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Potency and Toxicity of Bacterial Preparations from Trans-conjugants of *Bacillus thuringiensis* Against Larvae of *Spodoptera littoralis*

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Abstract: The potency of two *Bacillus* strains and four new isolates of transconjugants was determined against *Spodoptera littoralis.* Most of bacterial transconjugants revealed insignificant correlation between the average consumption of fed leaves with the time of treatment during the age of larvae if a mixture of relatively pure crystals and endospores was used, similar to that resulting from either fraction of crystals protein alone. This suggested that feeding was reduced by the *B. thuringiensis* products. The bacterial preparation containing crystals and endospores from transconjugant-D revealed high levels of toxicity for the negative correlation obtained between the average consumption of fed leaves with the larval age. Highly significant correlation was obtained between mortality and the larval age using bacterial preparations containing crystals alone from trans-conjugants A and D, as well as, that containing crystals and endospores from all bacterial strains and their transconjugants. In most cases, transconjugant preparations causing high levels of mortality than parental strains, because of high toxicity against *S. littoralis* larvae. The new product of transconjugant-B containing crystals and endospores appeared to have insignificant correlation between treatment time and the average weight of surviving larvae. It was indicated that this product increased pathogenicity and decreased both the growth and development of *S. littoralis* larvae. This new product from transconjugant-B decreased the average consumption of fed leaves and the average weight of surviving larvae and it would be preferable against *S. littoralis* in biological control. The results showed that higher levels of mortality, if a mixture of relatively pure crystals and spores was used as compared to either fraction of crystals protein alone.

Key words: *Bacillus thuringiensis*, biopesticide, conjugation, correlation coefficient, crystals, endospores, regression equation, *Spodoptera littoralis.*

Introduction

Cotton leafworm, *Spodoptera littoralis* (Boisd), is a major pest of field crops in Egypt, predominantly alfalfa, cotton, sugarbeet 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. Biological control of lepidopteran insect pests, affecting crop plants is possible by using *B. thuringiensis* subsp. HD-1 *Kurstaki* (Whiteley and Schnepf, 1986). Only a few bioassays with strains of *B. thuringiensis* Berliner have been carried out against this insect (Sneh *et al.*, 1981). Further screening of effective *B. thuringiensis* strains was therefore, undertaken (Navon *et al.*, 1983). Berliner (1915) reported the isolation of *B. thuringiensis* from diseased larvae of the Mediterranean flour moth, *Anagasta kuehniella* and subsequent investigators found that other Lepidoptera were also susceptible to this pathogen, though insects outside this order were not. From the data available, it was reasonable to conclude that *B. thuringiensis* was an order-specific pathogen and therefore its successful use as a possible microbiological insect control agent depended on dispersing enough spores in the environment to initiate an epizootic (Dulmage *et al.*, 1971). Hannay (1953) reported a crystalline body formed at sporulation in the cells of *B. thuringiensis* and suggested it might be associated with the insecticidal activity of this bacterium. Angus (1954) demonstrated that the crystals contained an alkaline soluble toxin for insects, and Heimpel (1967) named this agent the ó-endotoxin.

B. thuringiensis is an aerobic sporeformer which during the sporulation process produces some proteinaceous crystalline structures (delta endotoxins) that are toxic to a variety of insects (Jarrett *et al.*, 1987 and Ohba and Aizawa, 1986). After being ingested, the protein of the crystals dissolves in the alkaline gut of the insects; the epithelial gut wall loses integrity and gut contents mix with the blood, which becomes alkaline. These phenomena lead to paralysis of the insect. Since spores and crystals of *B. thuringiensis* are not known to be toxic to higher-order animals or plants, preparations of sporulating cells of *B. thuringiensis* have extensive applications as biological insecticides in agriculture (Karamanlidou *et al.*, 1991). The present investigation aimed to compare between wild type strains of *Bacillus* and their derived transconjugants through the relationship between mortality and the time of larvae feeding on leaves sprayed with *Bacillus* preparations.

Materials and Methods

Strains and media: *Bacillus thuringiensis* serovar *kurstaki* (NRRL HD-1), *Bacillus subtilis* (NRRL NRS-744) and their transconjugants used in this study are described in a previous work (Zaied, 2000b). All strains were maintained on L.B. slope medium, containing 5% peptone, 0.1% yeast extract and 0.5% NaCl, pH 7.5 (Puntambekar and Ranjekor, 1989). Larvae of *Spodoptera littoralis* were obtained from matured eggs presented on cotton leaves collected from cotton fields in El-Dakahlia governorate.

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 re-suspended in small volumes of distilled water. Crystal bands and spore pellets were collected and purified according to the method described previously by Karamanlidou *et al.* (1991).

Bioassay technique: Toxicity tests were performed on 4-day-old larvae (mean body weight = 10 mg). The larvae were fed on 2-3 gram of *Ricinus communis* leaves, added daily to a new breeding bottle in which the surviving larvae were transferred daily. The leaves added daily were sprayed with 200 μL of bacterial preparations (crystals and spores). The concentration of the *B. thuringiensis* component was approximately 10^9 crystals and spores per mL (Karamanlidou *et al.*, 1991). Control larvae were fed on leaves sprayed with 200 μl H_2O. Then these drops were evenly distributed over the diet surface of leaves with a sterile glass rod and the surface was air-dried. Surviving larvae and residual fed leaves were pooled and weighed daily (Ignoffo *et al.*, 1977 and Karamanlidou *et al.*, 1991). Larvae mortality was recorded daily. Evaporated water from the fed leaves in control breeding bottle through 24 hours was subtracted before recording the weight of residual fed leaves (Zaied, 2000a).

Statistical analysis: Data were subjected to regression equations procedure between each parameter recorded with the time of larvae feeding on leaves sprayed with bacterial preparations. The correlation coefficient between both of them was also calculated according to Steel and Torrie (1960).

Results and Discussion

Biological control lepidopteran insect pests, affecting crop plants has been possible using *B. thuringiensis* subsp. *Kurstaki* and their transconjugants with *B. subtilis*. As shown from Fig. 1, other than transconjugant-D and *B. subtilis* strain, all transconjugants revealed insignificant relationship between the time of treatment and average consumption of fed leaves (g/day). Because the bacterial preparation containing crystals of these transconjugants has been considered more toxic against the Egyptian cotton leafworm, *B. thuringiensis* produces an insecticidal protein toxin during sporulation. This toxin is effective against three orders of insect pests (Lepidoptera, Diptera and Coleoptera) (Travers *et al.*, 1987). The results presented in Fig. 2 describe the relationship between treatment time and the average consumption of fed leaves (g/day) sprayed with crystal + endospores. From these results, the data revealed other than transconjugant A, that all bacterial strains and most of their transconjugants showed insignificant correlation between both variables. This is due to the un-suitability of fed leaves sprayed with *Bacillus* preparations containing crystals + endospores, suggesting that feeding was reduced by the *B. thuringiensis* product. The present results are in agreement with those reported by Adams *et al.* (1992), who found that *B. thuringiensis* show remarkable specificity and toxicity towards number of economically important insect species, yet it has little or no effect on non-target organisms and is, therefore a safe, effective insecticide.

Although, *B.thuringiensis* is the major biological pesticide as shown in this study, it was used in the US and in other countries.The cost of *B.thuringiensis* and its modest activity towards certain agronomically important pests are undoubtedly factors contributing to its low usage relative to "harder" chemical pesticides. In an effort both to reduce the cost and to increase the performance of *B. thuringiensis*, this study was undertaken to improve; certain characteristics of *B. thuringiensis,* such as growth and sporulation, as well as, production of crystals protein. Transconjugant-D revealed negative correlation between both variables undertaken here, indicating that bacterial preparation containing crystals + endospores from these transconjugant possess high levels of toxicity against *Spodoptera littoralis*. 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). The same authors in 1983 have also suggested that insects which were not good targets for the toxins could lack the necessary receptor which could be exploited by soluble delta endotoxin.

The results presented in Figs. 3 and 4 revealed the relationship between treatment time and mortality percent related to the effect of bacterial preparation containing crystals or crystals + endospores, respectively. As shown from the results presented herein, all bacterial preparations containing crystals from transconjugants B and C revealed significant correlation between both variables. However, bacterial preparation containing crystals alone from transconjugants A and D, as well as, that containing crystals + endospores from all bacterial strains and of their transconjugants, revealed highly significant correlation between both variables. It is of interest to note that the specificities of the toxins of bacterial preparation containing crystals + endospores have more toxic effects on the larvae than which contained crystals alone. The development of bacterial transconjugants with desirable properties of *B. subtilis* and *B. thuringiensis* provides an interesting model system, for improving the toxicity of *B. thuringiensis* against *Spodoptera litura*, which is one of the more important pests infecting field crops of economic importance in Egypt. One of the important observations in this study was the time required to obtain 50% mortality which is appeared in these figures and were differed markedly from one strain to another and from bacterial preparations containing crystals alone to that containing crystals + endospores of the same strain. Increased susceptibility of *S. littoralis* to the bacterial preparation from *B. thuringiensis*, indicated by low LT_{50} observed. 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). In most cases, transconjugants preparation causing high levels of mortality than the parental strains and control experiment, because they were highly active against *S. littoralis* among crystals and crystals + endospores. This bioassay provided an effective treatment time / mortality response. The results indicated that delta-endotoxin-containing preparations of *B. thuringiensis* are at present increasingly

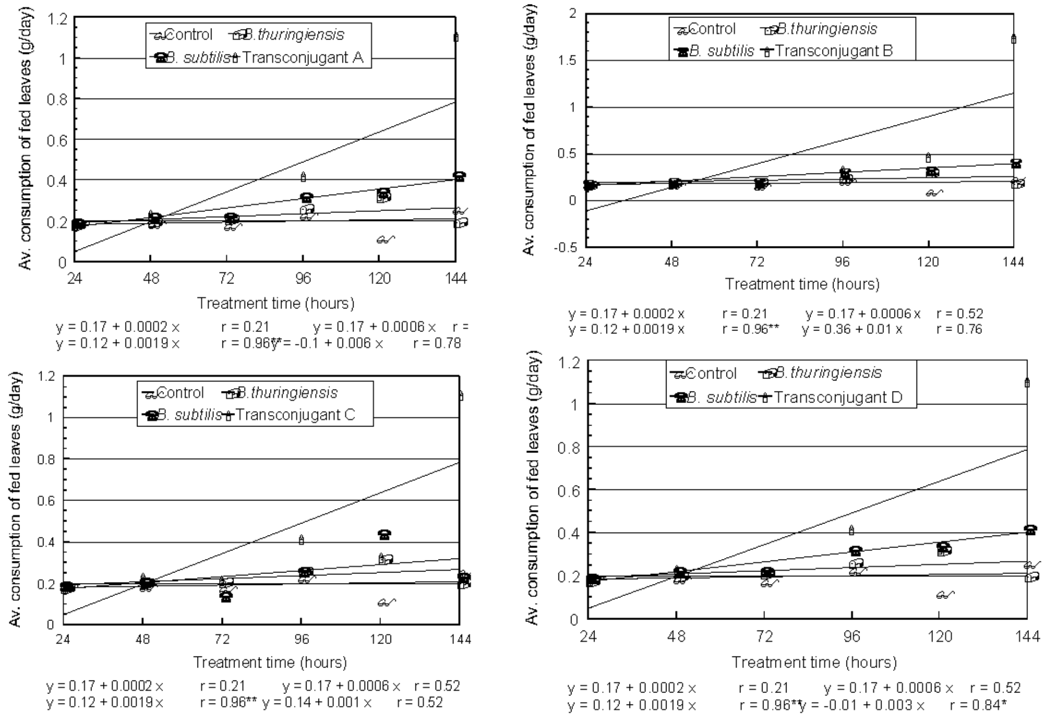

Fig. 1: Relationship between average consumption of fed leaves and the time of feeding on leaves, sprayed with crystals from *B. thuringiensis*

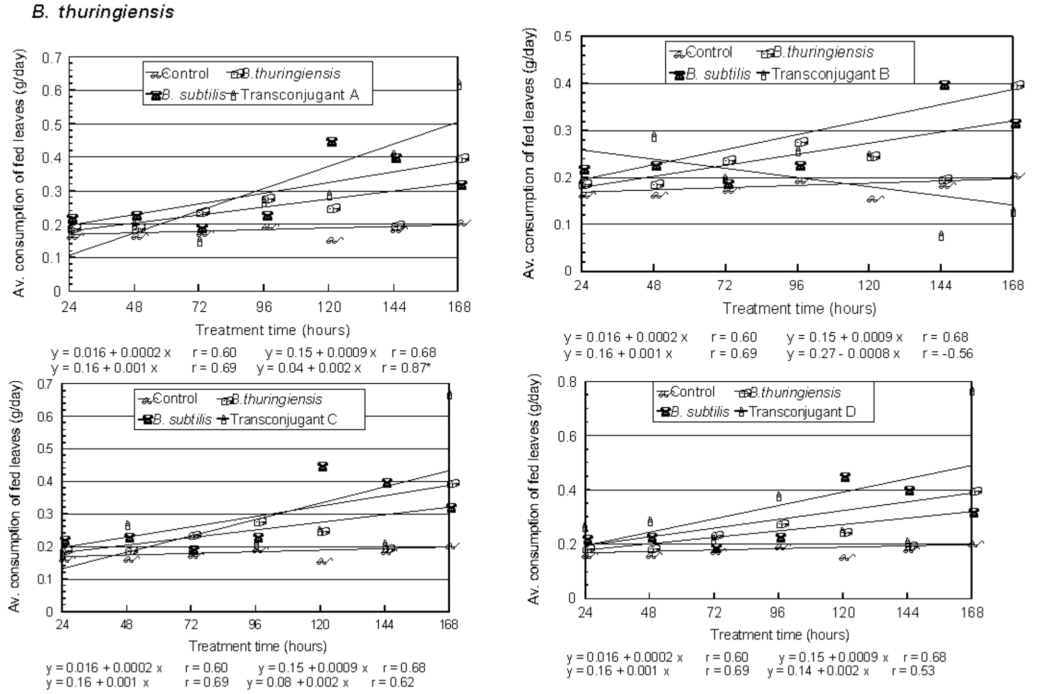

Fig. 2: Relationship between average consumption of fed leaves and time of feeding on leaves, sprayed with crystals from *B. thuringiensis*

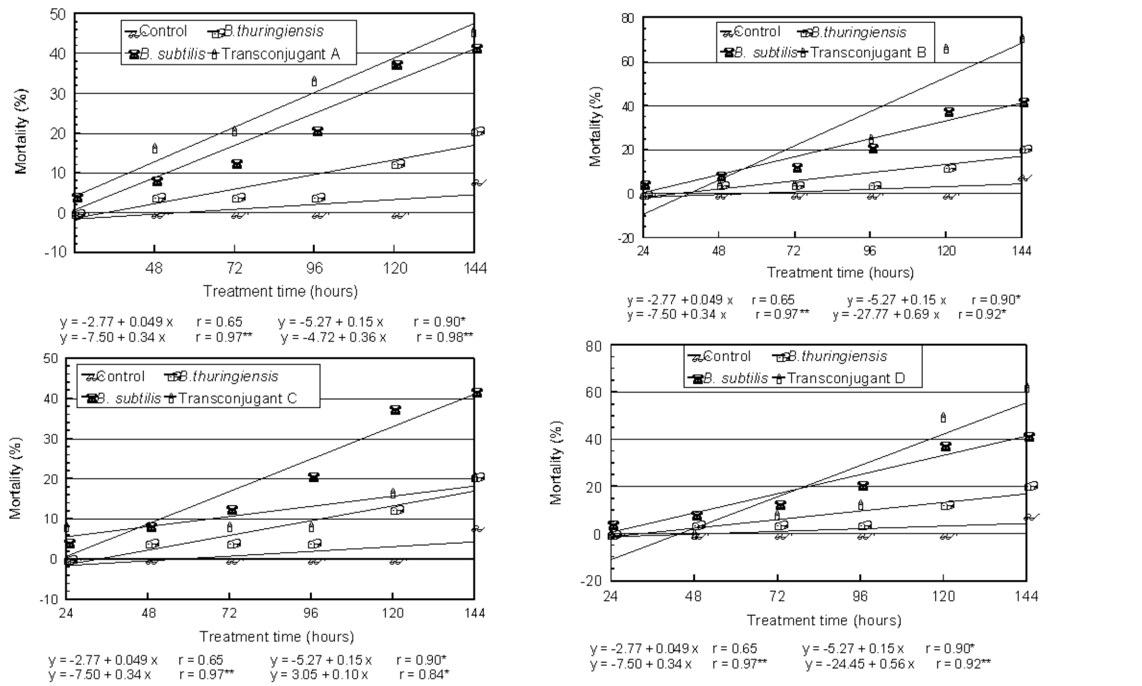

Fig. 3: Relationship between average weight of surviving larvae and the time of feeding on leaves, sprayed with crystals + endospore from *B. Thuringiensis*

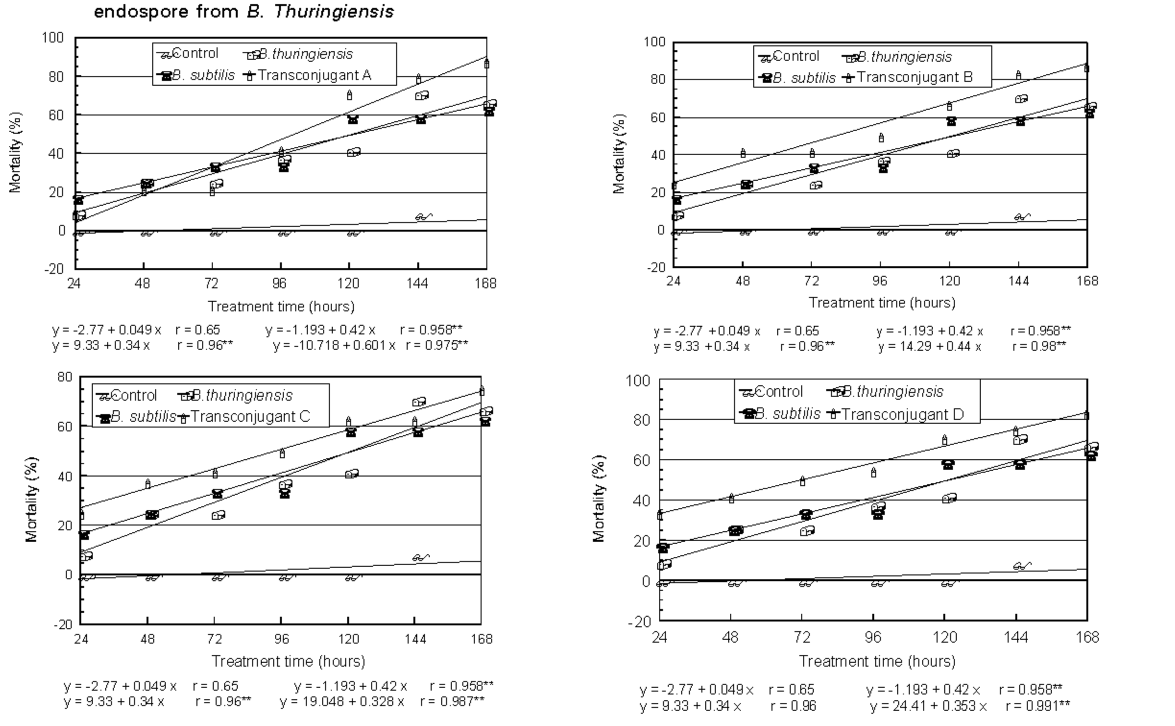

Fig. 4: Relationship between average weight of surviving larvae and the time of feeding on leaves, sprayed with crystals from *B. thruingiensis*

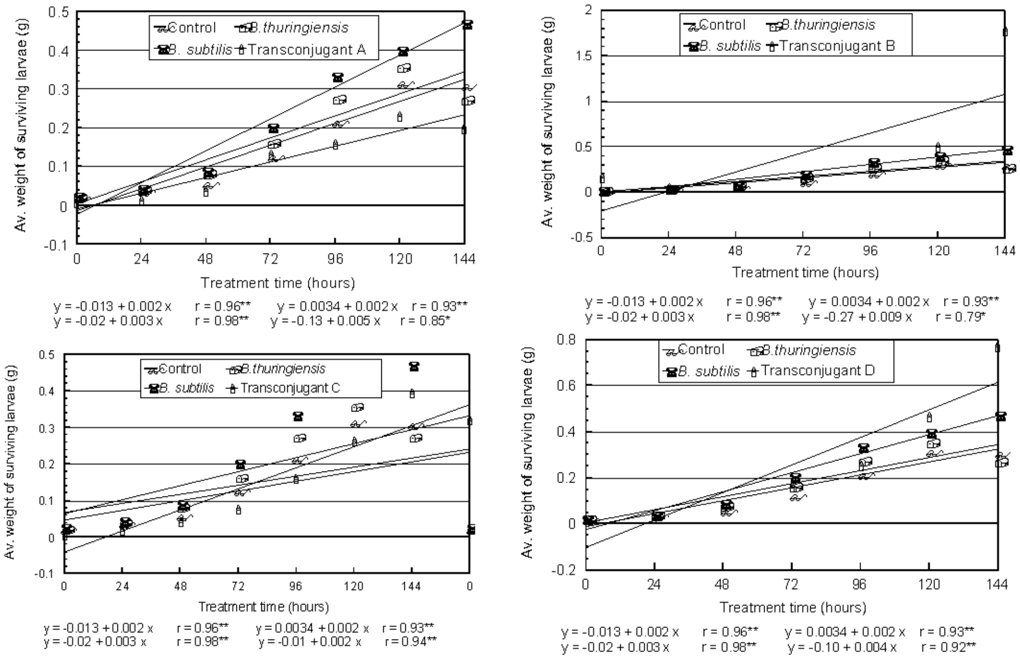

Fig. 5: Relationship between mortality of *Spodoptera littoralis* larvae and time of feeding on leaves, sprayed with crystals + endospores from *B. thuringiensis*

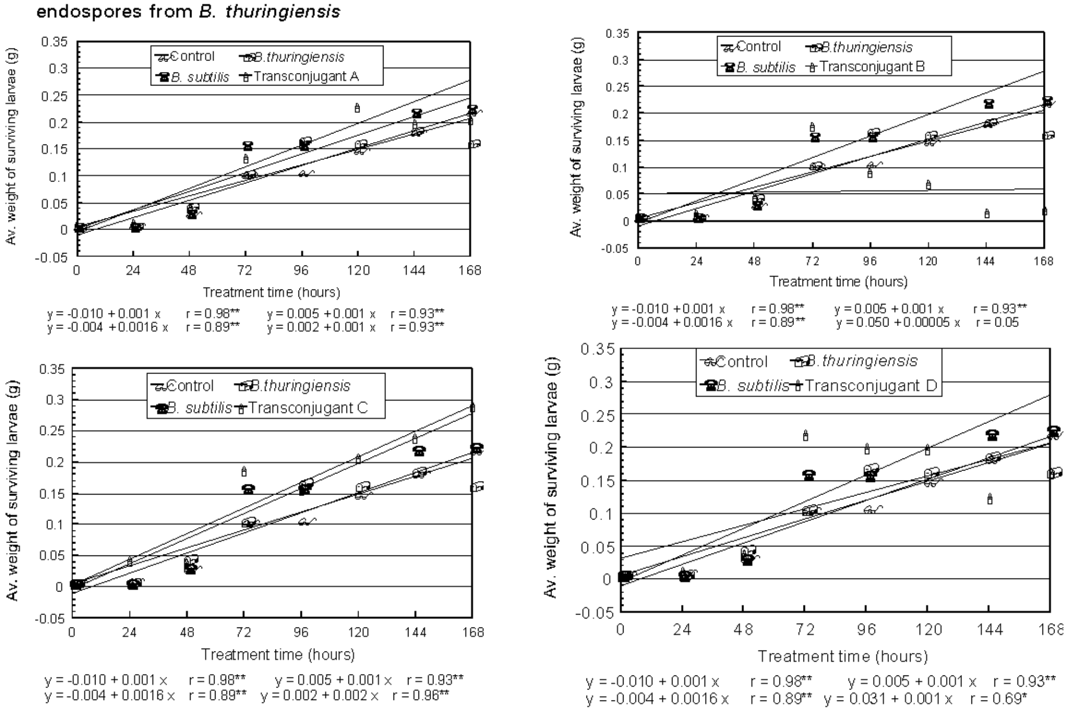

Fig. 6: Relationship between mortality of *Spodoptera littoralis* larvae and the time of feeding on leaves, sprayed with the crystals from *B. thuringiensis*

empolyed as insect controling agents. Up to now most investigations of the delta endotoxin action have been focused on the histopathology.

The results summarized in Figs. 5 and 6 demonstrated significant correlation between treatment time and average weight of surviving larvae, except for transconjugant B appeared insignificant correlation between both variables using bacterial preparation containing crystals + endospores. Furthermore, the significant correlation obtained here indicated that bacterial preparation would not affect growth and development of young *S. littoralis* larvae. It is possible that mature larvae are less affected by the *B. thuringiensis* product than newly hatched larvae, but this would not reduce the sensitivity of the potency bioassay, as shown from the previous Figs. 3 and 4 indicated mortality increased. It seems, therefore, that it is necessary to spray plants with *B. thuringiensis* against *S. littoralis* at an early stage of larval growth. On the other hand, the new products of transconjugant B containing crystals + endospores would be more effective than from other strains for fewer correlations obtained between both variables. This indicated that the product of transconjugant B increased pathogenicity and decreased both the growth and development of young *S. littoralis* larvae.

It seems, therefore, that the average consumption of fed leaves after the plants sprayed with this new product was decreased through the larval age, this relationship was appeared previously in Fig. 2. The new product of transconjugant B would be preferable against *S. littoralis* because of their probably more effective than other bacterial preparation in controlling *S. littoralis* on cotton plants. The present results are in agreement with Salama and Foda (1982) and Sneh *et al.* (1981), who found that *B. thuringiensis* var. *entomocidus* was also more effective than Dipel against insect larvae. Further selection of new strains of *B. thuringiensis* resulted from conjugation process as shown herein based on potency bioassays and effectiveness studies in the lab. or in the field would be useful to achieve microbial control of the pests.

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