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Full Length Research Paper

Biotechnological control of cotton leafworm via intoxication with recombinants of *Bacillus thuringiensis*

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In this study twelve *Bacillus* strains and 20 transconjugants resulted from nine matings between strains that having the opposite genetic markers were used in this work. This study aimed to evaluate accumulative mortality, as well as, body weight yielding of surviving larvae, after intoxication with *Bt* recombinants. The results appeared that recombinant crystals from transconjugants number Tr-B and Tr-D were more toxic if compared with other Bt toxins used in this study. This is because they were appeared in the lower LT_{50} which reached to 4.09 and 5.30 days, respectively. In addition, recombinant crystals + endospores derived from transconjugants; Tr- B and Tr- D were more toxic if compared with other Bt, this because they appeared the lower values of LT_{50} than other isolates of Bt. The LT_{50} of Tr-B and Tr-D were 2.87 and 2.44 day, respectively. The results indicated that mortality percentage increased with increasing feed period but decreased with increasing larval age. The yield in body weight of surviving larvae treated with Bt. increased above that in untreated larvae. In addition, the yield in mortality percentage increased above that in control experiment. Although, yield in mortality percentage was increased when the larvae was treated with crystals + endospores than that treated with crystals alone. The results indicated that wider application of this technology will necessitate the testing of a broad range of insects encompassing agriculturally important pests and also beneficial insects to ascertain the full efficacy and safety of these recombinant proteins. This article sets the stage for developing an alternative strategy via using transgenic *Bt* in controlling important insect pests in a sustainable manner. The results demonstrated that *B. thuringiensis* - induced accumulative mortality depending on the components of bioinsecticides.

Key words: Accumulative mortality percentage, *Bacillus thuringiensis*, body weight, LT_{50}, *Spodoptera littoralis*, toxicity curves.

INTRODUCTION

Biopesticides need not be reserved for organic crop production. Biopesticides can be particularly effective when incorporated into integrated pest and disease management programs and when used in conjunction with conventional pesticides. Biopesticide products typically have no pre - harvest intervals and very short reentry intervals, which permits a crop to be harvested immediately after the biopesticide is sprayed. This is particularly important in export crops that are shipped globally and are subject to international maximum residue levels. Biopesticides can be used as a management tool in rotations with other products to reduce pesticide resistance. Biopesticides contain multiple modes of action which makes them well - suited for rotation in pest management programs to help prolong the efficacy of conventional pesticides. *Bt* is a naturally occurring bacterium, that is found throughout most regions of the world. It occurs naturally in soil and in other common environmental habitats where insects are found. It has pesticidal properties when consumed by the larvae of specific insects. The primary toxic component of *Bt* is a crystalline protein (toxin). *Bt* must be eaten by the insect larvae to cause mortality. *Bt* has no effect on adult insects [Clifford et al., 2002; Gregory et al., 1999].

B. thuringiensis "Bt", first discovered in Japan in 1901, refers to a spore - forming bacterium, *B. thuringiensis*. Some leaf-feeding caterpillars (larvae of butterflies and moths) are killed when they eat very small amounts of leaves or other plant parts that have been coated (sprayed) with *Bt.* Thousands of strains of *Bt* bacteria

have been used to manufacture microbial or biological insecticides. Most of these commercial products contain crystal-shaped proteins and living spores from *Bt* bacteria. There are many commercial brands of *Bt* ; two *Bt* subspecies are commonly used against caterpillars which feed on cabbage and other vegetable crops [Gregory et al., 2006; Jerald et al., 2006].

B. thuringiensis is a species of bacteria that has insecticidal properties affecting a selective range of insect orders. There are at least 34 subspecies of *Bt* [Feitelson et al., 1992] (also called serotypes or varieties) and probably over 800 strain isolates. *Bt* was later isolated from Mediterranean flour moths and named *B. thuringiensis* in 1911. It was not until 1958 that *Bt* was used commercially in the United States. By 1989, *Bt* products had captured 90 to 95% of the biopesticide market [Feitelson et al., 1992]. *Bt* products are used to control moth pests in fruits, vegetables, and beehives; black fly and mosquito pests in ponds and lakes; and several beetle pests in vegetables and shade trees. Common brand names include Dipel, Foray, Thuricide (all *Bt* kurstaki), Vectobac, Mosquito Attack (all *Bt* israelensis), and M - Trak (*Bt* tenebrionis) [Farm Chemicals Handbook, 1992; Gregory et al., 2004].

B. thuringiensis must be eaten by lepidopteran larvae (caterpillars) to be effective. *Bt* is a great material for leaf roller control because it is specific and has little effect on natural enemies. However, it must be applied 2 to 3 times to be effective when leafroller populations are high. Experience has also shown that in the spring, the high temperatures need to be above 65°F for 3 or more days so that larvae have a chance to feed on it before sunlight breaks it down. *B. thuringiensis* (*Bt*) is an insect pathogen that produces one or more toxins that are utilized as bacterial insecticides. *Bt* is effective against lepidopteran larvae such as leaf rollers, peach twig borer and cutworms. *Bt* is not a contact insecticide and must be consumed by the larvae to be effective. When ingested, the *Bt* toxins are activated and cause holes in the insect's gut membrane. Gut bacteria then get into the insect's blood stream and poison it. Once it has consumed a toxic dose, the larvae stops feeding but may remain alive for several days. *Bt* is most effective against young larvae, as it takes a smaller dose to kill them than it takes for more mature larvae. *Bt* has a short effective life of 3 to 7 days. It breaks down in sunlight and high temperatures and must be applied more frequently than traditional insecticides to achieve adequate control [James et al., 2011; Jose, 1992].

B. thuringiensis subspecies are differentiated by their insecticidal activity. Generally, only insect species within an order are susceptible to a given insecticidal *Bt* δ endotoxin, also referred to as a Cry (crystal) protein. The toxicity of *Bt* proteins expressed by transgenic corn to larval stages of butterflies and moths is well known [Peacock et al., 1998; Johnson et al., 1995]. Field data from these studies indicated a temporary reduction in lepidopteran populations during periods of prolonged *Bt* use, although widespread irreversible harm has not been reported [Hall et al., 1999]. Based on such information, the United States Environmental Protection Agency (EPA) made the assumption that lepidopteran - active *B. thuringiensis* insecticides are likely to be hazardous to all lepidoptera, although exposure from agricultural uses was not expected to be as high as in forest spraying. In the initial assessments of transgenic corn, the EPA predicted that the impact of *Bt* corn pollen on non target butterflies and moths would be minimal because of low exposure [U.S. Environmental Protection Agency, 1995; Jerald et al., 2001].

There are different strains of *Bt*, each with specific toxicity to particular types of insects: *Bt* aizawai (*Bt a*) is used against wax moth larvae in honeycombs; *Bt israelensis* (*Bt i*) is effective against mosquitoes, black flies and some midges, *Bt kurstaki* (*Bt k*) controls various types of lepidopterous insects, including the gypsy moth and cabbage looper. To be effective, *Bt* must be eaten by insects during their feeding stage of development, when they are larvae. More than 150 insects, mostly lepidopterous larvae, are known to be susceptible in some way to *B. thuringiensis* forms asexual reproductive cells, called spores, which enable it to survive in adverse conditions. During the process of spore formation, *Bt* also produces unique crystalline bodies. When eaten, the spores and crystals of *Bt* act as poisons in the target insects. *Bt*. is therefore referred to as a stomach poison. *Bt* crystals dissolve in the intestine of susceptible insect larvae. They paralyze the cells in the gut, interfering with normal digestion and triggering the insect to stop feeding on host plants. *Bt* spores can then invade other insect tissue, multiplying in the insect's blood, until the insect dies. Death can occur within a few hours to a few weeks of *Bt* application, depending on the insect species and the amount of *Bt* ingested. Typical agricultural formulations include wet table powders, spray concentrates, liquid concentrates, dusts, baits, and time release rings [Anderson et al., 2004; Jerald et al., 2003; Mark et al., 2004].

Decreased larval feeding and weight of the monarch butterfly *Danaus plexippus* L., have been detected after 4 days of exposure in the laboratory to a high density of *B. thuringiensis* (*Bt*) - expressing anthers. One hypothesis is that larvae exposed to *Bt* anthers exhibit increased wandering, resulting in less feeding and lower weight gain. To test this hypothesis 2-d-old monarch butterfly larvae exposed to milkweed leaf disks with no anthers, anthers that express *Bt* (Cry1Ab, event MON810), or other non - *Bt* anthers were observed using a video-tracking system. As had been shown in previous studies, larvae exposed to *Bt* anthers fed less and gained less weight than larvae exposed to non-Bt or no anthers, yet there was no evidence of feeding on anthers. Total distance moved, maximum displacement from release point, percentage of time spent moving or near anthers,

Table 1. Bacterial strains used in this study.

Strains	Source of reference	Designation
*B. thuringiensis*NRRL - HD18	NCFAUR	BT-18
B. thuringiensis NRRL - HD64	NCFAUR	BT-64
*B. thuringiensis*NRRL - HD73	NCFAUR	BT-73
B. thuringiensis NRRL - HD110	NCFAUR	BT-110
B. thuringiensis NRRL - HD147	NCFAUR	BT-147
*B. thuringiensis*NRRL - HD153	NCFAUR	BT-153
*B. thuringiensis*NRRL - HD537	NCFAUR	BT-537
*B. thuringiensis*serovar *Kurstaki* NRRL HD - 1	NCFAUR	BT – 1
B. subtilis NRRL NRS -744	NCFAUR	BS- 744
B. licheniformis NRRL B - 571	NCFAUR	BL-571
B. licheniformis NRRL B - 1584	NCFAUR	BL-1584
B. licheniformis NRRL B - 358	NCFAUR	BL-358

NCFAUR = National Center for Agriculture Utilization Research, USA.

Table 2. Antibiotics and their abbreviations.

Antibiotics	Designation
Chloramphenicol	*Cm*
Streptomycin	*Str*
Tetracycline	*Tc*
Neomycinsulphate	*Nm*
Ampicillin	*Ap*
Erythromycin	*Erth*
Amoxycillin and flucloxacillin	*AmFluc*
Rifampicillin	*Rf*

Table 3. Heavy metals and their abbreviations.

Heavy metals	Designation
$ZnSO_4.7H_2O$	Zn
Pb SO4	Pb
CdSO4	Cd
$CoSO4.\ 7H_2O$	Co
HgCL	Hg
$K_2Cr_2O_7$	Cr

or mean turn angle did not differ across treatments. However, larvae exposed to *Bt* anthers spent more time off milkweed leaf disks than those exposed to no anthers and were more likely to move off the leaf than larvae exposed to non - *Bt* anthers. Results suggest that larvae exposed to *Bt* anthers behave differently and that ingestion may not be the only way Bt can affect nontarget insects like the monarch butterfly" [Prasifka et al., 2007; Martin et al., 1998].

Furthermore, Bt Cry3A had no detrimental effects on reproductive fitness of either beetle species, either in terms of fecundity or subsequent egg viability. Behavioral analysis revealed no significant impact of *Bt* Cry3A on beetle activity or locomoter behavior. Ligand blots indicated that this is due to either the absence of *Bt* - binding sites in brush border membrane vesicles (BBMV) isolated from *Nebria brevicollis*, or in the case of *Harmonia axyridis*, the binding did not functionally lead to behavioral or physical effects" [Ferry et al., 2007; Rakesh, 2009].

The present study was undertaken to focus on a biotechnology technique to evaluate weight reduction in body weight of surviving larvae in relation to untreated larvae which expressed as yield percentage, LT_{50} and mortality percentage in response to sprayed with recombinant preparations of *B. thuringiensis* and their recombinants.

MATERIALS AND METHODS

Bacterial strains

Thirteen bacterial strains listed in Table 1 were used in this study. These strains were genetically marking using eight antibiotics presented in Table 2, as well as, six heavy metals presented in Table 3 and crystal violet (*rfa* mutation).

Host plants

Fresh leaves of *Ricinus communis* were collected daily; squares and middle leaves were used for the experiments. Leaves were cleaned and three grams were weighted and placed in each container daily.

B. thuringiensis formulations used in the experiment

Two *B. thuringiensis* preparations were used; crystals + endospores, crystals, in liquid formulations using 200 µl of the suspension. The bio – insecticide were applied on 250 ml bottles as well as mixed with 3 g of leaves as diet for larvae.

Media

B. thuringiensis strains were grown on T_3 medium (L^{-1}: 3 and 2 g tryptose, 1.5 g yeast extract, 0.05 M sodium phosphate, pH 6.8 and 0.005 g $MnCl_2$, according to Ashfaq et al. [2001], until sporulation was complete. All strains were grown on L agar (per liter 10 g tryptone, 5 g yeast extract, 5 g NaCl and 15 g agar, according to Ashfaq et al. [2001] at 30°C until they sporulated. Strains were maintained on LB Slape medium consists of; 1% tryptone, 0.5% yeast extract and 0.5% NaCl, pH 7.5.

Antibiotic susceptibility assays

Heavy metals and antibiotics susceptibility was measured by plate diffusion method, according to Collins and Lyne [1985] with cultures grown to logarithmic growth phase in nutrient broth of LB medium. They are used with the concentration of 100 µg/ml. Bacterial suspension (0.2 ml) was mixed with 10 ml of LB agar medium in petri dishes. Wells (8 mm diameter) were punched in the agar, using a stainless steel borer, and were filled with 0.1 ml of the antibiotic concentration. The plates were incubated overnight at 37°C and the diameter of resulting zones of inhibition was measured, three replicates were used for each bacterial strain, and concentration of antibiotics used [Toda et al., 1989]. Different antibiotics were used with the concentration of 400 µg/ml, according to Roth and Sonti [1989]. The results of this experiment were recorded as (+) for resistant and (-) for sensitive strain. These markers were used in conjugation experiment between donor and recipient strains that having the opposite genetic markers.

rfa mutation

Strains having the deep rough (*rfa*) character should be tested for crystal violet sensitivity according to Ames et al. [1973]. For the test, nutrient agar plates are seeded with cultures of the strains to be tested and a sterile filter paper disc containing crystal violet is placed on the surface of each seeded plate by pipette 10 µl of a 1 mg / ml solution of crystal violet to the center of sterile filter paper discs (1/4 inch). Invert the plate and incubate at 37°C. After 12 h incubation, a clear zone of inhibition (approximately 14 mm) appears around the disc indicating the presence of the *rfa* mutation which permits large molecules such as crystal violet to enter and kill the bacteria. Wild - type strains or strains containing the *gal* deletion are not inhibited because the crystal violet cannot penetrate the cell.

Table 4. Matings between *Bacillus* strains that having the opposite genetic markers.

Mating	Relevant genotype
BL-571 × BT-147	RF^- Cd^+ X RF^+ Cd^-
BL-1584 × BT-147	RF^- Cd^+ X RF^+ Cd^-
BL-358 × BT-18	RF^+ $AmFluc^-$ X RF^- $AmFluc^+$
BL-358 × BT-64	Ap^- RF^+ Tc^- X Ap^+ RF^- Tc^+
BL-358 × BT-73	Ap^- RF^+ Tc^- X Ap^+ RF^- Tc^+
BL-358 × BT-110	Ap^- RF^+ Tc^- X Ap^+ RF^- Tc^+
BL-358 × BT-153	Ap^- Tc^+ X Ap^+ Tc^-
BL-358 × BT-537	Ap^- RF^+ Tc^- X Ap^+ RF^- Tc^+
Bt -1 × BS -744	$Hico^+$ rfa^- X $Hico^-$ rfa^+

Mating in liquid broth

Donor and recipient cells were grown in Luria Bertani broth – LB at 30°C [Sambrook et al., 1989], with aeration to logarithmic growth phase ($107cfu.ml^{-1}$). Enumeration of donor and recipients strains was done by plating on LB agar with appropriate antibiotic and heavy metals appeared as the opposite genetic markers between both mating strains. This was done by combined equal volumes of donor and recipient cells (250 ml) in 7 ml of prewarmed LB broth, and incubated at 30°C [Andrup et al., 1993]. After 2 h, 100 ml of the mixture was inoculated onto LB agar plates containing the same concentration of antibiotic markers. Single colonies appeared on selective medium were picked up and grown on Luria Bertani slant agar [Furlaneto et al., 2000]. Matings between *Bacillus* strains that having the opposite genetic markers were shown in Table 4. These matings were done according to Battisti et al. [1985] and Klier et al. [1983].

Separation of crystals and endospores

Bacteria were grown in petri dishes or in suspension cultures. The spores were collected from nutrient agar washed three times in ice-cold distilled water. Pellets (spores and crystals) were resuspended in small volumes of distilled water. The bacterial suspension cultures were prepared as follows: Loopfuls from bacterial colonies with spores and crystals were transferred to 1 ml of distilled water. Heat-shocked (70°C for 30 min.) suspensions were transferred to 250 ml of PWYE medium (5% peptone, 0.1% yeast extract, 0.5% NaCl, pH 7.5) and incubated at 30°C for 8 to 15 h with shaking at 180 rpm.

Two milliliters of the PWYE culture was used to inoculate 1 liter of CCY minimal sporulation medium (5 g peptone, 1.5 g yeast extract, 0.05 M sodium phosphate (pH 6.8), 0.005 g of $MnCl_2$ per liter) and was incubated at 30°C for 3 to 4 days with shaking at 180 rpm; at least 90% of bacterial cells were lysed releasing spores and crystals after this incubation. Spores and crystals were collected by centrifugation (10000 × g for 10 min). Pellets were washed three times with ice-cold distilled waters and final pellets were resuspended in 20 ml of water and stored at -5°C. To purify crystals from spores and cellular debris, samples were sonicated and centrifuged on discontinuous sucrose density gradients (67 to 72 to 79% [wt/vol] sucrose) at 15000 ×g for 2 h. Crystal bands and spore pellets were purified by three centrifugations and washed with distilled water. Final pellets were resuspended in small volumes of distilled water and stored at -5°C [Karamanlidou et al., 1991] .

Insects

Eggs of *Spodoptera litura* were collected from cotton fields during the summer season. Bioassay technique was assayed against 4 days - old larvae according to Dulmage [1971].

Bioassay of toxicity

The toxicity was bioassayed with *Spodoptera littoralis* second instar larvae (mean body weight = 10 mg) according to Klanfon and DeBarjac [1985] with some modifications. Bacterial cell component of *B. thuringiensis* was approximately 10^9 crystals and/or spores per milliliter were used with the dilution of 1:1. Larvae of *S. littoralis* were exposed to the appropriate dose of the component of *B. thuringiensis* using a Gentaur micropipette to dispense 200 µl of the suspension on 2 to 3 g of diet surface of *R. communis* [Ignoffo et al., 1968]. Then this drop was evenly distributed over the diet surface with a sterile glass rod, and the surface was air-dried. Mortality was recorded daily after 24 h for 6 to 7 days. Surviving larvae from each replicate were pooled and weighted daily [Inagaki et al., 1992].

Mortality

Mortality percentages were corrected by Abbott's formula [Abbott, 1925]. Results were illustrated graphically as log / probit regression lines, and LT_{50} values as well as the slope were obtained according to Finney [1971]. Abbott's formula was as follows;

$$\% \text{ Mortality} = \frac{\text{Control survival - Treatment survival}}{\text{Control survival}} \times 100$$

Body weight of surviving larvae

Weight of surviving larvae (g) = Total weight of all of surviving larvae per bottle / Number of survivors per bottle.

RESULTS AND DISCUSSION

B. thuringiensis is a unique bacterium in that it shares a common place with a number of chemical compounds which are used commercially to control insects important to agriculture and public health. Importantly, *Bt* is safe for humans and is the most widely used environmentally compatible biopesticide worldwide. Furthermore, insecticidal *Bt* genes have been incorporated into several major crops, rendering them insect resistant, and thus providing a model for genetic engineering in agriculture. This study highlights our understanding of what makes Bt distinctive in the microbial world and biological control of insects.

Lethal time LT_{50}

Data shown in Table 5 and Figures 1 to 8 appeared medium lethal time in response to crystal and endospore obtained from parental strains and their transconjugants against cotton leafworm, *S. littoralis* larvae. Transconjugants number T-3 and T-9 were more toxic if compared with other *Bt* toxins used in this Table. This is because they appeared in the lower LT_{50} than other *Bt* isolates, they reached 2.8 and 2.6 days, respectively. Also, slope values were 3.3 and 2.1 for T-3 and T-9, respectively, which proved the homogeneity of the tested larvae. The results indicated that accumulative mortality was increased with the larval development period Note about the number of lines in the aforementioned Figure: Figure 1 to 8 relates to conjugation between L571 × T 147; 1 = P2, 2 = T2, 3 = T1, 4 = P1, T = recombinant transconjugant.

The results obtained in this study agreed with Sneh et al. [1981], who found that among more than 50 isolates of *B. thuringiensis Berliner* (*Bt*) tested, 7 incited 100% mortality when 2nd instar larvae of *S. littoralis* Boisduval were fed on alfalfa leaves dipped in a spore-crystal suspension of 10^8 colony forming units/ml. Larvae of instars 1 and 2 were the most susceptible to *Bt* susceptibility decreased with larval development. Their weight relative to the controls was lower as the spore concentration on the leaves on which they fed was higher. Those data are important for the determination of spore concentrations in suspensions required for spraying.

Meanwhile, Gore et al. [2003] studied the larval developmental times, pupal weights and survival of *Helicoverpa zea* (Boddie). They found that on *Bt* cotton,

Table 5. Medium lethal time of bioinsecticides containing crystals and endospores against cotton leafworm, *S. littoralis*.

Bioinsecticides		Accumulative mortality % after feeding periods (day)										LT_{50}	Confidence limit		Slope
		3	5	6	7	8	9	10	11	12	13		Lower	Upper	
L571 × T 147	P1	30	30	51	72	86	93	96	97	98	99	5.0	3.9	5.7	4.5 ± 0.3
	P2	53	53	62	71	83	88	90	91	93	94	3.7	2.7	4.2	2.6 ± 0.2
	T1	40	52	62	76	88	95	96	97	98	99	4.2	3.4	4.7	3.8 ± 0.3
	T2	36	55	82	94	98	100	100	100	100	100	3.9	2.7	4.4	4.7 ± 0.4
L1584 × T147	P1	40	40	46	50	55	64	71	74	79	83	5.7	4.5	6.6	2.0 ± 0.2
	P2	53	53	62	71	83	88	90	91	93	94	3.7	2.7	4.2	2.6 ± 0.2
	T3	60	72	83	91	96	99	100	100	100	100	2.8	2.3	3.2	3.3 ± 0.4
	T4	30	37	43	56	69	81	89	92	95	97	5.5	4.5	6.1	3.9 ± 0.3
L358 × T18	P1	36	36	49	61	77	84	89	91	93	95	5.1	4.1	5.7	3.5 ± 0.3
	P2	40	70	88	95	98	99	100	100	100	100	3.5	3.2	3.8	5.1 ± 0.4
	T5	30	44	51	77	88	95	97	98	99	100	4.7	3.9	5.3	4.7 ± 0.3
	T6	33	33	40	46	73	84	90	95	97	98	5.5	4.1	6.3	3.9 ± 0.3
L358 × T64	P1	36	36	49	61	77	84	89	91	93	95	5.1	4.1	5.7	3.5 ± 0.3
	P2	43	54	72	86	93	97	98	99	100	100	3.7	3.0	4.3	4.2 ± 0.3
	T7	43	54	63	81	92	97	99	100	100	100	3.9	2.4	4.4	3.5 ± 0.3
	T8	36	42	59	74	84	89	92	94	95	96	4.5	3.6	5.1	3.4 ± 0.3
L358 × T73	P1	36	36	49	61	77	84	89	91	93	95	5.1	4.1	5.7	3.5 ± 0.3
	P2	1	10	28	52	76	86	92	94	96	97	6.9	6.7	7.1	7.9 ± 0.4
	T9	63	63	74	81	89	92	94	95	96	97	2.6	1.3	2.9	2.1 ± 0.3
	T10	16	41	59	75	87	95	97	98	99	100	5.1	4.8	5.4	5.4 ± 0.3
L358 × T110	P1	36	36	49	61	77	84	89	91	93	95	5.1	4.1	5.7	3.5 ± 0.3
	P2	30	44	61	80	92	98	99	100	100	100	4.6	3.5	5.2	4.9 ± 0.4
	T11	53	72	86	94	98	100	100	100	100	100	3.7	3.4	4.0	5.4 ± 0.5
	Tr12	33	53	72	87	96	98	100	100	100	100	4.1	2.7	4.7	4.1 ± 0.4
L358 × T153	P1	36	36	49	61	77	84	89	91	93	95	5.1	4.1	5.7	3.5 ± 0.3
	P2	30	37	50	65	82	91	94	96	97	98	5.0	4.2	5.6	4.1 ± 0.3
	T13	40	52	66	84	94	98	99	100	100	100	4.1	2.8	4.6	4.3 ± 0.4
	T14	33	53	67	80	92	96	97	99	100	100	4.3	3.2	4.8	4.0 ± 0.3
L358 × T537	P1	36	36	49	61	77	84	89	91	93	95	5.1	4.1	5.7	3.5 ± 0.3
	P2	16	33	46	71	88	94	96	97	98	99	5.4	4.8	5.9	5.6 ± 0.3
	T15	36	49	75	88	98	100	100	100	100	100	4.6	4.4	4.9	6.2 ± 0.5
	T16	46	62	77	91	97	99	100	100	100	100	3.6	2.3	4.0	4.3 ± 0.4

larvae from the corn colony had a higher level of mortality than larvae from the soybean and grain sorghum colonies.

However, Abd El-Salam et al. [2011] evaluated the biological activities of two species of bacteria isolated from soil of cotton fields identified as *Bacillus subtilis* strain NRC313 (BS NRC313) and *B. thuringiensis* strain NRC335 (BT NRC335) against the third larval instar of the cotton leafworm, *S. littoralis* (Boisd). The different entomopathogenic bacteria of BS NRC313 and BT NRC335 contained 10×10^8 cell / ml caused mortality of 100 and 97.3% for the aforementioned strains, respectively. LC50 were 3.3×10^8 and 3.9×10^8 cell / ml, respectively. The percentage of larvae that survived and succeeded to pupate increased by decreasing the concentration. These results indicated that *B. subtilis* was

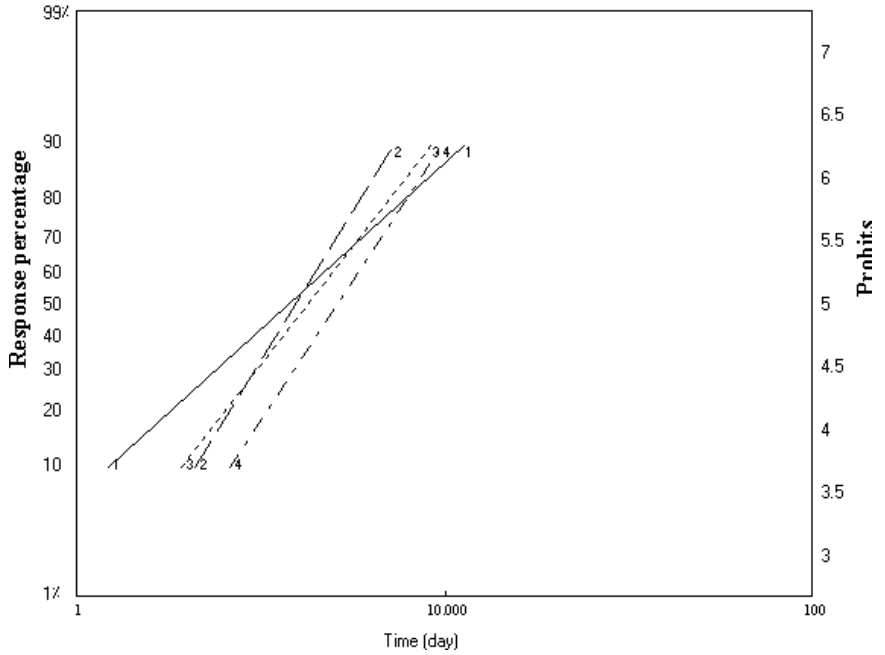

Figure 1. Lethal time curves of bioinsecticides containing crystals and endospores against cotton leafworm, *S.littoralis.*

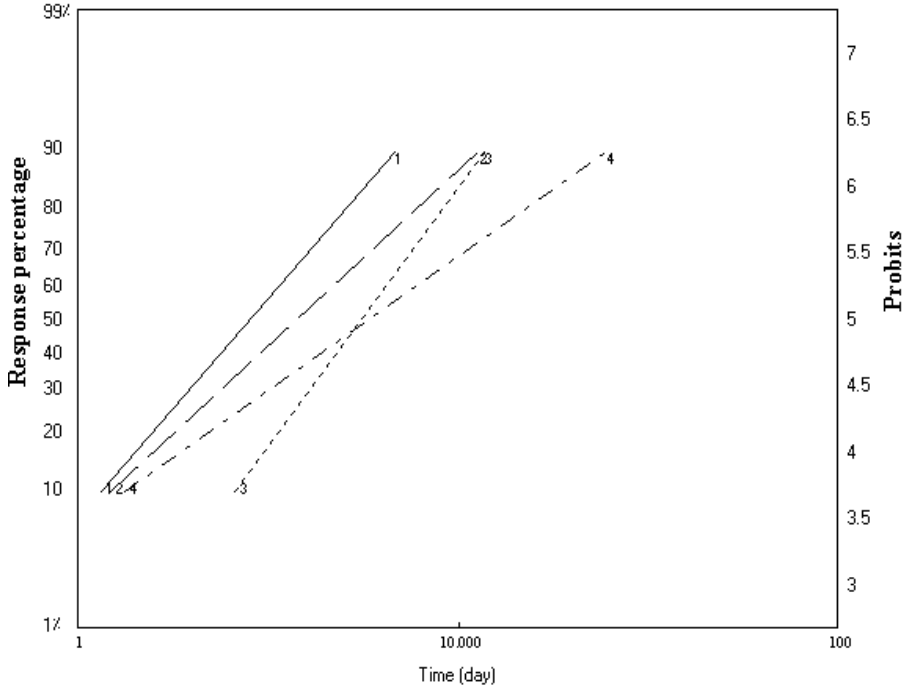

Figure 2. Note about the number of lines in the aforementioned figure: This figure related to conjugation between L1584 × T147, 1 = T3, 2 = P2, 3 = T4, 4 = P1, T = recombinant transconjugant.

more potent than *B. thuringiensis*. Field applications of *B. thuringiensis, B. subtilis* and Reldan achieved 55.6, 67.4 and 89.4% reduction of the cotton leafworm larvae *S. littoralis* in clover plants under field conditions.

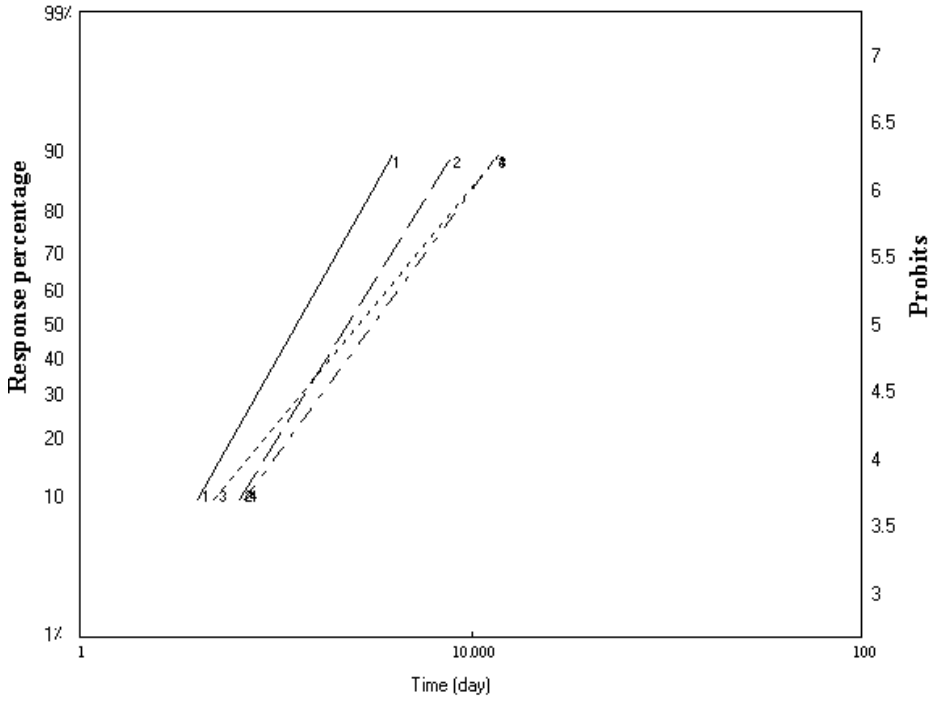

Figure 3. Note about the number of lines in the aforementioned figure: This figure related to conjugation between L358 × T18, 1 = P2, 2 = T5, 3 = P1, 4 = T6, T = recombinant transconjugant.

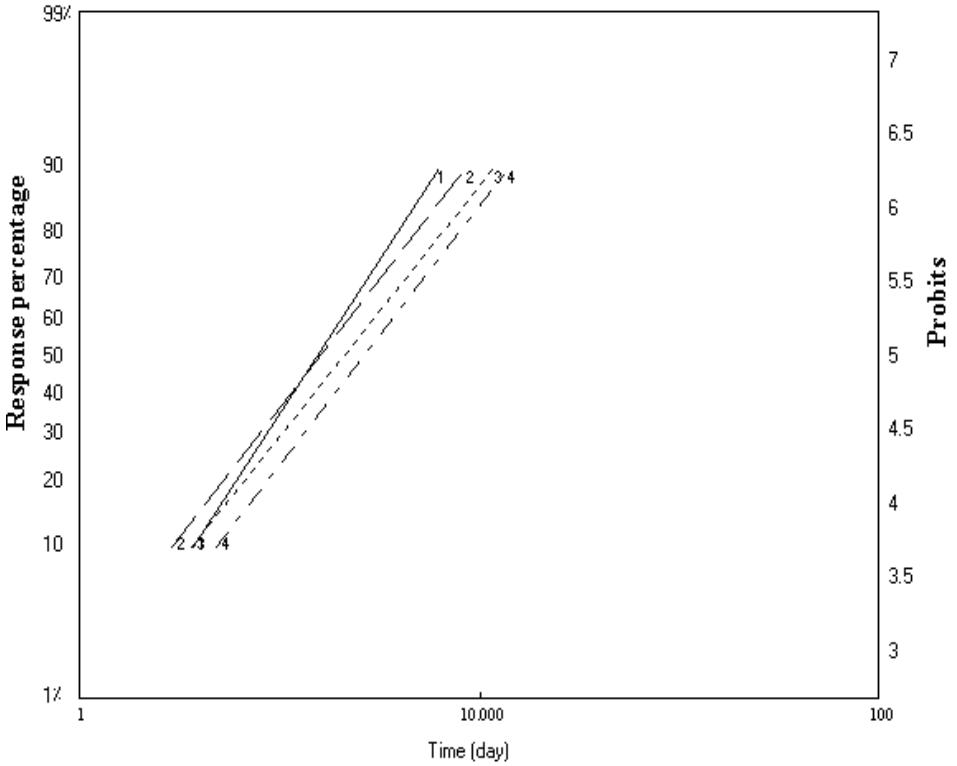

Figure 4. Note about the number of lines in the aforementioned figure: This figure related to conjugation between L358 × T64, 1 = P2, 2 = T7, 3 = T8, 4 = P1, T = recombinant transconjugant.

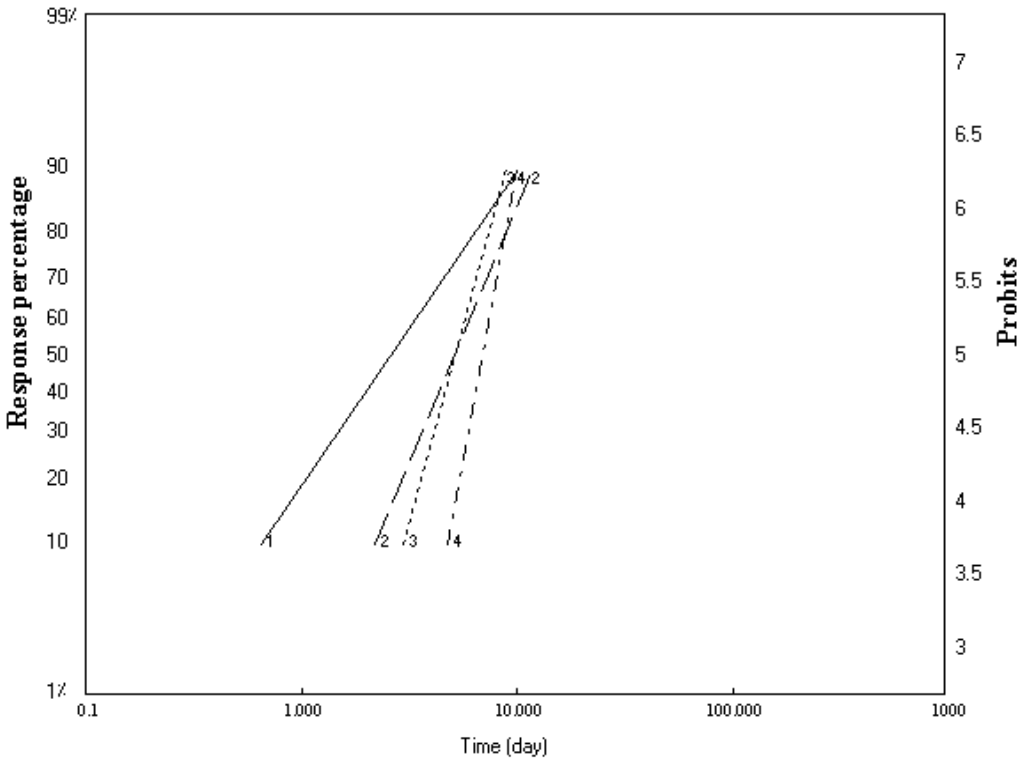

Figure 5. Note about the number of lines in the aforementioned Figure: This figure related to conjugation between L358 × T73, 1 = T9, 2 = P1, 3 = T10, 4 = P2, T = recombinant transconjugant.

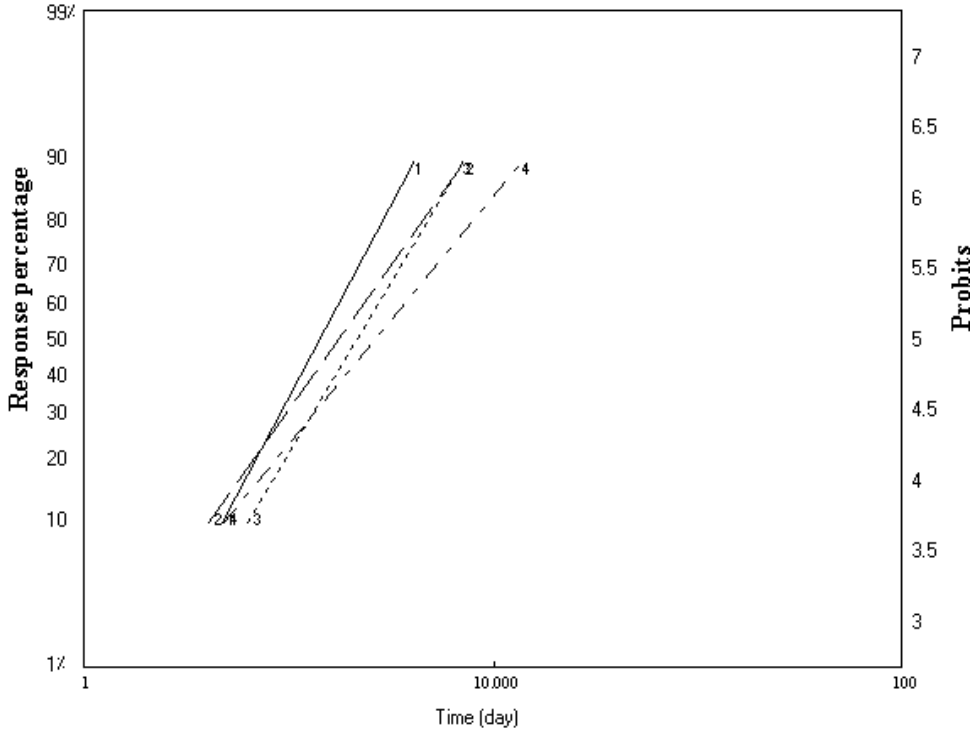

Figure 6. Note about the number of lines in the aforementioned figure: This figure related to conjugation between L358 × T110, 1 = T11, 2 = T12, 3 = P2, 4 = P1, T = recombinant transconjugant.

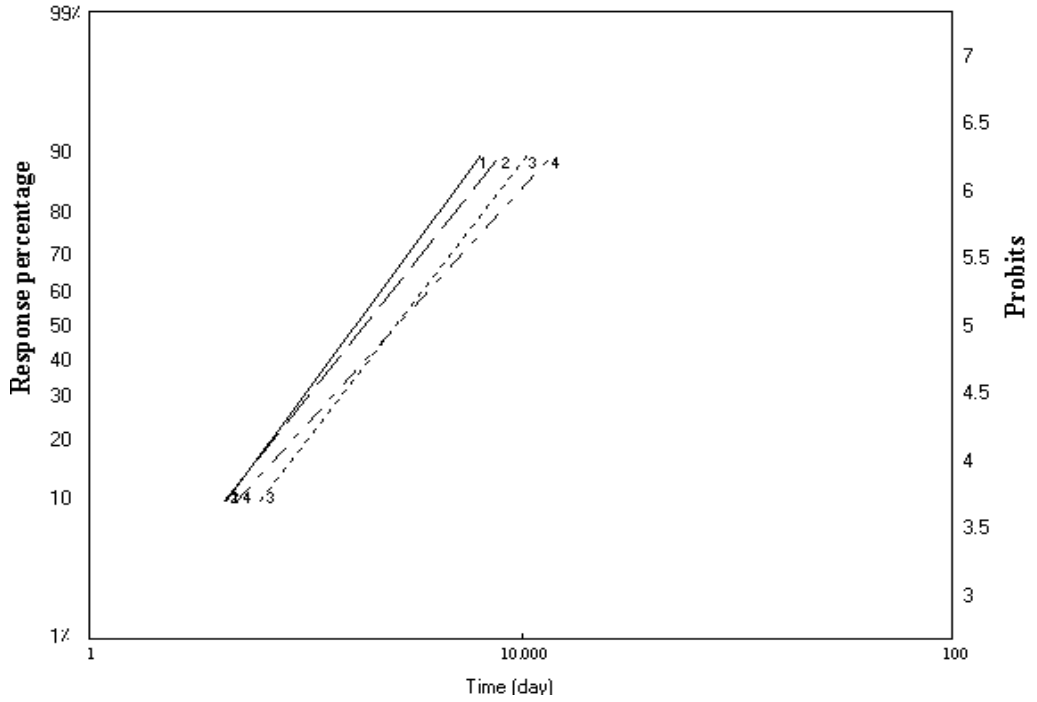

Figure 7. Note about the number of lines in the aforementioned figure: This figure related to conjugation between L358 × T153, 1 = T13, 2 = T14, 3 = P2, 4 = P1, T = recombinant transconjugant.

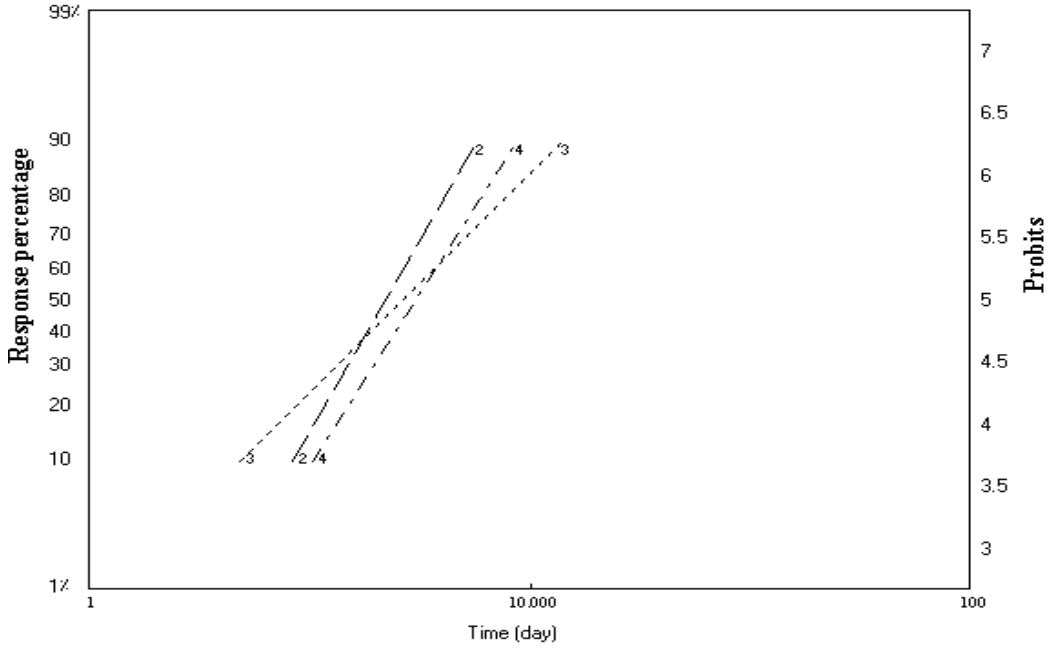

Figure 8. Note about the number of lines in the aforementioned figure: This figure related to conjugation between L358 × T537, 1 = T16, 2 = T15, 3 = P1, 4 = P2, T = recombinant transconjugant.

Data shown in Table 6 and Figure 9 appeared medium lethal time in response to crystal toxins obtained from parental strains and their transconjugants against cotton leafworm larvae *S. littoralis.* Transconjugants number Tr-B and Tr-D were more toxic if compared with other *Bt* toxins used in this study. This is because they appeared

Table 6. Medium lethal time of bioinsecticides resulted from conjugation between Bt -1 × BS -744, containing crystals against cotton leafworm, *S. littoralis*.

Treatments	Accumulative mortality % after feeding periods (day)						LT_{50}	Confidence limit (day)		Slope± SE
	1	2	3	4	5	6	(Day)	Upper	Lower	
Bt	0.10	4.17	4.17	4.17	12.5	20.83	15.30	38.92	10.26	2.33 ± 0.49
Bs	4.17	8.33	12.50	20.83	37.50	41.67	7.96	10.85	6.56	2.24 ± 0.31
TA	0.10	16.67	20.83	33.33	37.50	45.83	6.40	7.91	5.53	2.39 ± 0.29
TB	7.00	15.00	22.00	40.00	66.67	79.17	4.09	6.17	3.11	3.18 ± 0.29
TC	3.33	8.33	11.33	13.33	17.33	30.83	16.17	41.40	10.43	1.59 ± 0.31
TD	0.10	0.10	8.33	12.50	50.00	62.50	5.30	5.64	5.04	6.54 ± 0.67

T = Recombinant transconjugant.

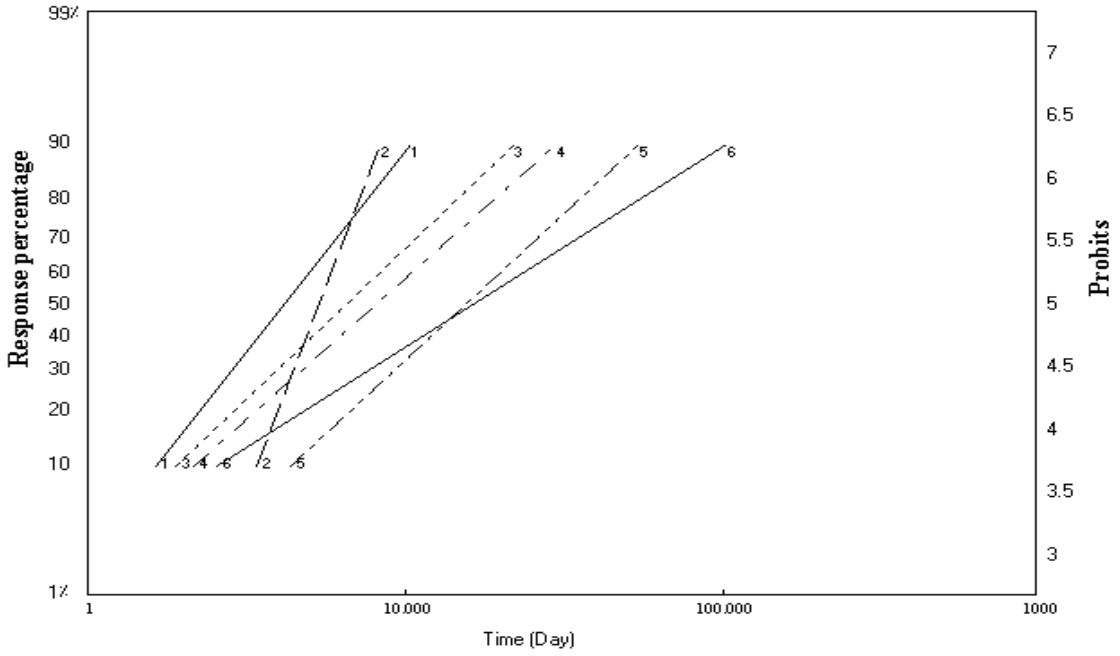

Figure 9. Lethal time curves of bioinsecticides resulted from conjugation between Bt -1 × BS-744, containing crystals against cotton leafworm, *S. littoralis.* Note about the number of lines in the Figure: 1 = TB, 2 = TD, 3 = TA, 4 = Bs, 5 = Bt, 6 = TC, T = recombinant transconjugant.

in the lower LT_{50} than other *Bt* isolates, they reached 4.09 and 5.30 days, respectively. Also, slope values were 3.18 and 6.54 for Tr-B and Tr-D, respectively, which proved the homogeneity of the tested larvae. The results indicated that accumulative mortality was increased with the larval development period.

The results obtained herein agreed with Mei et al. [2009], who found that *Photorhabdus* insecticidal toxin (Pit) killed *Galleria mellonella* (LD50, 30 ng / larvae) and *S. litura* (LD50, 191 ng / larvae) via hemocoel injection. This demonstrated that Pit possessed insecticidal activity.

In addition, Al - Deeb et al. [2001] conducted feeding study to determine the effect of *Bt* corn silk on mortality of immature *O. insidiosus.* No significant differences occurred in developmental time, body weight, or body length of mature *O. insidiosus* or mortality of immature *O. insidiosus* when reared on European corn borer larvae that had fed on a diet containing Dipel ES. The nymphs feeding only on *Bt* or non-*Bt* corn silk suffered 100 % mortality.

Data presented in Table 7 and Figure 10 indicated that the toxicity against *S. littoralis* increased in response to crystals + endospores obtained from parental strains and their transconjugants if compared with crystals. Transconjugants; T-B and T-D were more toxic if compared with other *Bt*, this because they appeared the lower values of LT50 than other isolates of Bt. The LT_{50} of T-B and T-D were 2.87 and 2.44 days , respectively.

Table 7. Medium lethal time of bioinsecticides resulted from conjugation between Bt -1 × BS -744, containing crystals + endospores against *S. littoralis*.

Treatments	Accumulative mortality % after feeding periods (day)							LT_{50}	Confidence limit (day)		Slope ± SE
	1	2	3	4	5	6	7	(Day)	Upper	Lower	
Bt	8.33	25.00	25.00	37.50	41.67	66.67	70.83	4.81	6.60	3.87	2.22 ± 0.22
Bs	16.67	25.00	33.33	33.33	58.33	58.33	62.50	4.85	5.76	4.21	1.64 ± 0.20
TA	8.33	20.83	20.83	41.67	70.83	79.17	87.50	3.77	4.98	2.81	3.23 ± 0.25
TB	25.00	41.67	41.67	50.00	56.67	83.33	87.50	2.87	4.16	1.59	1.90 ± 0.19
TC	25.00	37.50	41.67	50.00	62.50	62.50	75.00	3.33	3.87	2.85	1.49 ± 0.19
TD	33.33	41.67	50.00	54.17	70.83	75.00	83.33	2.44	2.82	2.03	1.57 ± 0.18

T = Recombinant transconjugant.

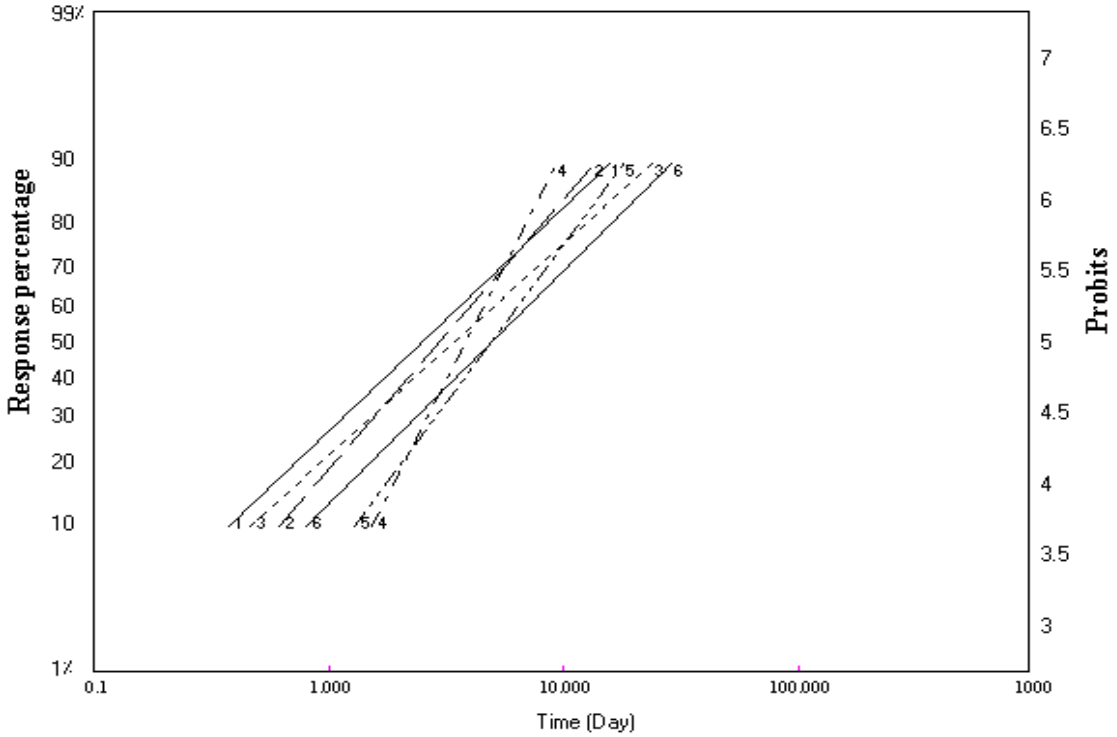

Figure 10. Note about the number of lines in the Figure: 1 = TD, 2 = TB, 3 = TC, 4 = TA, 5 = Bt, 6 = Bs, T = recombinant transconjugant.

Also, slope values were 1.90 and 1.57 for T-B and T-D, respectively, which proved the homogeneity of the tested larvae. These results indicated that toxicity increased in response to increasing feeding period on contaminated leaves with bioinsecticides. These results agreed with Moftah et al. [1990] and Taher et al. [1994], who found that mortality percentage increased with increasing feed period but decreased with increasing larval age. The results also agreed with Ashfaq et al. [2001], who found that the length of the larval developmental period increased linearly with an increase in feeding time on Bt-cotton in first and third instars; again, there was no significant response in the fifth instars. For both mortality and larval developmental time, the linear trend lines for the first and third instars were quite similar. Pupal weight declined linearly in the first and fifth instars in response to feeding time on Bt-cotton.

The results obtained herein agreed with He et al. [2003], who found that all the tissues of *Bt* transgenic corn expressing Cry1Ab protein, with the exception of pollen, contained sufficient insecticidal protein to kill > or = 95% of larvae within 7 days. Surviving larvae of Asian corn borer, *Ostrinia furnacalis* had also not grown beyond first instar and weighed < or = 0.1 mg. Although larvae

Table 8. Yield percentage in body weight of surviving larvae and in mortality larvae in relation to untreated control as a response to recombinant *Bt* bioinsecticides resulted from the mating between *B. thuringiensis* × *B. subtilis*.

Bioinsecticides	Yield % at different treatment times (h)											
	24 Body weight		Mortality		48 Body weight		Mortality		72 Body weight		Mortality	
	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End
B. thuringiensis	102	63	00	67	141	141	33	200	129	100	33	167
B. subtilis	105	54	33	133	149	93	67	200	159	150	100	234
TA	113	118	00	67	142	121	133	167	129	127	167	134
TB	118	127	33	200	124	134	33	333	122	169	33	300
TC	108	118	67	200	129	121	67	300	127	178	67	300
TD	82	100	00	267	110	118	00	333	130	208	67	367
	96 Body weight		**Mortality**		**120 Body weight**		**Mortality**		**144 Body weight**		**Mortality**	
	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End
B. thuringiensis	126	154	33	200	123	108	100	233	88	101	100	400
B. subtilis	152	144	167	167	125	196	300	367	152	119	266	300
TA	146	146	266	233	116	153	300	467	339	107	300	466
TB	146	83	200	300	157	46	533	433	56	008	566	399
TC	120	143	67	300	124	138	133	400	104	129	100	333
TD	117	181	100	333	147	131	400	467	251	668	433	433

T = Recombinant transconjugant.

feeding on *Bt* corn pollen were significantly smaller than those on non - *Bt* corn pollen, there was no significant difference in mortality. Damage ratings in field trials and number of larvae surviving per plant indicated that *Bt* corn was highly resistant to Asian corn borer (Figure 10). Lethal time curves of bioinsecticides resulted from conjugation between Bt -1 × BS -744, containing crystals + endospores against cotton leafworm, *S. littoralis* .

The results obtained herein agreed with Ambiente et al. [2001], who reported that entomopathogenic bacterium *B. thuringiensis* (Bt) produces a spore - crystal complex which is responsible for its biocide characteristic, and the bacterium can be obtained by fermentation, either in liquid or semi - solid substrates. They prepared the active complex (substrate plus spore - crystal of Bt) in order to obtain 2×10^6 spores / mL; and then sprayed the final suspension via tractor on corn fields. On the treated plants, mortality of neonate larvae was 100% within two days of spraying, and all larvae were found dead on leaves. During one maize crop cycle, two applications were made, and up until 70 days after emergence it was not necessary to apply any other insecticide for fall armyworm control.

In addition, Kim et. al. [2008] evaluated the effects of the pollens of transgenic Chinese cabbage (*Brassica campestris* subsp. *napus* var. *pekinensis* Makino), expressing *B. thuringiensis* Cry1Ac toxin (Bt cabbage), on *Bombyx mori* larvae. They found decreased survival rate and body weight of *B. mori* larvae when fed with an artificial diet containing *Bt* cabbage pollens. Taken together, the results suggested that *Bt* cabbage pollens adversely affect on non-target insect, *B. mori* larvae, when consumed.

Body weight and mortality of surviving larvae

B. thuringiensis is a live microorganism that kills certain insects and is used to kill unwanted insects in forests, agriculture, and urban areas. *B. thuringiensis* - crystal protein genes encode insecticidal δ-endotoxins that are widely used for the development of insect-resistant crops. The potency of recombinant *B. thuringiensis* preparations was determined against *S. littoralis* via calculated yield percentage in average weight of surviving larvae.

As shown from the results presented in Table 8 that the yield in body weight of surviving larvae treated with *Bt* was increased above that in untreated larvae. In contrast, the yield in mortality percentage was increased above that in control experiment. The yield in mortality percentage was increased when the larvae treated with crystals + endospores than that treated with crystals alone. This may be due to the higher efficient of larvae tolerated *Bt* for good feeding and food metabolism, as well as, larvae tolerated more toxic preparations of *Bt* were more effective in feeding and in food metabolism than that tolerated lower toxic effects of Bt preparations.

The results are in harmony with Roger et al. [2008]

who found that a high dose of snowdrop lectin Galanthus nivalis agglutinin (0.1%) in the larval diet of the solitary bee *Osmia bicornis* resulted in significantly increased development time and reduced efficiency in pollen food conversion of oilseed rape expressing the cysteine protease inhibitor oryzacystatin-1 into larval body weight.

The results agreed with Laura and Fiuza [2003], who carried out toxicity bioassays with *S. frugiperda* using purified proteins of *Bt aizawai* HD68 which indicated a LD_{50} of 0.95 µg/larvae. Two *Bt* isolates carrying the *cry3* genes (PCR detection) caused a 100% mortality to *Oryzophagus oryzae* larvae.

In addition, Xiao et al. [2004] found that the larval duration of the parasitoid was delayed, and the pupal weight, body weight of the newly emerged adult and adult longevity decreased significantly when the hosts larvae fed on diet containing Bt protoxin Cry1Ac at the concentrations of 0.58.0 µg/g in all time or from 12 h before parasitismtill pupation of the parasitoid. Compared with the control, the larval weight and pupal weight of *H. armigera* decreased significantly when the larvae fed on diet containing cry1Ac. Pupation rate of *H. armigera* decreased significantly in the treatment with diet containing Cry1Ac at 4.0 µg/g. When *H. armigera* larvae fed on the transgenic cotton leaves expressing both Cry1A and CpTI proteins, the mortality of the laboratory strain was 48.5, 95.8% in 25 days after treatment, which was significantly higher than that of the field strain. They concluded that feeding on diet containing *Bt* insecticidal protein have significantly negative effects on development of the parasitoid *M. mediator*.

However, Ul - Haq et al. [2009] revealed the individual and synergistic effects of destruxin B (DB) (mycotoxin from *Metarhizium anisoplae*), tea saponin (Ts) and *B. thuringiensis* (Bt) against *Spodoptera exigua*. DB, Ts and *Bt* reduced the growth of neonate larvae by up to 91.30, 89.17 and 77.17%. EC_{50} values of DB against 4th and 5th instars were 0.17 and 0.22 mg ml^{-1}, 0.35 and 0.41 mg ml^{-1} against 4th and 5th instars for Ts and 0.0031 and 0.0035 mg ml^{-1} after being treated with *Bt*. The synergism of DB and Ts with *Bt* resulted in the increased efficiency of these chemicals as mortality percentages significantly increased up to 94.2% with DB + Ts + Bt followed by DB + *Bt* with 91.99% and were significantly higher than individual treatments of Bt 65.81 and Ts 76.66%.

The results obtained herein agreed with Navon et al. [1983], who determined the potency of two new *B. thuringiensis* preparations (coded ABG 6104 and ABG 6105) and of Dipel (*B. thuringiensis* var.*kurstaki*) against *S. littoralis* (Boisd) 5th-instar larvae on a calcium-alginate diet. With this bioassay, the new *Bt* products were more than twice as potent as Dipel. They were also 2 to 3 times more active than Dipel on alfalfa and cotton leaves in the laboratory. When applied in an alfalfa field at the rate of 312 mg / m^2, ABG 6104 and ABG 6105 caused 40% mortality of 5^{th} – instar larvae and reduced the weight of the survivors to 30 to 40% of the control; only half of this activity was obtained with Dipel.

The results also are in harmony with Zhang et al. [2004], who found that, the total consumption on transgenic cotton was lower than that of the non-transgenic cotton. More larvae were found off diet in the treatments with leaves than that of buds, and the number of injured leaf discs by per fourth instar was significantly higher than that of buds in choice tests, suggesting that leaf is a less preferred organ for *H. armigera* larvae, elicited more larval movements. When cotton line was considered, compared with non-transgenic cotton, significantly lower feeding time and higher resting time occurred on the two transgenic cottons.

The results agreed with Fangneng et al. [2006] , who found that larval growth of the three species of the European corn borer, *Ostrinia nubilalis* (Hübner), southwestern corn borer, *Diatraea grandiosella* Dyar; and sugarcane borer, *Diatraea saccharalis* (F) ; on Cry1Ab - treated diet was inhibited, but the inhibition was greater for *Ostrinia nubilalis* and *D. grandiosella* than for *D. saccharalis*. The lower susceptibility of *D. saccharalis* to Cry1Ab protein suggested that it is necessary to verify if a high - dose Bt corn for *O. nubilalis* and *D*. *grandiosella* is also a high dose for *D*. *saccharalis* .

In addition, the results also agreed with Ostlie et al. [1997], who found that plants transformed with genetic material from the bacterium *B. thuringiensis* (*Bt*) are generally thought to have negligible impact on non - target organisms. In a laboratory assay John et al. [1999], found that larvae of the monarch butterfly, *Danaus plexippus,* reared on milkweed leaves dusted with pollen from *Bt* corn, at less, grew more slowly and suffered higher mortality than larvae reared on leaves dusted with untransformed corn pollen or on leaves without pollen. *Bt* corn plants might represent a risk because most hybrids express the *Bt* toxin in pollen [1997], and corn pollen is dispersed over at least 60 m by wind. Corn pollen is deposited on other plants near corn fields and can be ingested by the non-target organisms that consume these plants [Raynor et al., 1972; Henneberry et al., 2003].
Also, John et al. [1999] found that larval survival (56%) after four days of feeding on leaves of milkweed dusted with *Bt* pollen was significantly lower than survival either on leaves dusted with untransformed pollen or on control leaves with no pollen (both 100%, P = 0.008). All of the mortality on leaves dusted with *Bt* pollen seems to be due to the effects of the *Bt* toxin.

The low consumption rates of larvae fed on leaves with *Bt* pollen led to slower growth rates, the average weight of larvae that survived to the end of the experiment on *Bt*-pollen leaves was less than half the average final weight of larvae that fed on leaves with no pollen [Henneberry et al., 2003].

Young et al. [2008] evaluated the effects of the pollens of transgenic Chinese cabbage (*Brassica campestris* subsp. *napus* var. *pekinensis* Makino), expressing *B.*

thuringiensis Cry1Ac toxin (*Bt* cabbage), on *Bombyx mori* larvae. Decreased survival rate and bodyweight of *B. mori*larvae were observed when fed with an artificial diet containing *Bt* cabbage pollens.

The results of John et al. [1999] have potentially profound implications for the conservation of monarch butterflies. Monarch larvae feed exclusively on milkweed leaves [Malcolm et al., 1993]; the common milkweed. A. syriaca, is the primary host plant of monarch butterflies in the northern United States and southern Canada [Malcolm et al., 1989]. With the amount of *Bt* corn planted in the United States projected to increase markedly over the next few years [Andow and Hutchison, 1998]. Bienvenu and Danel [1997] conducted laboratory tests to determine the effect of two varieties of *B. thuringiensis* on food consumption and survival of diamondback larvae, *Plutella xylostella* L. Third instar larvae were allowed to feed for 48 h on cabbage disks treated with a series of concentrations (0.006 to 100 µg AI/ml water) of the formulations Dipel 2X (6.4% AI; *Bt* var *kurstaki*) and Xentari (10.3% AI; *Bt* var *aizawai).* Surviving larvae were transferred to untreated leaves to complete their life cycle until pupation or death. After two days of feeding on treated leaves, *Bt* subsp. *aizawai* with LC50 (0.82 µg/ml) was less efficacious than *Bt* subsp. *kurstaki* with LC50 (0.45 µg/ml). Moreover, mortality of larvae on treated leaves increased, whereas food consumption was reduced with increasing concentrations of delta ‑ endoxin [Brian, 2003].

The results obtained in this study also agreed with Henneberry et al. [2003], who found that tobacco budworm (TBW) , *Heliothis virescens* (F), larvae were highly susceptible to feeding on *Bt* cotton leaves or flower buds with 100 and 96% mortality occurring within 4 days, respectively, compared to an average mortality of 95% for cabbage looper (CL), *Trichoplusia ni* (Hübner), and 57% for beet armyworm (BAW), *Spodoptera exigua* (Hübner), after 14 days feeding on *Bt* leaves. Larval weights, of CL and BAW after 7, 10, or 14 days of feeding on *Bt* leaves were lower compared with those feeding on non - *Bt* cotton leaves. BAW, CL, and TBW larvae consumed significantly less *Bt* leaf area per feeding day compared with DPL 5415.

In addition , Wang et al. [2008] found that food consumption of striped stem borer on *Bt* transgenic rice and body weight growth, as well as, the survival of both 3rd and 5th instar larvae fed on *Bt* rice were significantly reduced, as compared with those on the control. There was a linearly positive relationship between the corrected mortality of larvae and the accumulative amount of *Bt* rice tissues ingested by larvae.

The results also are in harmony with Le et al. [2007], both the original transgenic oilseed rape and hybrid plants had a negative effect on body - weight gain of *Helicoverpa armigera* larvae. It was assumed that Bt Cry1Ac toxin concentration was similar in hybrids compared to the original transgenic OSR at the investigated developmental stages.

However, Shi - Gui et al. [2001] found that food consumption and body weight, as well as, the survival of striped stem borer (SSB), *Chilo suppressalis*, both 3rd and 5th instar larvae fed on *Bt* rice were significantly reduced, as compared with those on the control. There was a linearly positive relationship between the corrected mortality of larvae and the accumulative amount of *Bt* rice tissues ingested by larvae.

The results obtained in this study suggested that *B. thuringiensis* toxicity depends on an interaction between crystals and endospores. This finding contrasts sharply with established models that assume that *B. thuringiensis* itself induces mortality through starvation or direct septicemia. Thus, much of the research on *B. thuringiensis* since its discovery in 1918 and its development for commercial use in the 1950s has been predicated on a mechanism that is quite similar to that present here. Studies of the mode of action of *B. thuringiensis* in the last 25 years have clarified the molecular specificity of toxin binding and pore formation but have not addressed the subsequent events that lead to insect death [Schnepf et al., 1998].

A number of published results that are inconsistent with the starvation and *B. thuringiensis* - induced septicemia models are easily reconciled with the gut microbial community model. *B. thuringiensis* spores, for example, are typically absent from the hemocoel until very late in the infection process and frequently do not appear until well after the death of the insect. This finding challenges the idea that *B. thuringiensis* induces death by septicemia but is consistent with the model that proposes that septicemia is initiated by the enteric bacteria in the insect gut [Toumanoff and Vago, 1953].

The results leading to development of *Bt* crops which is one of the most significant advances in crop protection technology of the past fifty years. Current *Bt* crops are based on highly specific insecticidal cry proteins of *B. thuringiensis*. Foliar *Bt* insecticides based on the same proteins have been used for more than forty years and have a remarkable safety record, with no known detrimental effects reported on vertebrate or non-target invertebrate populations. *Bt* cotton and *Bt* corn have been widely adopted by farmers in the United States, with acreage averaging 40 to 50% of the area planted with cotton or corn in 2002. Concern has been raised about the safety of *Bt* crops. However, most evidence shows that these crops, like foliar *Bt* insecticides, are safe for non - target organisms, especially in comparison to chemical insecticides. Evidence for safety comes from knowledge of cry protein mode of action, as well as, from studies of the effects of cry proteins on non-target organisms tested in the laboratory and under operational growing conditions. This indicated that *Bt* crops, owing to their high degree of safety, can serve as the cornerstone for more environmentally sound integrated pest management programs [De Barjac and Frachon, 1990;

Yves et al., 2001].

Current and future developments

Biotechnological studies in this work focused on problems facing Arabic agriculture. The immediate objectives are to utilize cellular and molecular biology methodologies to develop and deliver transgenic *Bt* have the potential to cause a significant impact on mortality in cotton and vegetables leaf worm which reducing crop productivity and negatively affecting on the economy and the environment. However, millions of dollars are spent annually on the purchase of imported pesticides to combat insect pests in cotton and vegetables growing areas. The production of *Bt* bioinsecticides expressing insecticidal toxin gene (*Bt*) is one of the main targets of this study. Different *Bt* genes expressing toxicity against Lepidoptran, coleopteran and dipteran insects have been used to induced *Bt* recombinants of specific *Bt* genes for maximum expression of the toxic protein in addition to optimization of the regeneration and transformation system.

It can be concluded that recombinant bioinsecticides increased mortality yielding of *S. littoralis* larvae, as well as, reduced the weight of surviving larvae. The lower yielding in body weight due to lower consumption rates of larvae fed on leaves sprayed with *Bt* led to slower growth rates. The yield in mortality percentage was increased when the larvae treated with crystals + endospores than that treated with crystals alone. The results recommended that the use of endospores with crystals in all bioinsecticides preparations was better in controlling insects than that containing crystals alone.

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