; pink bollworm *Pectinophora gossypiella*, spiny bollworm *Earias insulana* and the American bollworm *Helioverpa armigera*.

The present work was devoted to evaluate the biological efficacy of some Egyptian cotton varieties (Giza 80, Giza 90 and Giza 89) which genetically modified by *Bacillus Thuringiensis* (*Bt*) genes against the cotton leafworm *S. littoralis*. These attempts were elucidate to rationalize the using of insecticides via IPM program on cotton crop in Egypt.

2. Material and Methods

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 (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 *Gossypium barbadense* by crossing between the American cotton and the Egyptian cotton varieties.

The transgenic Egyptian cotton varieties plants (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 Sedes Research Station (Beni Soef Governorate) and Giza 89 at Sakha Research Station (Khafer Elshekh Governorate).

Cotton leaves (*Bt* cotton and non *Bt*) were transfer to the laboratory for fed the *S. littoralis* instars larvae from hatching until pupation take place.

Cotton leafworm *S. littoralis* Rearing technique:

The cotton leafworm, *S. littoralis* were collected from fields as a egg, larvae and pupae and reared in the laboratory from 2-4 generations on the fresh leaves of castor oil plant (*Ricinus communis*) as natural food which resemble the natural food in the field (i.e. cotton leaves) before started the evaluation in laboratory.

For strain establishment, the eggs were maintained at $25 \pm 1^\circ\text{C}$ and 65-70 % R.H until hatching. Newly hatched larvae were transferred to clean 5 pound glass-Jars covered with muslin and secured with rubber bands. They were provided with fresh castor-oil leaves which were renewed daily until the larvae show the signs of pupation. A thin layer of fine saw-dust was spread on the bottom of every glass-Jar to help the successful pupation. Pupae were kept individually in a vial until moth emergence. Ten pairs of newly - emerged moths were confined into oviposition cages. This consists of a conventional mating glass bells (16cm. high and 8cm.diam.) opened at each end. Each mating-glass bell was supplied with a small fresh branch of *Nerium oleander* to serve as an oviposition site, and placed on its wide end on a half Petri-dish. Tops of the glass bells were covered with muslin and secured with rubber bands. Cages were examined daily to replace *N. oleander* branches with new ones and renew the adult feeding solution (a small piece of absorbent cotton wool previously soaked in 10% sucrose solution). The cages were maintained at the same conditions of temperature and % R.H. Deposited egg- masses were kept in Petri-dishes, and then were available to achieve the different experiments.

Experimental design:

All stages (from egg to adults) were kept under the constant temperature of $25 \pm 1^\circ\text{C}$ and 65-70 % R.H.

to determine all the biological parameters of each stage. Eggs were collected from the breeding cages daily. The collected eggs were transferred to glass vials (2.0 x 7.5 cm), subsequently the incubation took place under the required combination of temperature and relative humidity.

Larval stage:

To study the larval instars development of *S. littoralis*, 50 larvae from each instar (from 1st to 6th instar) were transferred to 5 replicates in 5 glass-Jars (10 larvae / replicate) and covered with muslin and secured with rubber bands. The same number of *S. littoralis* larvae from each instar (from 1st to 6th instar) was taken place as a control. They were provided with fresh cotton leaves (*Bt* cotton and non *Bt*) which were renewed daily until the larvae show the signs of pupation. A thin layer of fine saw-dust was spread on the bottom of every glass-Jar to help the successful pupation. The larvae were left in the glass-Jar until pupation. Daily observations were made to count the pupated larvae and larval duration. Actual values for larval mortality percent were estimated using the following equation (Abbot, 1925).

$$\% \text{ Effect} = (1 - N_t / N_c) \times 100$$

Where: N_t and N_c is the number of alive larvae in treatment (*Bt* cotton) and check (non *Bt*), respectively.

Pupal stage:

Newly formed pupae which resulted from the 2nd instar larvae experiment were collected on the same day of pupation and placed in the glass tube (2.0x7.5 cm.) (one pupae for each tube) and plugged tightly with a piece of cotton. The pupae were placed at the same temperature (25±1°C) and observed daily till adult emergence. Pupal weight could also be considered.

Adult stage:

Ten of newly emerged moths were transferred on the same day of emergence to a glass mating-cage as mentioned before and also held on the same conditions of temperature and % R.H. Five replicates, each has 2 adults (1 ♂ +1 ♀), were placed at each same condition. Daily observations were made to record the adult survival, collect and count the number of deposited eggs. The eggs were incubated at the same conditions

in order to calculate the fecundity, fertility and adult longevity.

Duration of different stages, pupation, adult emergence %, sex ratio, pre-oviposition, oviposition and post-oviposition periods, adult longevity, fecundity and fertility were calculated.

3. Results

Effects of *Bt* cotton on the herbivore *S. littoralis*

Giza 80:

Data in Table (1) indicated that the mortality % for *S. littoralis* 1st instar larvae started with 42.0 % after the first day from feed on *Bt* cotton leaves and reached to 100 % at the fifth day from feed on the *Bt* cotton leaves. The same parameter (mortality %) started with Zero % and increased gradually to 6% (maximum case) after 31 days with 94.0 % pupation %. For the 2nd instar larvae the mortality % reach to 8 % after 19 days for non *Bt* and it reached 94.0 % after 35 days for *Bt* cotton.

The larval mortality % were (92.0 & 12.0), (100.0 & 8.0), (100.0 & 10.0) and (100.0 & 10.0 %) for 3rd, 4th, 5th and 6th instar larvae of *S. littoralis* when fed on (*Bt* cotton & non *Bt*), respectively.

Giza 90:

Data in Table (2) show the same trend of larval mortality % was observed for Giza 90. Larval mortality % were (100.0 & 8.0), (96.0 & 4.0), (100.0 & 4.0), (92.0 & 10.0), (100.0 & 8.0) and (98.0 & 0.0 %) for 1st, 2nd, 3rd, 4th, 5th and 6th instar larvae when the larvae fed on (*Bt* cotton & non *Bt*), respectively.

Giza 89:

The Data in Table (3) cleared that the mortality % for *S. littoralis* instars larvae were (100.0 & 6.0), (98.0 & 6.00), (96.0 & 4.0), (100.0 & 8.0), (100.0 & 2.0) and (100.0 & 0.0 %), for 1st, 2nd, 3rd, 4th, 5th and 6th instar larvae when the larvae fed on (*Bt* cotton & non *Bt*), respectively.

Generally, data in Table (4) indicate the mean *S. littoralis* larval mortality % for the three Egyptian cotton varieties (*Bt* cotton & non *Bt*) were (97.7 & 9.0 %) for Giza 80, (97.7 & 5.7%) for Giza 90 and (99.0 & 4.3 %) for Giza 89, respectively.

Table (1): Accumulated larval mortality and pupation % for *S. littoralis* instars fed on Giza 80 (*Bt* cotton and non *Bt*).

Instars	Varieties	Accumulated larval mortality% after																	Pupation %	
		1 Day	3	5	7	9	11	13	15	17	19	21	23	25	27	29	31	33		35 Days
1 st	<i>Bt</i> Cotton	42	92	100																0.0
	Non <i>Bt</i>	0	0	2	2	4	4	4	4	4	4	6	6	6	6	6	6	6		94.0
2 nd	<i>Bt</i> Cotton	2	2	2	2	2	4	6	6	6	16	30	48	62	68	74	76	90	94	6.0
	Non <i>Bt</i>	0	4	4	4	4	4	4	6	6	8									92.0
3 rd	<i>Bt</i> Cotton	0	6	6	10	14	26	32	40	40	54	56	62	72	76	78	82	82	92	8.0
	Non <i>Bt</i>	2	6	8	8	10	10	10	12											88.0
4 th	<i>Bt</i> Cotton	0	12	12	16	30	44	66	70	84	92	96	100							0.0
	Non <i>Bt</i>	2	4	4	4	6	6	6	8											92.0
5 th	<i>Bt</i> Cotton	26	42	54	56	90	98	100												0.0
	Non <i>Bt</i>	0	6	10																90.0
6 th	<i>Bt</i> Cotton	0	0	12	62	84	94	98	100											0.0
	Non <i>Bt</i>	2	6	8	10															90.0

Table (2): Accumulated larval mortality and pupation % for *S. littoralis* instars fed on Giza 90 (*Bt* cotton and non *Bt*).

Instars	Varieties	Accumulated larval mortality% after																	Pupation %			
		1 Day	3	5	7	9	11	13	15	17	19	21	23	25	27	29	31	33		35	37 Days	
1 st	<i>Bt</i> Cotton	32	76	88	92	100															0.0	
	Non <i>Bt</i>	0	6	8	8	8	8	8	8	8	8	8	8	8	8							92.0
2 nd	<i>Bt</i> Cotton	36	58	68	70	72	72	72	74	74	74	76	80	82	86	86	86	90	94	96		4.0
	Non <i>Bt</i>	0	0	0	4	4	4	4	4	4	4	4										96.0
3 th	<i>Bt</i> Cotton	0	0	4	8	12	14	20	32	52	58	68	76	80	88	96	100					0.0
	Non <i>Bt</i>	0	0	0	0	0	0	0	2	4	4											96.0
4 th	<i>Bt</i> Cotton	2	8	24	40	46	54	66	72	80	86	88	92									8.0
	Non <i>Bt</i>	0	0	2	4	6	6	8	8	10												90.0
5 th	<i>Bt</i> Cotton	58	66	68	68	82	86	96	100													0.0
	Non <i>Bt</i>	0	2	4	6	8	8	8														92.0
6 th	<i>Bt</i> Cotton	2	2	4	28	38	66	80	86	98												2.0
	Non <i>Bt</i>	0	0	0	0	0																100.0

Table (3): Accumulated larval mortality and pupation % for *S. littoralis* instars fed Giza 89 (*Bt* cotton and non *Bt*).

Instars	Varieties	Accumulated larval mortality% after																	Pupation %			
		1 Day	3	5	7	9	11	13	15	17	19	21	23	25	27	29	31	33		35	37	39 Days
1 st	<i>Bt</i> Cotton	80	100																			0.0
	Non <i>Bt</i>	0	0	0	0	2	2	4	4	4	4	6	6	6	6	6	6					94.0
2 nd	<i>Bt</i> Cotton	0	0	4	8	8	12	16	18	24	34	48	58	60	62	64	64	70	76	88	98	2.0
	Non <i>Bt</i>	0	0	0	0	0	2	2	4	4	4	4	4	4	6							94.0
3 th	<i>Bt</i> Cotton	0	8	8	24	24	32	36	42	46	46	50	50	64	78	88	94	96				4.0
	Non <i>Bt</i>	0	0	0	2	2	2	2	4													96.0
4 th	<i>Bt</i> Cotton	0	0	2	10	12	18	46	70	74	80	86	88	100								0.0
	Non <i>Bt</i>	0	2	8	8	8	8	8	8													92.0
5 th	<i>Bt</i> Cotton	49	58	64	70	70	76	88	100													0.0
	Non <i>Bt</i>	0	0	0	2	2	2															98.0
6 th	<i>Bt</i> Cotton	14	32	32	44	62	78	100														0.0
	Non <i>Bt</i>	0	0	0	0	0																100.0

Table (4): Larval mortality % of *S. littoralis* instars when feed on Giza 80, Giza 90 and Giza 89 for both *Bt* cotton and Non *Bt*.

Instars	Varieties	Larval mortality%			
		Giza 80	Giza 90	Giza 89	Mean
1 st	<i>Bt</i> Cotton	100	100	100	100
	Non <i>Bt</i>	6	8	6	6.7
2 nd	<i>Bt</i> Cotton	94	96	98	96.0
	Non <i>Bt</i>	8	4	6	6.0
3 th	<i>Bt</i> Cotton	92	100	96	96.0
	Non <i>Bt</i>	12	4	4	6.7
4 th	<i>Bt</i> Cotton	100	92	100	97.3
	Non <i>Bt</i>	8	10	8	8.7
5 th	<i>Bt</i> Cotton	100	100	100	100
	Non <i>Bt</i>	10	8	2	6.7
6 th	<i>Bt</i> Cotton	100	98	100	99.3
	Non <i>Bt</i>	10	0	0	3.3
Total larval stage	<i>Bt</i> Cotton	97.7	97.7	99.0	98.1
	Non <i>Bt</i>	9.0	5.7	4.3	6.3

Corrected mortality:

The mortality % was corrected by **About Formula** (as shown in Table (5)). The mean corrected

mortality % for *S. littoralis* larvae after fed on the three Egyptian *Bt* cotton varieties Giza 80, Giza 90 and Giza 89 were 97.5 , 97.6 and 99.0 %, respectively.

Table (5): Corrected mortality % of *S. littoralis* instars when feed on Giza80, Giza 90 and Giza 89 for both *Bt* cotton and Non *Bt*.

Instars	Corrected mortality%			
	Giza 80	Giza 90	Giza 89	Mean
1 st	100	100	100	100
2 nd	93.4	95.8	97.9	95.7
3 th	93.2	100	95.8	96.3
4 th	100	91.1	100	97.0
5 th	100	100	100	100
6 th	100	98	100	99.3
Total larval stage	97.5	97.6	99.0	98.0

Effect of *Bt* toxins on pupal stage:

Data in Table (6) indicate to the effect of *Bt* cotton varieties on *S. littoralis* pupal stage. There are a decrease in *S. littoralis* pupal weight values for pupae resulted from larvae feed on *Bt* cotton varieties compare with the pupae resulted from larvae feed on non *Bt* varieties. The pupal weight for *Bt* cotton & non *Bt* was 0.1668 & 0.2678 gm for Giza 80, 0.1594 & 0.2716 gm for Giza 90 and 0.1374 & 0.2492 gm for

Giza 89, respectively. The same table also, shows the variation between pupal mortality percent for pupae which resulted from larvae feed on *Bt* cotton and non *Bt* varieties. On the other hand, there are decreases in *S. littoralis* moths emergence percent as latent effect of *Bt* toxins on pupal stage. The emergence percent for *Bt* cotton & non *Bt* was 78.6 & 94.9 % (for Giza 80), 61.4 & 94.7 % (for Giza 90) and 77.8 & 86.0 % (for Giza 89), respectively.

Table (6): The biological efficacy of *Bt* cotton and non *Bt* against *S. littoralis* pupal stag

Varieties	Pupal weight (gm)		Pupal duration (days)		Pupal mortality %		Emergence %	
	<i>Bt</i> cotton	Non <i>Bt</i>	<i>Bt</i> cotton	Non <i>Bt</i>	<i>Bt</i> cotton	Non <i>Bt</i>	<i>Bt</i> cotton	Non <i>Bt</i>
Giza 80	0.1686	0.2678	9.39	8.38	21.4	5.1	78.6	94.9
Giza 90	0.1594	0.2716	8.77	8.48	38.6	5.3	61.4	94.7
Giza 89	0.1374	0.2492	10.4	9.3	22.2	14.0	77.8	86.0

Effect of *Bt* toxins on adult stage:

The main effect of *Bt* toxin on adult stage for *S. littoralis* concentrated on malformation percent for male and female moths as shown in Table (7). There are increases in *S. littoralis* malformation moths which resulted from larvae feed on *Bt* cotton as latent effect of *Bt* toxins on adult stage. The malformation percent

were (27.3 & 4.3 %), (37.1 & 5.6 %) and (28.6 & 8.4 %) for Giza 80, Giza 90 and Giza 89 (*Bt* & non *Bt* varieties), respectively.

Data in Table (8) show that the female longevity was affected by *Bt* toxins as latent effect for the three *Bt* cotton varieties.

Table (7): The biological efficacy of *Bt* cotton and non *Bt* against *S. littoralis* adult stag

Varieties	Sex ratio				Malformed adult %	
	<i>Bt</i> cotton		Non <i>Bt</i>		<i>Bt</i> cotton	Non <i>Bt</i>
	♂	♀	♂	♀		
Giza 80	0.46	0.54	0.27	0.73	27.3	4.3
Giza 90	0.57	0.43	0.32	0.68	37.1	5.6
Giza 89	0.59	0.41	0.49	0.51	28.6	8.4

Table (8): The biological efficacy of *Bt* cotton and non *Bt* against *S. littoralis* Female longevity

Varieties	Fecundity (Number of egg / ♀)		Fertility %	
	<i>Bt</i> cotton	Non <i>Bt</i>	<i>Bt</i> cotton	Non <i>Bt</i>
	Giza 80	257.5	726.3	68.5
Giza 90	440.0	585.3	78.7	92.0
Giza 89	317.0	491.7	71.0	94.0

The changes in fecundity and fertility parameters for *S. littoralis* male and female are important parameters which resulted from exposure all stages of *S. littoralis* to release the *Bt* toxins from Cry 1Ac and Cry 2Ab which transfer to the three Egyptian *Bt* cotton varieties (Giza 80, Giza 90 and Giza 89).

Data in Table (9) indicated that the fecundity (no. of eggs/female) for *Bt* cotton & non *Bt* were (257.5 & 726.3) for Giza 80, (440.0 & 585.3) for Giza 90 and (317.0 & 491.7 eggs) for Giza 89, respectively.

Table (9): The biological efficacy of *Bt* cotton and non *Bt* against *S. littoralis* Fecundity and Fertility

Varieties	Female longevity (days)							
	<i>Bt</i> cotton				Non <i>Bt</i>			
	Pre-Ovi-	Ovi-	Post	Total	Pre-Ovi-	Ovi-	Post	Total
Giza 80	2.5	4.5	4.5	11.5	2.3	5.0	3.0	10.3
Giza 90	2.0	7.0	2.7	11.7	2.0	5.7	0.33	8.03
Giza 89	2.0	5.0	2.0	9.0	1.7	4.0	0.5	8.2

Effect of *Bt* toxin on egg stage:

Egg fertility resulted from moths of *S. littoralis* which their larvae fed on *Bt* cotton varieties were less than another one which their larvae fed on non *Bt* varieties. The fertility percent as indicate in Table (9) were 65.5 and 97.0 % for Giza 80 *Bt* cotton and non *Bt*, 78.7 and 92 % for Giza 90 *Bt* cotton and non *Bt* and 71.0 and 94.0 % for Giza 89 *Bt* cotton and non *Bt*.

4. Conclusion and 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. In addition, Egyptian cotton leafworm, *S. littoralis* is one of the most important pests in cotton fields (Hosney *et al.* 1986). Among the alternatives for controlling this pest, 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). Moreover, laboratory and field evaluations of *Bt* transgenic soybean for control of Lepidopteran pests was confirmed (Macrae *et al.* 2005). 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 *S. littoralis*. Our findings confirm that the transgenic cotton containing a Cry 1Ac and Cry 2Ab genes have significantly more efficacy against *S. littoralis* than the conventional cotton, for all things measured. The effect of Egyptian *Bt* cotton varieties on growth, development and metamorphosis of *S. littoralis* 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, *Helicoverpa 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 Vojtech *et al.* 2005, in a laboratory study on effect *Bt* toxins against *S. littoralis* they reported that the *S. littoralis* larvae when reared for their whole lifespan on a mixture of leaves and stems from 2–4week old *Bt* maize plants, the *S. littoralis* survival, developmental times and larval weights were significantly affected by *Bt* maize. Also, Dutton *et al.* 2005, reported that the insects fed on either transgenic or *Bt* sprayed plants were negatively affected. Young *S. littoralis* larvae (1st and 2nd instars) were found to be the most sensitive to the *Bt* toxin. This was represented by a higher mortality and a slower developmental time of larvae maintained on transgenic or sprayed plants when compared to insects maintained on control plants.

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