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Screening of the Insecticidal Activity of New Recombinant Preparations of *Bacillus thuringiensis* var. *kurstaki* Against the Egyptian Cotton Leafworm

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Abstract: In the present work, two cultures of *Bacillus* strains belonging to two serotypes and four of their transconjugants were screened with respect to their activities against lepidopterous cotton pest. Two strains of *Bacillus* were screened for their drug resistance to be used as a genetic markers to identify bacterial strains in the conjugation process. *B. subtilis* was found to be sensitive to all antibiotics and drugs, and also resistant to crystal violet. Although, *B. thuringiensis* was found to be sensitive to all antibiotics, drugs and crystal violt, except for it was resistant to hiconcil. Bacterial transconjugants isolated from conjugation between both strains were more resistant to both crystal violet and hiconcil. Two groups of crystals and spores have been isolated within *Bacillus* strains and their transconjugants. This because δ-endotoxin gene of *B. thuringiensis* codes for the insecticidal crystal protein (ICP) specific for lepidopteran insects, since the N-terminal half of the toxin is sufficient both for insect specificity and toxicity. The potency of two *Bacillus* preparation was determined against *Spodoptera littoralis*. The toxicities of these two groups against larvae of *Spodoptera littoralis* expressed as; average consumption of fed leaves, average weight of surviving larvae, survival percent of the larvae and mortality percentage are also differed. Mortality levels of larvae caused by the different isolates varied from 20.83 to 79.17 (crystals alone) and from 58.33 to 83.33 (crystal and spores). Higher levels of mortality were observed if a mixture of relatively pure crystals and spores was used compared with the mortality resulting from either fraction of crystal alone. δ-endotoxin recombinant crystalline protein and endospores from bacterial transconjugants were highly toxic effective against *S. littoralis* than from their parental strains.

Key words: antibiotic markers, *Bacillus thuringiensis*, *Spodoptera littoralis*, spore-crystal complex.

Introduction

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.), is a major pest of field crops in Israel, predominantly alfalfa, cotton, sugar beet and vegetables (Avidov and Harpaz, 1969). The pest is being controlled by insecticides only. This causes environmental hazards and the insect becomes resistant to the chemicals. Screening of effective microbial agents against the larvae, to replace chemicals, is therefore needed urgently. Only a few bioassays with strains of *Bacillus thuringiensis* Berliner (B.t.) have been carried out against this insect (Sneh *et al.*, 1981). The bacterium *Bacillus thuringiensis* is used widely to control insect pests in the orders Lepidoptera, Coleoptera, and Diptera. During sporulation in nutrient media, the bacterium produces a parasporal protoxin (delta-endotoxin) which, when ingested by a susceptible insect larva, is cleaved into toxic subunits of various molecular weights by alkaline proteases. These toxins are bound to specialized receptor cells in the gut epithelium and cause disruption of the gut epithelium. The disruption leads to morphological and pathological changes in many cellular organelles, increases in haemolymph pH, disturbance of ionic balance, and eventually, gut paralysis and death of the host (English and Slatin, 1992).

Bacillus thuringiensis is an insect-pathogen with an unusual but highly specific mode of action. During the sporulation cycle, it lays down a parasporal protein crystal which is rendered toxic on ingestion by susceptible insect larvae. The major component of crystals toxic lepidoptera is a protein (protoxin) with a molecular mass of approximately 130 KDa (Höfte and Whitely, 1989). Treatment with thiol reagents at basic pH solublizes the protoxin by cleaving the disulfide bonds which stabilize the crystal. Incubation of the solubilizied protoxin with proteolytic enzymes or insect gut juice produces a 58-70-KDa proteinase-resistant toxin derived from the N-terminal portion of the molecule (Bietlot *et al.*, 1989). The toxin then binds to receptors in the midgut epithelium, causing cell lysis and eventual larval death (Jaquet *et al.*, 1987). The details of the lytic mechanism are not yet established but it appears that the toxin generates small pores or localized perturbations in the plasma membrane, causing disruption of homeostatic ion regulation (Haider and Ellar, 1987). The delta endotoxin of *Bacillus thuringiensis* is a protein-aceous, crystalline insecticide, toxic to the larval stages of several important pest insects in agriculture and forestry. *In vitro*, the protoxin needs proteolytic activation to exhibit toxicity against insect cell cultures (Hofmann and Lüthy, 1986). *In vivo*, the protoxin is enzymatically activated within the digestive tract. Furthermore, the toxins of various strains differ in their host spectrum (Jaquet *et al.*, 1987). The delta endotoxin destroys the gut epithelia of susceptible insect larvae (Lüthy and Ebersold, 1984). In the present work, gene transfer through conjugation between related bacterial genera leading to change phenotypic properties has been reported in this study. The potency and activity of new recombinant (*Bacillus thuringiensis* x *Bacillus subtilis*) products were compared with that of their parental strains. The products were assayed against larvae of *Spodoptera littoralis*, a serious pest of Egyptian cotton.

Materials and Methods

Microbial strain: *Bacillus thuringiensis* serovar *Kurstaki* (NRRL HD-1) and *Bacillus subtilis* (NRRL NRS-744) were obtained from Dr. L.K. Nakamura, U.S. Department of Agriculture, Agricultural Research Service, U.S. Department of Agriculture, Peoria, I llinois. The strains were maintained on L.B. Slape medium, containing 5% peptone, 0.1% yeast extract and 0.5% NaCl, pH 7.5 (Puntambeker and Ranjeker, 1989).

Antibiotic susceptibility assays: Antibiotic 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. 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), they were the product of Hoechst Orient S.A.E., Cairo, Egypt.

Conjugation procedures: Nutrient broth cultures, in the late-exponential growth phase, were used. Quantitative spot mating of conjugal transfer was carried out, according to Lessl *et al.* (1993), by inoculating 10 μl samples of the donor cultures onto the surface of selective medium, previously seeded with 100 μl of the recipient culture. A single colony of transconjugants was picked up and transferred to LB slant agar medium.

***rfa* mutation:** Strains having the deep rough (*rfa*) character should be tested for crystal violet sensitivity (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 pipet 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.

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, 1.5g 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 x 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 xg 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).

Bioassay of toxicity: The toxicity was bioassayed with *Spodoptera littoralis* second instar larvae (mean body weight = 10 mg) to estimate the biological insecticidal effect on cotton leafworm, according to Klanfon and DeBarjac (1985) with some modifications. The bacterial cell component of *B. thuringiensis* was approximately 10^9 crystals and/or spores per milliliter was used with the dilution of 1:1. Larvae of *Spodoptera littoralis* were exposed to the appropriate dose of the component of *B. thuringiensis* by using a Gentaur micropipette to dispense 200 μl of the suspension on 2-3 gram of diet surface of *Ricinus communis* (Ignoffo *et al.*, 1977). 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 6-7 days. Surviving larvae from each replicate were pooled and weighted daily (Ignoffo *et al.*, 1977; Karamanlidou *et al.*, 1991 and Inagaki *et al.*, 1992).

Statistical analysis: The data were statistically analyzed using the analysis of variance procedure. Significance of the differences between sample means was recorded using LSD values, according to Steel and Torrie (1960).

Results and Discussion

Prevalence of intrinsic antibiotic resistance markers: Both *Bacillus thuringiensis* and *Bacillus subtilis* were tested for their antibiotic and drug resistance using 18 compounds (Table 1). *B. thuringiensis* was found to be more resistant to Hiconcil, and intermediate resistant to streptomycin, this are due to insignificant diameter of inhibition zone. In addition, *B. thuringiensis* was found to be sensitive to all other antibiotics and drugs, this might be due to the appear of significant diameter of inhibition zone. Similarly *B. subtilis* was found to be more sensitive to all antibiotics and drugs this also due to the appear of significant diameter zone of inhibition, except for crystal violet, for their disappearance of significant diameter of inhibition zone. *B. subtilis* was found to be more resistant to crystal violet, for disappearance of inhibition zone. This have often relied upon resistance as a genetic marker to identify bacterial strains (Schwinghamer and Dudman, 1973). Resistance to antibiotics and chemical compounds has proved to be a stable marker with a high level of resistance available. Crystal violet resistance in *B. subtilis* is also similar to that of hiconcil in *B. thuringiensis* and provides a second potential marker for use in conjugation process to isolate bacterial transconjugants, who showed significantly differ from their parental strains in this respect. The results obtained here are in agreement with those reported by Garg *et al.* (1985), who screened more than 200 wild type isolates of chikpea for their drug resistance, they found that only 15 were resistant to one or more antibiotics at concentration of 5 μg/ml (units/ml). In addition, some isolates were found to be resistant to more than one antibiotic.

Conjugation: Conjugation process (i.e. direct transfer of DNA from cell to cell was carried out between *Bacillus subtilis* (*Hico⁻ rfa⁺*) and *Bacillus thuringiensis* serovar *Kurstaki* (*Hico⁺ rfa⁻*), which have the opposite genetic markers. The results obtained here are in accordance with those found by Wooley *et al.* (1992), who found that virulent avian *E. coli* isolates transferred a single large molecular weight R plasmid to two recipient *E. coli* strains, antibiotic resistances transferred included streptomycin (two isolates) and streptomycin-tetracycline-sulfa (one isolate). On the other hand, Koh *et al.* (1984) found in *E. coli* strains of avian, bovine and porcine origins, three were resistant
to one antibiotic and five were resistant to more than one antibiotic, all eight were resistant to tetracycline, and five to gentamicin, streptomycin and kanamycin. Three of them were able to transfer all or part of their resistance to an *E. coli* K12 recipient by conjugation.

Comparative potencies of spore-δ-endotoxin preparations of various cultures: Table 2 shows the average consumption of fed leaves of *Ricinus communis* (gram / day) of the tested *Spodoptera littoralis* after feeding for 7 d on a leaves to which endotoxin preparations were added. It is of interest to note that the rate of leaves consumption after 6 d was greater when the leaves sprayed with crystals than that sprayed with crystals + endospores. The development of a strains with desirable properties of *Bacillus thuringiensis* and *B. subtilis* provides an interesting model system.

Table 1: Bactericidal effect of antibiotics, drugs and crystal violet as measured by the diameter of inhibition zone (cm).

	Bactericidal strains		F	L.S.D.	
	B.thuringiensis	*B.subtilis*	test	0.05	0.01
Tetracide	2.65	3.05	*	5.81	17.04
Kanamycin	2.62	2.30	*	13.54	39.70
Streptomycine	0.58	1.89	*	5.50	16.12
Flucamox	1.22	4.87	**	2.38	6.97
Ampicillin	1.22	2.47	**	4.60	13.50
Duricef	3.38	4.87	**	10.08	29.56
Vibramycin	2.55	2.37	**	0.51	1.50
Flummox	1.23	4.78	**	4.43	12.99
Erythromycin	3.08	2.15	**	8.42	24.70
Velosef	3.37	5.38	**	15.49	45.41
Cidocteine	3.30	2.78	**	2.71	7.94
Cloxapen	1.55	5.40	**	5.81	17.04
Septazole	2.27	3.98	**	1.02	3.00
Hostacycline	2.48	2.30	*	2.05	6.00
Tetracycline	2.92	2.20	**	2.71	7.94
Hiconcil	0.00	2.48	**	4.88	14.31
Chloramphenicol	3.73	2.48	**	10.74	31.51
Crystal violet	2.03	0.00	**	1.35	3.97

* = Significant at 0.05 probability level. ** = Significant at 0.01 probability level.

Table 2: Average consumption of fed leaves of ricinus communis (gram/day) by spodoptera littoralis larvae after sprayed with *Bacillu thuringiensis* prepatations.

Source of bacterial preparation	Treatment time (h)												
	24		48		72		96		120		144		168
	Cry	Cry+	Cry	Cry+	Cry	Cry+	Cry	Cry+	Cry	Cry+	Cry	Cry+	Cry+
Control	0.19	0.17	0.19	0.17	0.18	0.18	0.23	0.20	0.12	0.16	0.26	0.19	0.21
B. thuringiensis	0.18	0.19	0.20	0.19	0.21	0.24	0.26	0.28	0.32	0.25	0.20	0.20	0.40
B.subtilis	0.19	0.22	0.22	0.23	0.22	0.19	0.32	0.23	0.34	0.45	0.42	0.40	0.32
Transconjugant-A	0.18	0.19	0.23	0.20	0.22	0.15	0.42	0.27	0.33	0.29	1.11	0.41	0.62
	0.19	0.19	0.19	0.29	0.18	0.20	0.34	0.26	0.48	0.25	1.75	0.08	0.13
Transconjugant-B	0.19	0.18	0.21	0.27	0.14	0.19	0.26	0.21	0.44	0.25	0.23	0.21	0.67
Transconjugant-C	0.17	0.27	0.17	0.29	0.21	0.22	0.25	0.38	0.34	0.25	0.69	0.21	0.77
Transconjugant-D													
F-test	NS	**	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	*
L.S.D. 0.05		0.0004							0.01				0.41
0.01		0.0006							0.02				0.57

Cry = Crystals end = Endospores. NS = Not significant. *.** = significant at 0.05 and 0.01 probability levels, respectively

The specificities of the toxins were by means correlated to the variety or the serotype examined. This observation is in accordance with Dulmage (1971), who stated that the extent of potency and host range depend upon the isolate itself rather than upon the variety or biotype. Thus, with *Spodoptera littoralis*, the spore-endotoxin preparations (crystals) from *B. thuringiensis* caused rate of leaves consumption that ranged between 0.18 and 0.32 (g/d), whereas *B. subtilis* caused the rate ranged between 0.19 and 0.42. In addition, the rate of leaves consumption when sprayed by crystals of bacterial transconjugants A, B, C and D was ranged between; 0.18-1.11, 0.18-1.75, 0.14-0.44 and 0.17-0.69 (g/d), respectively. On the other hand, the spore-endotoxin preparations (crystals + endospores) from *B. thuringiensis* caused rate of leaves consumption ranged between 0.19 and 0.28, whereas *B. subtilis* caused the rate ranged between 0.19 and 0.45. However, the rate of consumption by bacterial transconjugants A, B, C and D was ranged between; 0.15-0.62, 0.19-0.29, 0.18-0.67 and 0.21-0.77. It is of interest to note that a highly potent strains for leaves consumption belonging to bacterial transconjugants at 7 d, which gaves 0.62, 0.67 and 0.77 (g/d) leaves consumption, this will increase mortality percent as shown in Table (6). One of the important observations in this study was to select the isolates required to obtain cells with maximum insecticidal activity.The average weight of surviving larvae is recorded in Table 3. The results indicated that feeding the larvae on leaves sprayed with crystals + endospores caused, i most cases, less weight than that feeding on leaves sprayed wit crystals only. The weight of surviving larvae feeding on leave sprayed with Cry + End. was markedly reduced. It is of interes because *Spodoptera litura* is one of the more important pest infecting field crops of *B. thuringiensis* HD-1 preparation exhibit poor mortality against this pest (Inagaki *et al.*, 1992). In th present work, bioassays with neonate larvae of *S. litura* have shown mortality of the order that ranged between 75-87% at 7 days of larval feeding by preparations
of Cry + End resulted from bacterial transconjugants than thei parental strains (Table 6). The results obtained here indicated tha the effect of microbial product containing crystals + endospores caused a severe weight loss in the surviving larvae than tha containing crystals alone.
Table 4 shows the percentage survival of the tested larvae after feeding on leaves to which endotoxin preparations were added for 7 days. It is of interest to note that endotoxin preparations containing crystals + endospores was more effective against *Spodoptera litoralis* than that containing crystals only, because *B.* economic importance in Egypt. It is resistant to conventional insecticides (Ramkrishnan *et al.*, 1984). The commercially available *thuringiensis* has been considered an insect pathogen and most strains have been isolated in association with insects (Martin and Travers, 1989). Among both endotoxin preparations the results

Table 3: Average weight of surviving larvae (g) of spodoptera littoralis fed on leaves of ricinus communis sprayed with *Bacillus thuringiensis* preparations.

Source of bacterial preparation	Treatment time (h)														
	00		24		48		72		96		122		144		168
	Cry	Cry+ End	Cry	Cry+ End	Cry	Cry+ End	Cry	Cry+ End	Cry	Cry+ End	Cry	Cry+ End	Cry	Cry+ End	Cry+ End
Control	0.020	0.008	0.039	0.011	0.059	0.032	0.126	0.105	0.217	0.106	0.317	0.150	0.308	0.184	0.221
B.thuringiensis	0.0179	0.006	0.040	0.007	0.083	0.045	0.163	0.105	0.275	0.168	0.356	0.163	0.272	0.186	0.163
B.subtilis	0.023	0.006	0.041	0.006	0.088	0.030	0.201	0.158	0.332	0.157	0.339	0.295	0.470	0.219	0.225
Transconjugant-A	0.019	0.007	0.044	0.013	0.084	0.039	0.163	0.133	0.317	0.160	1.369	0.230	0.047	0.198	0.205
Transconjugant-B	0.017	0.006	0.046	0.014	0.073	0.043	0.154	0.178	0.317	0.091	1.498	0.069	0.780	0.016	0.021
Transconjugant-C	0.019	0.006	0.042	0.013	0.076	0.039	0.160	0.187	0.262	0.156	0.396	0.207	0.320	0.239	0.291
Transconjugant-D	0.018	0.006	0.032	0.011	0.065	0.038	0.164	0.219	0.255	0.198	0.469	0.197	0.774	0.123	0.161
F-test	NS	NS	NS	**	NS	NS	NS	**	NS	NS	**	NS	NS	NS	NS
L.S.D. 0.05				0.000008				0.061			0.063				
0.01				0.000011				0.086			0.088				

Cry = Crystals end = Endospores. NS = Not significant. ** = significant at 0.01 probability levels.

Table 4: Average weight of surviving larvae (g) of spodoptera littoralis fed on leaves of ricinus communis sprayed with *Bacillus thuringiensis* preparation.

Source of bacterial preparation	Treatment time (h)												
	24		48		72		96		120		144		168
	Cry	Cry+ End	Cry	Cry+ End	Cry	Cry+ End	Cry	Cry+ End	Cry	Cry+ End	Cry	Cry+ End	Cry+ End
Control	100.00	100.00	100.00	100.00	100.00	95.83	100.00	87.50	100.00	87.50	91.67	79.17	75.00
B. thuringiensis	100.00	91.67	95.83	75.00	95.83	75.00	95.83	62.50	87.50	58.33	79.17	33.33	29.17
B.subtilis	95.83	83.33	91.67	75.00	87.50	66.67	79.17	66.67	62.50	41.67	58.33	41.67	37.50
Transconjugant-A	100.00	91.67	83.33	79.00	79.17	79.17	66.67	58.33	62.50	29.17	54.17	20.83	12.50
	95.83	75.00	95.83	58.33	95.83	58.33	75.00	50.00	33.33	33.33	20.83	16.67	12.50
Transconjugant-B	91.67	75.00	91.67	62.50	91.67	58.33	91.67	50.00	83.33	37.50	79.17	37.50	25.00
Transconjugant-C	100.00	66.67	100.00	58.33	91.67	50.00	87.50	45.83	50.00	29.17	37.50	25.00	16.67
Transconjugant-D													
F-test	NS	**	NS	NS	NS	NS	NS	NS	**	*	*	**	**
L.S.D. 0.05		14.57							32.27	32.45	36.11	24.20	19.92
0.01		20.45							45.29	45.55	50.69	33.96	27.96

Cry = Crystals end = Endospores. NS = Not significant. *.** = significant at 0.05 and 0.01 probability levels, respectively.

shows a time response for decreasing survival of larvae. For the purpose of this study, it only did toxicity screening to show that most of new recombinants were toxic to *Spodoptera littoralis* than their parental strains. As ohbo and Aizawa (1978) have also shown, however, no correlation could be made between crystal type and toxicity. Toxicity to certain types of insects appeared to be clustered in some samples. That is, some samples that consisted entirely of *B. thuringiensis* strains which formed bipyramidal crystals were toxic to Lepidoptera. In other soil samples, the *B. thuringiensis* isolates formed irregular crystals that were toxic to mosquitoes. Some soil samples also consisted of *B. thuringiensis* isolates that were not toxic to any insect tested. Other samples contained isolates toxic to both Lepidoptera and Diptera (Martin and Travers, 1989). Even more interesting was the information of the same authors which are in agreement with the present results, who found that almost 40% of the crystal formers tested for toxicity were not toxic to any of the insects that were tested. The first thought was that these crystals may be toxic to insects that are not normally susceptible to *B. thuringiensis*. The previous investigation (Martin and Travers, 1989) revealed that soil samples with high levels of insect activity were found to be no more likely to have high numbers of *B. thuringiensis* than was a soil sample obtained at random. *B. thuringiensis* is not normally toxic to insect larvae that live in the soil, such as black cutworm, corn root worm, Japanese beetles, or wireworms; but it is toxic to insects that have aerial or water-borne larvae, such as cabbage loopers, gypsy moths, and mosquitoes. So one is left with the dilemma that either the bacteria make a crystal toxin for insects that it very rarely contacts or it makes the crystal for some other purpose than to kill these insects. Although at present the results demonstrated what this purpose might be, this hypothesis may lead to answers about the fundamentals toxicity of the *B. thuringiensis* crystal.

As shown from the results presented in Table 5, several transconjugants were highly active for toxicity against the larvae of *Spodoptera lituralis*. An interesting finding is that toxicity shows a time response. It has been shown that the toxins of these strains and their transconjugants possess various levels of activity when tested for toxicity against the Egyptian cotton leafworm. The activity of some of the transconjugants is significant than the mid parents. In addition, crystals + endospores possess high levels of activity as being caused by crystals alone. The fact that the toxicity of these materials is consistently higher at all times tested than the activity of the crystals alone supports the view that the endospores are, on their own, carriers of activity that is different from that of the crystalline inclusions. This view is supported by the findings of Bone and Bottjer (1988), who distinguish two toxic activities of *B. thuringiensis*, each being specific for larvae or adults of the nematode *Trichostrongylus colubriformis* (Bone, 1989). Alternatively, the increased toxicity of the crystalline inclusions in the presence of the spores might be due to the possibility that the spore itself provides the necessary factors that are required for the processing of the protoxins into active toxins (Thomas and Ellar, 1983).

Table 6 summarizes the percentage mortality of the tested *Spodoptera littoralis* larvae after feeding for 7 days on a leaves sprayed with endotoxin preparations. The results indicated that the highest larval mortalities were obtained using endotoxin preparations from crystals + endospores than that from crystals alone. The percentage of mortality was increased with increases in the time of feeding on sprayed leaves with endotoxin preparations. The variable toxicity of the delta endotoxins of *B. thuringiensis* and their transconjugants against *Spodoptera littoralis* has been interpreted by the different mechanisms that lead to the

Table 5: Toxixity of crystals, crystals and endospores isolated from bacillus thuringiensis against spodoptera littoralis larvae.

Source of bacterial preparation	Mean number+of mortality larvae at the following times (h)												
	24		48		72		96		120		144		168
	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry + End
Control	0.00	0.00	0.00	0.00	0.00	0.33	0.00	1.00	0.00	1.00	0.67	1.67	2.00
B.Thuringiensis	0.00	0.67	0.33	2.00	0.33	2.00	0.33	3.00	1.00	3.33	1.67	5.67	5.33
B.subtilis	0.33	1.33	0.67	2.00	1.00	2.67	1.67	2.67	3.00	4.67	3.33	4.67	2.67
Transconjugant-A	0.00	0.67	1.33	1.67	1.67	1.67	2.66	3.33	3.00	5.67	3.67	6.33	7.00
A	0.33	2.00	0.33	3.33	0.33	3.33	2.00	4.00	5.33	5.33	6.33	6.67	7.00
Transconjugant-B	91.67	2.00	0.67	3.00	0.67	3.33	0.67	4.00	1.33	5.00	1.67	5.00	6.00
Transconjugant-C	0.00	2.67	0.00	3.33	0.67	4.00	1.00	4.33	4.00	5.67	5.00	6.00	6.67
Transconjugant-D													
F-test	NS	**	NS	NS	NS	NS	NS	NS	**	*	*	**	**
L.S.D. 0.05		1.17							2.58	2.60	2.89	1.93	2.59
0.01		1.63							3.62	3.64	4.06	2.72	3.63

+ = Initial number of larvae per each replicate was equal eight. Cry = Crystals end = Endospores.
NS = Not significant. *.** = significant at 0.05 and 0.01 probability levels, respectively.

Table 6: Mortality percentage of Spodoptera littoralis neonate larvae of Ricinus communis sprayed with Bacillus thuringiensis preparations.

Source of bacterial preparation	Mean number+of mortality larvae at the following times (h)												
	24		48		72		96		120		144		168
Cry	Cry +	Cry End		Cry + End	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry	Cry + End	Cry + End
Control	0.00	0.00	0.00	0.00	0.00	4.17	0.00	12.50	0.00	12.50	8.33	20.83	25.00
B.Thuringiensis	0.00	8.33	4.17	25.00	4.17	25.00	4.17	37.50	12.50	41.67	20.83	66.67	70.83
B.subtilis	4.17	16.67	8.33	25.00	12.50	33.33	20.83	33.33	37.50	58.33	41.67	58.33	62.50
Transconjugant-	0.00	8.33	16.67	20.83	20.83	20.83	33.33	41.67	37.50	70.83	45.83	79.17	87.50
A	4.17	25.00	4.17	41.67	4.17	41.67	25.00	50.00	66.67	56.67	79.17	83.33	87.50
Transconjugant-B	8.33	25.00	8.33	37.50	8.33	41.67	8.33	50.00	16.67	62.50	20.83	62.50	75.00
Transconjugant-C	0.00	33.33	0.00	41.67	8.33	50.00	12.50	54.17	50.00	70.83	62.50	75.00	83.33
Transconjugant-D													
F-test	NS	**	NS	NS	NS	NS	NS	NS	**	*	*	**	**
L.S.D. 0.05		14.56							32.27	32.45	36.12	24.20	19.92
0.01		20.45							45.29	45.55	50.69	33.96	27.96

Cry = Crystals End = Endospores NS = Not significant. *, ** = Significant at 0.05 and 0.01 probability levels, respectively.

activation of the delta endotoxin. The activation of the protoxins requires the action of proteolytic enzymes (Fast, 1981) after their solubilization in alkali (Thomas and Ellar, 1983). The results of these studies indicated that *B. thuringiensis* strains and their transconjugants can be successfully utilized in the field as part of an integrated control program for Egyptian cotton insects in Egypt., The neonate bioassay provided an effective time / mortality response. Bacterial transconjugants had a higher toxicity effect than the parental bacterial strains. Insecticidal activity of *Bacillus thuringiensis*, could be ascribed to its production of protein crystals (δ-endotoxin), which were encoded by, at least, four gene families (Cry-I, Cry-II, Cry-III and Cry-IV), as reviewed by Arnonson *et al.* (1986) and Whiteley and Schnepf (1989). A genetic recombination and gene product interaction occurred between those genes in the transconjugants due to conjugation process, which might be modified by the gene action of one or more of them. The gene product interaction could be based on the expectation that, in transconjugants complementation, it would normally occur between alleles of the same gene (Schaeffer *et al.*, 1976).

In conclusion, new recombinant products of *Bacillus thuringiensis* would probably be more effective in controlling *S. littoralis* on Egyptian cotton, especially that containing crystals and endospores. Further selection of new strains of *B. thuringiensis* based on potency bioassays and effectiveness studies in the field would be useful to achieve microbial control of the pests.

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