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EVALUATION OF *BACILLUS thuringiensis* (BERLINER) FORMULATIONS AGAINST THE PINK BOLLWORM *PECTINOPHORA gossypiella* (SAUND.)

[62]

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ABSTRACT

Laboratory experiments were carried out to investigate the effect of two bacterial commercial products of *B. thuringiensis* (Dipel 2x and Protecto) on eggs and the newly hatched larvae of pink bollworm, *P.gossypiella*. Different concentrations of both commercial products of *B. thuringiensis* were tested. The results showed that the percentage of larval mortality increased by increasing concentration and the period after treatment, calculated LC50s values after 3-7 days of treatment. Treatment of eggs did not affect, significantly, the hatchability. While the percentage mortality of newly hatched larvae produced from the treated eggs was high according to the concentrations used.

Key words: Pink bollworm, *Bacillus thuringiensis* (Dipel 2x , Protecto)

INTRODUCTION

Cotton is one of the most important crop in Egypt as well as some other world countries. This crop suffers from insect pests complex over all the growing cotton season (**Abdel-Halim *et al* 2002**).

The pink bollworm, *Pectinophora gossypiella* (Saund.) is one of the most important insect pests that attacks cotton in Egypt and other countries of the world (**Hossain *et al* 1999**). The chemical control of this insect pest faced serious difficulties because this insect developed resistance to most of the common commercial pesticides and caused harmful effect on environment.

One of the most recent and yet promising approaches in this respect is using of microbial control agents such as bacteria (**Bai and Degheele, 1992; Abdel-Hafez *et al* 1994**). For several decades since its discovery, formulations of *B. thuringiensis* proved to be an ideal means of controlling Lepidopterous insects pests in agriculture rather than the use of synthetic chemical formulations. (**El-Sorady 1998**)

This work aim to study the bioassay of two commercial formulations of *B. thuringiensis* against eggs and newly hatched larvae of pink bollworm.

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MATERIAL AND METHODS

1- Bioassay experiments

In this study, two commercial products of *Bacillus thuringiensis* (Dipel 2x and Protecto) were tested for their bioinsecticidal activities against eggs and newly hatched larvae of the pink bollworm, *P. gossypiella*. The characteristics of formulations as following:

A- Dipel 2x : Wettable powder produced. Contains parasporal crystals and spores of *B. thuringiensis*. subsp. *kurstaki* (32,000 international units pf potency per mg, IU/mg), produced by USA.

B - Protecto: Wettable powder produced. Contains parasporal crystals and spores of *B. thuringiensis*. subsp. *kurstaki* (32,000 international units pf potency per mg, IU/mg), The product was obtained from the Ministry of Agriculture, Egypt, Cairo.

2- Egg treatment

Two different commercial compounds of *B. thuringiensis* (Dipel 2x and Protecto) were used in this work to treat the deposited 1 day old eggs. The egg masses of *P. gossypiella* for different tests were collected from the oviposition jars. The concentrations used in this experiment were 1-2-3-4 and 5 g/lit. The check treatment was dipped only in water **(Darwish *et al* 1998)**. The number of unhatched eggs and empty egg shells were counted four and seven days after treatment. Each test was replicated three times (30-50 eggs/replicate). The freshly hatched larvae from each treatment (20 larvae), were transferred to glass tubes containing untreated diet. Then the mortality and alive larvae were calculated, and corrected by Abbott's formula **(Abbott, 1925)**

3- Larvae treatment

Six concentrations of the commercial products (2, 1.5, 1, 0.5, 0.25 and 0.125 g/lit) were used on newly hatchet larvae**,** then 5 ml of each concentration was homogenately mixed with 100g. of artificial diet according to **Zidan *et al* (1998)**. The treated diet of each concentration of
the tested compounds were transferred into glass tubes. The larvae were fed on treated diet for 48 hours and then transferred to untreated diet till pupation. The same number of larvae that fed on untreated diet were used as control. In all experiments, mortality was counted after 2, 3, 4, 5, 7 and 10 days. Each treatment was corrected by the use of Abbott's formula **(Abbott, 1925)**. The percentages of mortality were statistically computed according to **Finney, (1952)**. Computed mortality percentages were plotted versus log, concentrations on logarithmic probit paper to obtain the corresponding

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regression mortality lines, the required concentrations to give 50% kill were estimated from the established regression lines **(Jyoti and Brewer, 1999)**. Each concentration test included four replicates each of 20 larvae (80 larvae / concentration, 320 larvae / treatment). Artificial diet used in the present work for the pink bollworm, *P. gossypiella*, was mainly consists of kidney bean diet prepared according to **Abdel-Hafez *et al* (1982).**

4- Biological studies

Newly hatched larvae were treated in the same manner with the determined LC50 s to study the effect of Dipel 2x and Protecto on certain biological aspects of this insect pest. Three replicates of newly hatched larvae (40 larvae/replicate) were used for each compound. Larval and pupal mortalities, their durations and deformation as well as pupal weight, adult emergence, and moth were recorded. The experiments were carried out under laboratory conditions of 26±1C° and 70±5% R.H.

Statistical analysis

Statistical analysis of bioassay resulted by ANOVA test, in the computer. Program Sigma plots for Windows (version 2) was used to calculate the LC50 values.

RESULTS AND DISCUSSION

1- Effect of eggs treatment of pink bollworm *P. gossypiella* with different concentrations of Dipel 2x and protecto

The effect treatment of *P. gossypiella* eggs with different concentrations of *B. thuringiensis* products (Dipel 2x, Protecto) is shown in Table (2). The hatching percentage were 52.1% in control, averaged between 62.2 and 42.7% for highest concentration, and 54.5 and 58.8%, for lowest concentration of both products respectively, after four days of eggs' treatment. On the other hand, after seven days of eggs' treatment the hatching percentage was 87.6% in control, while they averaged between 82.8 and 75.9%, for highest concentration, and 88.6 and 71.3%, for lowest concentration of both products, respectively.

Similar results were reported by **Salama *et al* (1985)** when sprayed *Spodoptera littoralis* egg masses with *B. thuringiensis* hatched normally. while **Azab, (2003)** found that treatment of the *Agrotis ipsilon* eggs was susceptible to bacterial infection.

On the other hand, data in Table (2) indicated that treatment of eggs affected the survival on hatching. the percentages of mortality larvae after 2 days of hatching reached 37.8 and 24.0% for both products respectively. Seven days later, these percentages increased to 86.3 and 80%, mortality of larvae pink bollworm for both products respectively.

The previously mentioned results indicate, however, that the severest effect due to treatment of pink bollworm eggs by Dipel 2x and Protecto was the high larval mortality percentages after exclusion until pupation. This may be due to the part of treated egg-shell swallowed by the larvae at the time of exclusion, the aforementioned results coincide with **Salama *et al* (1985); Abd El-Hafez *et al***

(1994); Darwish, *et al* (1998) and Azab, (2003).

2- Bioassay of Dipel 2x and protecto, on the pink bollworm, *P. gossypiella*

Results in Table (1) showed the mortality percentages of pink bollworm in newly hatched larvae after feeding them on artificial diet treated with different concentrations of both commercial products of *B. thuringiensis* (Dipel 2x and Protecto). All the tested biocides had a great efficacy against pink bollworm larvae.

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Table 1. Hatchability percentages of *Pectinophora gossypiella* eggs treated with different concentrations of Dipel 2x, Protecto

Treatment Concentration g/lit	Dipel 2			
	%Mean hatching of eggs		% mortality of larvae	
	After 4days	After 7days		
	% hatched ± S.E	% hatched ± S.E	After 7 days	After 2 days
5	62.2 ±2.36 a	82.8 ±3.29 a	86.3	37.8
4	58.7 ±1.89 a b	84.4 ±1.89 a	82.5	38.8
3	63.4 ±2.83 a	86.5 ±2.36 a	80	24.5
2	59 ±1.88 a	83.7 ±1.74 a	80	16.1
1	54.5 ±2.12 b	88.6 ±2.17 a	67.5	19.5
check	52.1 ±2.87 b	87.6 ±1.70 a	10	6.6
L.S.D 0.05	6.71	7.55		
	Protecto			
5	42.7 ±2.50 b	75.9 ±2.36 b	80	24
4	56.2 ±1.98 a	70 ±1.89 c	62.5	13.9
3	57.8 ±2.69 a	71.2 ±1.18 c	35	6
2	54.8 ±2.27 a	79.8 ±1.42 b	32.5	17.6
1	58.8 ±1.79 a	81.3 ±2.15 b	22.5	12.2
Check	52.1 ±2.87 a	87.6 ±1.70 a	10	6.6

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Table 2. Corrected mortality percentages among the newly hatched larvae of pink bollworm *Pectinophora gossypiella* fed on artificial diet treated with Dipel 2x and Protecto

Mean of Corrected mortality %						
Treatment	Concentration g/lit	Days after treatment				
		2	3	5	7	10
Dipel 2x	2	67.4	71.1	99.8	100	100
	1.5	55.3	65.8	74.2	83.5	89.0
	1	43.7	52.6	64.3	85.2	85.2
	0.5	31.6	38.4	50.5	76.9	76.9
	0.1	26.3	39.5	45.1	45.1	64.3
	0.05	17.4	17.4	34.1	36.8	36.8
	0.025	15.8	16.8	22.0	25.8	28.6
Protecto						
	2	42.1	59.5	69.8	74.2	76.9
	1.5	41.1	42.1	53.3	56.0	57.7
	1	33.2	39.5	45.1	50.5	50.5
	0.5	31.6	35.8	47.8	47.8	50.5
	0.1	13.2	26.3	42.3	49.5	49.5
	0.05	5.3	21.1	25.8	27.5	28.6
	0.025	9.5	15.8	16.5	23.1	25.8

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Results indicate that the mortality rates increased with increasing the concentrations and the period after treatment. Mortality percentages were 16.8, 15.8 % within three days when the lowest concentrations (0.025 g/l) of Dipel 2x and Protecto were used, respectively. While, these percentages were increased to 25.8, 23.1% 7days after treatment, respectively. Using the highest concentration (2.0g/l), mortality percentages recorded 71.1 and 59.5% among after three days, while it were 100, 69.8% after 7 days for above mentioned larvae, respectively. The concentration mortality lines were graphically illustrated in Figs (1&2). It could be noticed that the highest percentage of mortality occurred within the first five days after treatment when highest concentration (2.0 g/l) was used, while mortality continued among larvae many days after treatment with different rates according to the concentration of the biocide products used, at high concentrations. These results are in agreement with those obtained by **Shalaby *et al* (1986) and Zidan *et al* (1998).**

The results of this study demonstrated that commercial products of *B. thuringiensis* used were effective in causing mortality of the pink bollworm. Such results coincide with **Watson (1995) and Romeilah and Abdel-Meguid (2000).** Morality among larvae was increased by increasing either the concentration or the period after treatment.

The concentration-mortality relationship of an insect to a toxin is typically expressed as an LC50 value, of the population in a specified period. Dipel 2x had the lowest LC50 value after 3, 7 days, (1.03, 0.29 gm/l), while for Protecto it as 1.60, 0.90gm/l after 3, 7 days, respectively, Table (1).

3- Effect of LC50 concentration of two commercial products of *B. thuringiensis* (Dipel 2x and protecto) on larval, pupal and adult stages of pink bollworm *P. gossypiella*

Data presented in Table (3) show that the concentrations of the two tested commercial products of *B. thuringiensis* (Dipel 2x and protecto) which kill 50% of the treated larvae (LC50). The untreated larvae and pupae required an average of 18.6and 9.1 days, respectively, (larval and pupal periods). Dipel 2x and Protecto treatment, showed retardation in larval and pupal development. Larval period reached 22.7, 21.1 days, after treatment with LC50 concentration of Dipel 2x and Protecto respectively, while pupal period increased significantly to 10.4, 9.6, days, after the aforementioned treatments, respectively.

The effect of treatment of the newly hatched larvae with LC50 concentrations of Dipel 2x and Protecto on the percentage of pupal deformities (5, 2.5%) was recorded. The latent effect of the same treatments reflected on the pupal weight, whereas the untreated pupa weighed 22 mg., while the pupae reared

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on diet containing LC50 concentrations of Dipel 2x and Protecto were 15 and 17 mg. respectively. These results revealed that Dipel 2x was more effective than Protecto, as it resulted significantly the longest larval and pupal periods. The present results agree with those obtained by **Radwan (2002)**.

The earlier treatment effect of the newly hatched larvae with LC50 of Dipel 2x and Protecto extended till the produced adults, as an external deformation in the resulted moths (Table, 3). Moths deformation averaged 5% in the control

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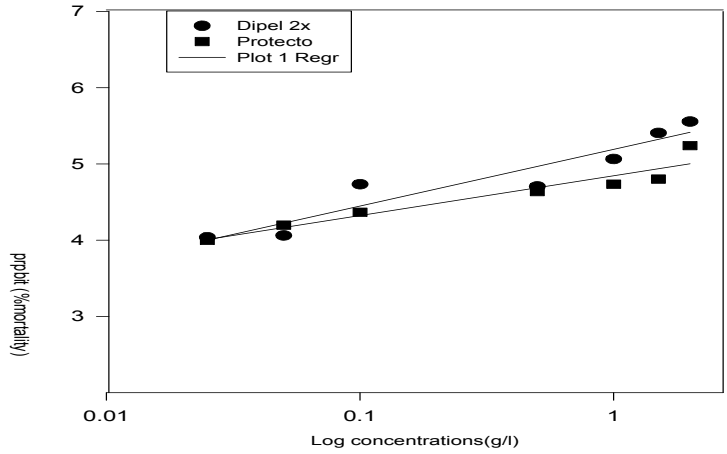

Fig. 1. Toxicity lines of Dipel 2x and Protecto on pink bollworm after three days following treatment

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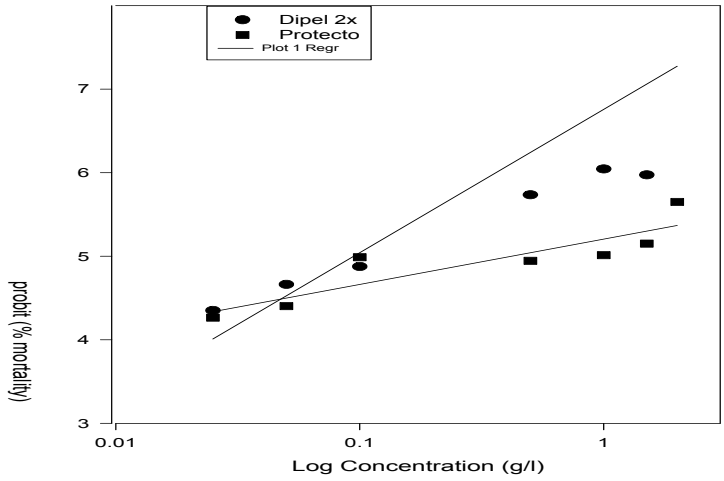

Fig. 2. Toxicity lines of Dipel 2x and Protecto on pink bollworm after seven days following treatment

Table 3. Certain biological aspects of the pink bollworm, *Pectinophora gossypiella* after treating the newly hatched larvae with LC50 concentration of the Dipel 2x and Protecto

Biological aspects (Means ± S.E)	Treatment				
	Dipel 2x	Protecto	Untreated check	F. value	L.S.D at 0.05
Larval duration (days)	22.7 ± 0.81	21.1 ± 0.86	18.6 ± 0.56	7.11	2.25
Larval weight after 15 days (mg)	51 ± 1.26	62 ± 1.95	81 ± 3.51	16.9	7.25
Pupal duration (days)	10.4 ± 0.51	9.6 ± 0.52	9.3 ± 0.38	1.3	1.45
Pupal weight (gm)	15 ± 0.58	17 ± 1.00	22 ± 0.97	15.3	2.68
Adults long days	12.1 ± 1.04	13.9 ± 0.99	17.6 ± 1.12	6.35	3.24
Percentage of pupal %	22.5 ± 1.39	30 b ± 2.30	92.5 ± 2.59	8.05	4.30

Pupal deformation %	5 ± 0.34	2.5 ± 0.23	0 ± 0	4.5	1.29
Adults deformation %	12.5 ± 0.58	7.5 ± 0.47	5 ± 0.09	6.46	5.00

- No. of insects used = 40 larvae.

while it increased significantly in the two commercial products to 12.5, 7.5%, respectively.

The adult longevity averaged 14.6 days in the control, while this value varied insignificantly to 12.1, 13.9 days after treatment with LC50 concentrations of Dipel 2x and Protecto, respectively. Such results coincide with **Mohamed *et al* (2000)** on first instar larvae of *Spodoptera littoralis* and *Agrotis ipsilon* treated with *B. thuringiensis*, while longevity of the moth was affected by larval treatment. While **Radwan, (2002)** who found that response of *Erias insulana* larvae to certain biopesticides caused shortened the adult life span and prolonged the larval and pupal durations.

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تقييم تحضيرات التبكتيريا ثلاثة بيوريا جنوسيس ضد دودة اللوز القرنفلية

]62[

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أجريت تجارب معملية لدراسة تأثير المبيدات الميكروبية (LC50) بعد المعاملة ببعض الثبات النسبة عند اثنين من المستحضرات البكتيرية التجارية أيام، وكان لهذا التركيز تأثير على زيادة فترة التحول للباسيلس ثيورنجينسيس (2 أكس، لل بيوبيوكس) على طول الرقص وعاء الزراعة بالبيض والرقائق تأثير الفراشات ونقص في وزن البيض وكذلك بروتكتيت الطور البيضي تختلف المعاملات والأخلاقيات للبيض في الرقائق بعد الزراعة الحيوية ونسبة البيض الذي أظهرته المعاملة كما أظهرت النتائج ووجود أي تأثير للمستحضرين التجاريين على دودة الزراعة.

وقد أظهرت النتائج المبكرة تأثير بعض المعاملات على بيض البيض والسكريات الحية كفاءة اليرقات التي لم تنضج البيض الثلاث والتي تقرب تحولها البيض الداخلي ونمو في الأغذية كفاءة البيض وتجاوز المحللات الداخلية بنسب الموت البيضي بيض زيادة التركيزات المستخدمة طول فترة المعالجة، وتختلف فالكثافة المستخدمة في التراكيز الدراسة وقد حسب قيمة التركيز المستخدمة.

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