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Efficacy and Residual Effect of *Bacillus thuringiensis* against Larvae of the Cotton Leaf worm, *Spodoptera littoralis* (Boisd.) in Egyptian Clover Fields

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ABSTRACT

The efficacy of Dipel-2x (*Bacillus thuringiensis* var. *kurstaki*) against 2nd larval instar of the cotton leaf worm, *Spodoptra littoralis* (Boisd.) was studied under both laboratory and field conditions. Four concentrations, 1,05, 0.25, 0.125% were used from the commercial product for the laboratory study. The highest (95%) and lowest (76%) mortalities were obtained at the 1 & 0.125%. There were reductions in pupation and adult emergence percentages at all used concentrations as compared with untreated larvae. The lowest reduction percentages recorded 5% for pupation and 1% for adult emergence at concentration 1%, while booth the highest percentages of failure in pupation and adult emergence recorded 2% at the same concentration. The longest larval period post treatment was 13.3 ± 0.12 days at concentration 1%, while for pupal duration it was 12.8 ± 0.22 days at the same concentration. Under field conditions, the mortality rate ranged between 18.84, 95.65 %, and 37.14, 17.14 after one and nine days respectively at two seasons. The accumulative residual effects of Dipel-2x compound under field conditions on *S. littoralis* were 95.65 and 97.14% in seasons 2002-2003 and 2003-2004 at Fayoum Governorate, after[illegible]s respectively.

Key Words: *Bacillus thuringiensis*, Cotton leaf worm, *Spodoptera littoralis*, Egyptian clover.

INTRODUCTION

Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.) is a major one among the many serious pests of vegetable, field and ornamental crops in Egypt. On the other hand, problems associated with indiscriminate insecticides usage, like poisonings, contamination of the environment, and residues in foods continue to be some of the critical problems facing the agriculture and agrochemical industries. An integrated control approach is the only possibility for the management of pest under which biological control through the use of *Bacillus thuringiensis* var. *kurstaki* (Dipel-2x) against it is a useful component in the integrated management program as mentioned by El-Husseini (1981), Salama, *et al.*, (1981), Hosny *et.al.*, (1983), Zaz 1989), Kares (1990), Abd El-Haleem (1997), El-moursy *et al.* (2000), Ben-Dov *et al.* (2003), Dutton *et al.* (2003) and Hosny *et. al.* (1983).

The aim of the present work is to evaluate and study the effectiveness of Dipel-2x (*B. thuringiensis* var. *kurstaki*) against the cotton leaf worm, *S. littoralis* .

MATERIALS AND METHODS

Dipel–2x (2000 IU/mg) based on *Bacillus thuringiensis* var. *kurstaki* was used in the present study to evaluate its efficacy under laboratory and field conditions. The used concentrations were 1, 0.5, 0.25 and 0.125% of the commercial formulation against 2nd larval instar of *S. littorallis* and at the rate of 300gm/fed. in clover field. A laboratory strain of the cotton leafworm, *S. littorallis* which has been reared in the laboratory for ten generations away from any insecticide contamination was used in this study. The larval instars were fed on castor bean leaves, *Ricinus communis* (L.). After pupation, the pupae were placed in wide glass jars until adult emergence. Then, adults were supplied with a piece of cotton wetted with 10% sugar solution and branches of Tafla (*Nerium oleander*) as an ovipositional site (El-Defrawi *et al.*, 1964). All stages were reared and treated under controlled conditions of 25±2°C & 65±5% R.H. Castor oil leaves were dipped in each concentration of Dipel-2x, for 10 seconds, then left to dry in air. One hundred larvae were used for each concentration divided into four replicates (25 each). Another group of larvae were fed on untreated leaves and kept as control. The tested larvae were starved for a period of 4–6 hrs before feeding on treated leaves to ensure rapid ingestion of the pathogen. The tested larvae were fed on treated leaves for 24hrs. The survivors were transferred to another clean jar and supplied daily with clean untreated castor oil leaves until pupation. The mortality was recorded daily. Mortalities after 4 days were calculated and corrected according to Abbott's formula (Abbott, 1925). For clover fields, the experimental area was divided into plots (1/24 feddan each) and every tested compound was applied at the recommended dose of 300gm/fed. As for clover, treatments and their control, one square meter (5 replicates) was inspected for each and larval population was recorded. For determining the effectiveness of the tested compound, percentage reduction in larval population of *S. littorals* was calculated according to Henderson and Tilton (1955).

Data were analyzed for determination of LC_{50}, and relative pathogenicity using Logprobit analysis software (LPD) developed by Dr. Ehab Bakr, Plant Protection Research Institute htt://www.Ehabsoft.Com according to Finney, 1971.

RESULTS AND DISCUSSIONS

Effect of *B. t.* (LC_{50}) on some biological parameters

1. Effect of Dipel-2x on larval mortality

The data presented in Tables (1&2) showed that increasing the bac. conc. in the suspension from 0.125 to 1% led to the increase of mortalities for 76 to 94.55% the LC_{50} value was 0.023 , while LC_{90} value was 0.487 g/100 ml water. The mortality among the check

groups was 2%.

2. Effect of Dipel-2x on pupation

The data presented in Tables (1&2) indicated that there were a reduction in pupation percentages in all tested concentrations compared with that resulted from untreated larvae. The pupation percentage decreased with the increase of the concentration of the microbial insecticide. It decreased from 24.0 to 5 when concentrations increased from 0.125 to 1%, while the pupation percentage of untreated larvae reached 98.0%.

3. Effect of Dipel-2x on failure of pupation

The data showed that the percentage of both of the larvae survived and those which were not able to continue their development after exposure to the formulation, were relatively high especially at the concentration 1% (Figs. 1&2). The value of LC_{50} was 0.0023, and LC_{90} was 0.000037g/100ml water. The percentage of deformed pupae reached 2%, while at the concentrations of 0.5, 0.25 and 0.125% it was 6, 7 and 10%, respectively opposed to 0.0% in the control.

4. Effect of Dipel-2x on adult emergence

There was a reduction in adult emergence from developed pupae due to the latent effect of the Dipel–2x. The lowest emergence percentage (1%) was recorded at concentration 1%, while the highest percentage (6%) was at the concentration 0.125% (Figs. 1&2). At the concentrations 0.5 and 0.25% the emergence percentages were 3, and 5%, respectively compared with 98.0% for the check.

5. Effect of Dipel-2x on failure of adult emergence

The data recorded in Tables (1&2) showed that the value of LC_{50} was 0.0018, and LC_{90} was 0.000047g/100ml water. The inhibition of adult emergence was 2% recorded for the highest concentration (1%) and the lowest was 8% at concentration 0.125% while the inhibition in adult emergence reached 2, 3% at the concentrations 0.5 and 0.25% compared with 0.0% in check.

6. Effect of Dipel-2x on durations of different developmental stages

The latent effect of *B. thuringiensis* extended from 2nd larval instar to adult. Data explaining such phenomenon were presented in Table (3) and could be summarized in the following points:

(i) Larval period post treatment was prolonged, at the lowest concentration of 0.125%. A slight increase was detected to reach 11.04±0.08 compared with untreated larvae (9.51±0.036). Increase in the Dipel-2x concentrations showed corresponding increases in the larval period to reach a maximum of 13.3±0.12 at the highest concentration of 1% Dipel-2x.

(ii) The pupal duration seemed to be relatively longer at treated larvae than the check and the averages were 10.78±0.06 and 12.8±0.22 days at the lowest and the highest concentrations (0.125 and 1%), respectively. On the other hands the concentrations 0.5 and 0.25% showed slight difference in the pupal duration between each other where they recorded 11.9±0.18 and 12.4±0.4 days, respectively compared to 10.47±0.038 days at the control ones.

(iii) The adult longevity of both sexes was markedly increased due to the increase of the concentration of bacteria. The average longevity of male and female moths resulted from treated larvae was 8.2±0.32 & 8.1±0.25 and 7.2±0.15 & 7.0±0.32 days at concentrations 0.5 and 0.25%, respectively compared with 7.03± 0.055 & 6.70± 0.047 days for the control ones, while the other concentrations showed slight difference. Generally, the elongation of the larval period, pupal duration and adult longevity of *S. littoralis* increased as the concentration of Dipel-2x increased compared with the corresponding check groups.

Efficacy of Dipel-2X , in Egyptian clover fields against *S. littoralis*

Data in Table (4) showed that the survivals of *S . littoralis* larvae generally decreased strongly as increasing in the time elapsed after spraying of the different concentrations. The initial mortality rate ranged between 18.84 and 37.14 %. The LT_{50} and LT_{90} values for Dipel-2x 1.782, 13.707 days, respectively.

Residual effect under field conditions

Taking into account, residual toxicity after 9 days of application, the used concentrations gave highest mortality percentage of 95.65%. Data in Table (4) indicated that the percentage of accumulative mortality increased with increasing the time of feeding

Table (1): Corrected mortality % of the second larval instar of *S. littoralis* after 24 hrs feeding on castor oil leaves treated with different concentrations of Dipel-2x (*B. thuringiensis* var. *kurstaki*).

Concentration %	Biological parameters				
	mortality%	pupation%	failure of pupae%	adult emergence%	failure adult emergence%
1	95.00	5.00	2.00	1.00	2.00
0.5	89.00	11.00	6.00	3.00	2.00
0.25	85.00	15.0	7.00	5.00	3.00
0.125	76.00	24.00	10.00	6.00	8.00

Table (2): LC_{50}, LC_{90} and slope values post 24 hrs feeding 2nd larval instar of *S. littoralis* on castor oil leaves treated with Dipel - 2x .

Biological parameters	LC_{50}	lower limit %	upper limit %	LC_{90}	lower limit %	upper limit %	slope
Mortality	0.023	0.0019	0.056	0.487	0.335	0.967	0.97
Failure of pupation	0.0023	-	-	0.000037	-	-	- 71815 E -5
failure adult emergence	0.0018	-	-	0.000047	-	-	- 8.571 E - 5

Table (3): Effect of Dipel - 2X on the larval period, pupal duration and adult longevity resulted from treated 2nd larval instar of *S. littoralis* .

Concentration %	Average period in days			
	larval period	pupal duration	adult longevity	
			female	male
1	13.3 ± 0.12 (12 - 14)*	12.8 ± 0.22 (12 - 14)	0	0
0.50	12 ± 0.09 (11 - 14)	11.9 ± 0.18 (9 - 13)	8.2 ± 0.32 (8 - 9)	8.15 ± 025 (6 - 8)
0.25	11.82 ± 0.05 (10 - 12)	12.4 ± 0.4 (9 - 14)	8.1 ± 025 (7 -9)	7.87 ± 022 (7 -9)
0.125	11.04 ± 0.08 (9 - 12)	10.78 ± 0.06 (9 - 11)	7.8 ± 0.22 (6 - 8)	7.54 ± 0.39 (5 - 9)
check	9.51 ± 0.036 (9 - 10)	10.47 ± 0.038 (10 - 11)	7.03 ± 0.056 (6 - 8)	6.70 ± 0.046 (5 - 7)

* range

Table (4): Effectiveness of Dipel - 2X at rate of 300 gm / fed. on the cotton leaf worm, *S. littoralis* expressed as percentage reduction of larval population and residual toxicity after spraying on Egyptian clover at El-Fayoum Governorate , Egypt, seasons 2002 -2003 and 2003-2004.

Season	No. larvae before spraying	No. larvae and reduction % in population												Total mortality
		one days		2 days		3 days		5 days		7 days		9 day		
		M.%	No. larvae	M.%	No. larvae	M.%	No. larvae	M.%	No. larvae	M.%	No. larvae	M.%	No. larvae	
2002 - 2003	95.65	95.65	3	9.86	7	86.96	9	71.01	20	4.78	45	8.84	56	69
2003 - 2004	70	44	37.14	25	64.25	13	81.43	6	92.43	5	92.86	2	97.14	97.14

on the treated clover leaves. This was depending on the activated toxin which resulted from lysis of δ-endotoxin in the mid-gut of the larvae. Fast and Regniere (1984) mentioned that the larvae may have a greater relevance to field efficacy of Dipel-2x, because larvae that live for a longer time will have better changes of recovery as the environmental degradation of the material progresses.

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