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THE ROLE OF *BACILLUS THURINGIENSIS* IN INCREASING THE SUSCEPTIBILITY OF PROFENOFOS - RESISTANT STRAIN OF *SPODOPTERA LITTORALIS* (BOISD.) TO CERTAIN INSECTICIDES.

Mohamady, Aziza , H .

Central Agricultural Pesticides Laboratory, Agricultural Research Center, Dokki, Giza, Egypt.

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ABSTRACT: Laboratory experiments were conducted to evaluate the effectiveness of *Bacillus thuringiensis* (Dipel 2X and Xentari) on reducing resistance in the cotton leafworm *S. littoralis* and their biological effects.

Results indicated that treatments of the first instar larvae of profenofos - resistant strain of *S. littoralis* by 150 and 300 g / feddan of Dipel 2X and Xentari caused high mortality percentages during the larval stage. Treatments greatly reduced pupation. The activity of Dipel 2X was slightly higher than Xentari treatments. *B. thuringiensis* treatments reduced the fecundity of emerged adults, the hatchability of deposited eggs and shortened the adult life span while larval and pupal durations were prolonged. On the other hand, 300 g / feddan of Dipel shortened the larval duration but 300 g /feddan of Xentari prolonged the adults life span.

Selection of profenofos - resistant strain with *B. thuringiensis* products for one generation reduced the resistance levels to profenofos, es-fenvalerate, and chlorfluazuron, whereas slight increased in resistance took place with methomyl. The lower application rate of *B. thuringiensis* induced a higher decrease in resistance ratios to some of insecticides than the higher one.

Key words: *Spodoptera littoralis* - biocides - delayed effects - cross resistance.

INTRODUCTION

The resistance problem is the greatest single challenge facing applied entomologists today, because of the widening circle of cross and multiple resistance among insect pests, the diminishing number of effective commercial insecticides and the exponentially-increasing costs of development of new insecticides (Metcalf, 1980).

Spodoptera littoralis (Boisd.) is one of the most economically important pest in Egypt. Control of the cotton leafworm has depended exclusively on insecticides. As a result, this pest has developed resistance to major classes of insecticides.

Excessive and indiscriminative use of conventional insecticides has resulted in the development of pest resistance to insecticides and resurgence of new pests, decreased of farmer's income, pesticide residue and environment pollution. Cases of resistance had been reported by several investigators (El-Guindy *et al.*, 1982; Ayad *et al.,* 1989 and Ghoneim *et al.*, 1994). Globally, formulated sprays of microbial insecticides such as *Bacillus thuringiensis* has bean widely used as an alternative to chemical insecticides. These new plant protection products are termed reduced-risk insecticides because of their narrow spectrum of activity and low toxicity to humans and non-target organisms, and are considered IPM-compatible (Maxwell and Fadamiro 2006).

B. thuringiensis is a gram positive bacterium that produces crystalline inclusions during sporulation. These inclusions consist of either one or a subset of related insecticidal crystal (Cry) proteins. The crystal inclusion is dissolved by the alkaline larval midgut of insect species to release a 130 kDa protoxin that is cleaved and activated by midgut proteases. Once activated, the toxin interacts with an appropriate midgut epithelial cell receptor and inserts into the gut membrane, producing spores that disturb the osmotic balance causing cells to swell and eventually lyse and as a result the larvae stop feeding and die (Manyangarirwa *et al.* 2006).

Many Lepidopterous species have been successfully controlled by microbial agents, e.g. *S. littoralis* (El-Hamaeky *et al.*, 1990; Salama *et al.*, 1993; Salem 1995, El-Gahr *et al.*,1995 and Romeilah and Abdel Megeed (2000) and *Heliothis virescens*, *Plutella xylostella*(L.), *Pieris* *rapae*(L.), and *Trichoplusia ni* (Hubner) (White *et al.*, 1999 and Maxwell and Fadamiro 2006).

The object of this study was to evaluate the effect of two *Bacillus thuringiensis* (Dipel 2X and Xentari) on resistance level of a resistant strain of *S. littoralis*. Effect of these products on some biological activities of a resistant strain was also evaluated.

MATERIALS AND METHODS

Strains

A laboratory strain of *Spodoptera littoralis*, reared free from any insecticide contamination under laboratory constant conditions, as described by El-Defrawi *et al.*, (1964).

An organophosphorus resistance strain, the parents were brought to the laboratory from the cotton fields of Kafr–El–Shikh Governorate and selected for resistance to the OP compound profenofos for 16 generations. The 4^{th} instar larvae were selected with LC_{50} of profenofos by using leaf dipping technique every generation until the resistant strain reached to 54.29-fold compared with the laboratory strain.

Chemicals Used

Bio-insecticides

Dipel 2X (6.4 WP): a formulation of *B. thuringiensis* subspecies *kursatki*.

Xentari (10.3 WG): a formulation of *B. thuringiensis* subspecies *aizawai*.

Two concentrations (150 and 300g/ feddan) were used from each product.

Insecticides Used

Profenofos (72% EC), es-fenvalerate (10% EC), methomyl (90%EC) and chlorfluazuron (5%EC). Six concentrations of each tested insecticide were used.

Method of Application

Treatments were applied by the use of leaf dipping technique (Shepared, 1958). Fresh castor bean leaves were dipped in each concentration of the tested chemical for 20 seconds and left for air dryness before being offered to the larvae.

With the bio- insecticides, first instar larvae of *S. littoralis* was used. About 260 larvae were put in three glass jars (1liter) and provided with treated leaves (the concentrations used were 150 and 300g / feddan). The larvae were confined with the treated leaves for 48h. The survived larvae were transferred to feed continuously on untreated leaves until pupation. For the conventional insecticides, the larvae were left to feed on treated leaves for 24 h after which mortality counts were recorded. In case of the chitin synthesis inhibitor chlorfluazuron the larvae were exposed to treated leaves for 48h. The survived larvae were transferred to feed on untreated leaves for another 24h; with this way the holding period, after which mortality percentages were recorded, was 72h. Mortality percentages were recorded and corrected using Abbott's formula (Abbott, 1925). The LC_{50} values were calculated according to the method of Finney (1971).

Effect of Selection for One Generation with Tested Bio-insecticides on The Biotic Potential, Susceptibility and Cross-Resistance Pattern of a Profenofos-Resistant Strain of *S. littoralis*

The effect of treatment of the first instar larvae of R-strain with 150 and 300 g /feddan of Dipel 2X and Xentari for only one generation on certain biological criteria as well as the sensitivity of the resulting 4^{th} instar larvae to certain insecticides were determined. The resistance ratio (RR) for the different insecticides were estimated and compared with the parent R-strain.

The resistance ratios (RR) of the different insecticides were estimated according to the following equation: Resistance ratio (RR) = LC_{50} of insecticides following treated resistant strain with *B. thuringiensis* / LC_{50} of insecticides to laboratory strain.

RESULTS AND DISCUSSION

Effect of Dipel 2X and Xentari on the First Instar Larvae of *S. littoralis*

Data in Table (1) revealed that the percentage of larvae which died before reaching the pupal stage due to treatment with Dipel 2X and Xentari were 88.46 and 93.08% and 83.46 and 90.77% at the two tested rates 150 and 300 g / feddan, respectively.

Results also indicate that treatments reduced obviously normal pupation. Pupation percentages ranged between 6.92 and 16.15 compared with 78.5 in the control.

On the other hand, slight mortality took place in the formed pupae and adult; mortality percentages ranged between 1.15 and 3.46, and 0.38 and 1.15 with pupal and adult stages, respectively.

In this respect, Hussein *et al.* (1990) reported that *B. thuringiensis* caused remarkable reduction in larval populations of *Pectinophora gossypiella* in Egyptian cotton fields, and these reductions were positively correlated with the concentrations of *B. thuringiensis* products.

Yamamoto *et al.* (1990) revealed that, a significant reduction in the pupal population of *Alabama argillacea,* 6 days after application of *B. thuringiensis* due to some secondary effects in the larvae before pupation. El - Halim (1993) evaluated the acute and latent effects of Dipel 2X against 2nd instar larvae of *S. littoralis*. Dipel 2X had slight acute toxicity. Both larval and pupal durations were remarkably prolonged with dose increase, while pupation percentage was reduced.

Remarkable latent adverse effects were detected on adult emergence, fecundity and egg viability, particularly with doses above 320IU/ ml

Salama *et al.* (1995) investigated the efficiency of *B. thuringiensis* and some insecticides to control *Agrotis ipsilon and S. littoralis.* With *B. thuringiensis* 96.1 and 88.3% larval mortality was obtained, respectively as compared to 96.8% with Lannate. Salama *et al.* (1999) assessed the efficacy of the commercial product Dipel (*B. thuringiensis* var. *kurstaki*) for the control of *S. littoralis* in *Trifolium alexandrium* in Egypt. The application of 700g Dipel 2X/ feddan produced similar level of pest control as Lannate application. Recently, Maxwell and Fadamiro (2006) evaluated several reduced – risk insecticides Dipel, Xentari, Dipel + Xentari, Entrust and Novaluron for management of three lepidopteran cole crop pests. They found that all tested insecticides formulations were effective in reducing infestations of the three lepidopteran pests and in providing marketable cabbage and collards in Alabama.

As shown in Tables 2 and 3 the larvae duration was adversely affected in all treatments with Dipel 2X and Xentari. The larvae and pupal durations were prolonged in all treatments compared with the untreated check (control). Prolongation percentages in all larvae duration showed 34.29 and 31.81 and 29.52 and 31.43 for

Table 1. Effect of Dipe 2X and Xentari (*B. thurinigensis* bio-insecticides) at rate of 150 and 300 g /feddan on the biotic- potential of the first instar larvae of resistant strain *S. littoralis*

Compounds	Rate of application gm / Feddan	Percentage mortality (%)				Pupation (%)	Normal adult emergence (%)	Total mortality (%)
		Larva	Prepupa	Pupa	Adult			
Dipel 2X	150	88.46	-	3.46	-	11.54	8.08	91.92
	300	93.08	-	1.15	1.15	6.92	4.62	95.38
Xentari	150	83.46	0.38	2.69	0.38	16.15	13.08	86.91
	300	90.77	-	2.31	-	9.23	6.92	93.08
Control	-	21	0.5	6	1	78.5	71.5	28.5

Dipel 2X and Xentari at 150 and 300g /feddan, respectively. All treatments reduced the male pupal duration and prolonged the female pupal duration (except female pupae resulted from treatment with 150 g / feddan of Xentari). Prolongation in larval duration and female pupal duration and shortening in male pupal duration may be attributed to the low and slow or fast metabolic rate of larvae treated with bio-insecticides. Dutton *et al.* 2003. found a significant increase in mortality, prolongation of developmental time and a slight decrease in weight were observed for *Chrysoperla. carne*a fed with *B. thuringiensis* contaminated *S. littoralis* larvae.

The obtained results indicated a decrease in female pupal weight in all treatments except in Xentari treatment at 300g / feddan. The rate of decrease in female pupal weight ranged from 7.65 to 24.47%, while the male pupal weight was increased in all treatments except with the high rate of Dipel 2X treatment as the pupal weight was slightly decreased to reach 2.64%. The rate of increase ranged from 19.97 % for low rate of Dipel 2X and 45.93 for high rate of Xentari.

The decreased effect of the bio-insecticides on adult life span, fecundity and hatchability of deposited eggs occurred in all treatments. The fecundity of adult females was highly reduced with

Dipel 2X and Xentari at the higher rate than the lower one.

The reduction in fecundity was decreased to 35.36 and 66.08% for Dipel 2X and 52.35 and 62.58% for Xentari at rates of 150 and 300g /feddan, respectively. The highest percentages of sterility were gained from treatment with high rate of Dipel 2X (72.07%) followed by the low one of Xentari (69.13%).

Grove *et al.* 2001. reported that the proteolytically activated insecticide crystal proteins significantly reduced the life span of *Heliothis viresens* and *Spodoptera exigua* adults at the concentration of 500 μg/ml.

The previous results indicated that treatment by the higher rate of Dipel 2X produced highest deleterious effect on development of first instar larvae of *S. littoralis* followed by Xentari.

The Effect of Selection of Profenofos - Resistant Strain with 150 and 300 g / feddan of *B. thuringiensis* (Dipel 2X and Xentari) for One Generation on

Susceptibility to Certain Insecticides.

The insecticides tested were the organophosphorus insecticide profenofos, the carbamate insecticide methomyl, the synthetic pyrethroid insecticide es- fenvalerate, and the chitin synthesis inhibitor chlorfluazuron. The resistant ratios (RR) to the different insecticides were calculated and compred to those obtained for the parental generation of the profenofos-resistant strain (Tables 4 and 5).

The profenofos – resistant strain when treated with Dipel 2X and Xentari for one generation decreased obviously the resistance levels to some insecticides, while increased the level of resistance of the others. The resistance ratios (RR) of the untreated parental generation of the profenofos – resistant strain were 54.29, 2.18, 44.03 and 1.98 – fold, respectively, for the insecticides profenofos, methomyl, es- fenvalerate and chlorfluazuron. Some of these values were decreased to 36.78 and 10.97 – fold, and 40.69 and 36.32 – fold for profenofos and es - fenvalerate after treating the parent larvae with 150 and 300 g / feddan of Dipel 2X, respectively. These treatments exhibited lower increase in resistance ratio to chlorfluazuron to reach 2.35 and 2.73 – fold, respectively. As for Xentari treatments, the resistant ratios for profenofos, es – fenvalerate and chlorfluazuron were decreased to reach 31.97, 26.92 and 1.42 – fold and 36.77, 35.62 and 1.72 – fold) after treating the parent larvae with 150 and 300 g / feddan, respectively. On the other hand, the resistance ratios to methomyl increased slightly.

Results show that *B. thuringensis* applied at the rates of 150 and 300 g / feddan of Dipel 2X and Xentari for one generation to profenofos - resistant strain decreased resistance under laboratory conditions. This means that *B. thuringensis* prevented the cross resistance between profenofos – resistant strain and the tested insecticides.

In order to delay the rapid development of insecticides resistance, a rotational application of various groups of insecticides is recommended. To achieve this, the target of the used insecticides must be different (Georghiou 1980). In the context of characteristics of insecticide resistance as mentioned above, (organophosphorus and pyrethroid insecticides) and (B. *thuringensis* bio-insecticides) are

Table 2. Effect of *B. thurinigensis* (Dipel 2X and Xentari) on some biological aspects of first instar larvae of the cotton leafworm, *S. littoralis*.

Compounds	Rate of application gm / F	Mean larval duration ± S.E. (days)	Mean pupal duration ± S.E. (days)		Mean pupal weight ± S.E. (mg.)		Mean moth life span ± S.E. (days)		Mean no of eggs/ female ± S.E. fecundity	Hatchability %	Sterility %
			female	male	female	male	female	male			
Dipel 2X	150	35.25 ± 1.49	14.5 ±1.34	15.2 ±0.6633	195.28 ±15.50	237.33 ±27.36	13.13 ±1.77	13 ±1.24	232.17 ±64.22	52.22	40.04
	300	34.6 ± 2.18	12.25 ±0.4787	14.25 ±0.4787	159.7 ±9.07	192.6 ±6.78	10 ±1.98	7 ±1.86	121.83 ±30.59	64.51	72.07
Xentari	150	34 ± 1.70	13.87 ±0.5084	14.88 ±0.8070	210.83 ±10.33	202.23 ±14.94	13.18 ±1.67	12.57 ±2.01	171.17 ±30.98	50.75	69.13
	300	34.5 ± 1.48	14 ±0.4470	15.33 ±0.4216	227.5 ±10.68	288.68 ±5.13	13.75 ±0.9774	12.38 ±1.08	134.42 ±45.47	66.43	68.27
Control	-	26.25 ± 1.93	13.6 ±0.4761	16 ±0.4438	211.45 ±6.81	197.82 ±4.98	13.33 ±1.25	13.18 ±1.59	359.2 ±36.11	78.35	-

Table 3. Change percent in some biological aspects in *S. littoralis* treated with 150 and 300 g / feddan of Dipel 2X and Xentari

Insecticides	Rate of application gm/ Feddan	Change in Larval duration %	Change in pupal duration %		Change in pupal weight %		Change in moth span %		Change in fecundity %	Change in hatchability %
			female	male	female	male	female	male		
Dipel 2X	150	(+) 34.29	(+) 6.62	(-) 5	(-)7.65	(+) 19.97	(-)1.5	(-)1.37	(-)35.36	(-)33.35
	300	(+) 31.81	(-)9.93	(-)10.94	(-)24.47	(-)2.64	(-)24.98	(-)46.89	(-)66.08	(-)17.66
Xentari	150	(+) 29.52	(+) 1.98	(-)7	(-)0.2932	(+) 2.23	(-)1.13	(-)4.63	(-)52.35	(-)35.23
	300	(+) 31.43	(+) 2.94	(-)4.19	(+) 7.59	(+) 45.93	(+)3.15	(-)6.07	(-)62.58	(-)15.21

Table 4. Resistance ratio of certain insecticides against *S. littoralis*

Treatments	Laboratory strain		Resistant strain		
	LC_{50} in ppm	Slope ±S.E.	LC_{50} in ppm	Slope ± S.E.	RR
Profenofos	47.345 (32.24-59.84)	1.495 ±0.31	2570.47 (2447.87-2716.46)	6.03 ±0.923	54.29
Methomyl	360 (315.86-410.31)	2.79 ±0.336	784.46 (743.93-826.50)	5.95 ±0.641	2.18
Es- fenvalerate	7.46 (5.70-9.68)	1.16 ±0.155	328.48 (307.52-359.70)	4.92 ±0.691	44.03
Chlorfluazuron	2.47 (2.08-2.97)	1.58 ±0.152	4.89 (3.89-6.14)	1.37 ±0.146	1.98

Table 5 . Cross resistance of tested Bt products with certain conventional insecticides

Treatments	D1			D2			X1					
	LC_{50} in ppm	Slope ±S.E.	RR	LC_{50} in ppm	Slope ±S.E.	RR	LC_{50} in ppm	Slope ±S.E.	RR	LC_{50} in ppm	Slope ±S.E.	RR
Profenofos	1741.58 (1652.36-1859.91)	5.82 ±0.775	36.78	1926.65 (1809.55-2056.21)	5.63 ±0.904	40.69	1513.631 (283.51 -1666.31)	3.63 ±0.661	31.97	1741.01 (1645.8-1851.69)	6.23 ±1.26	36.77
Methomyl	788.11 (740.77-837.53)	5.26 ±0.519	2.19	700.66 (565.70-858)	5.41 ±0.448	1.95	964.31 (896.40-1067.09)	4.44 ±0.634	2.68	861.92 (824.35-905.43)	6.82 ±0.775	2.39
Es-fenvalerate	81.86 (46.78-161.08)	1.103 ±0.36	10.97	270.99 (247.77-297.51)	3.93 ±0.624	36.32	200.86 (189.29-214.05)	5.88 ±1.06	26.92	265.71 (244.42-293.55)	3.59 ±0.47	35.62
Chlorflumuron	5.81 (4.77-7.14)	1.38 ±0.147	2.35	6.74 (5.56-8.74)	1.80 ±0.314	2.73	3.50 (3.01-4.25)	2.37 ±0.332	1.42	4.25 (3.57-5.04)	1.95 ±0.164	1.72

D1: Dipel 2X at the rate of 150g / feddan **D2: Dipel 2X at the rate of 300g / feddan**

X1: Xentari at the rate of 150g / feddan **X2: Xentari at the rate of 300g / fedda**

recommended for rotational application.

Zhao *et al.* (1993) tested the susceptibility of *Plutella xylostella,* collected from a Wuhan population (Hubei, China) to deltamethrin and 3 other insecticides. It was found that resistance was increased to 362.79, 30.78 and 10.91 times than that of a sensitive strain from the U S A. No cross resistance to *Bacillus thuringiensis* or chlorfluazuron was detected.

In this respect, Sannaveerappanavar and Viraktamath (1997) found that using the novel insecticide teflubenzuron was highly effective in suppressing *Plutella xylostella,* followed by four *B. thuringiensis* products; Biobit, Delfin, Dipel 8L and Xentari.

Wu and Guo (2004) reported no positive cross-resistance between Cry1Ac toxin and the insecticides used against *Helicoverpa armigera* (Hübner). Evidence of the lack of cross-resistance to three commonly used synthetic insecticides in laboratory-derived Cry1Ac-resistant population may suggest that growers can confidently use these insecticides if and when resistance to Cry1Ac cotton does occur.

Decreased the level of resistance is one of the goals of entomologists. For that it is recommended to change immediately an insecticide that has been used with another appropriate insecticide when any sign of resistance is detected. For effective rotational system, the intervals between applications need to be long enough so that organophosphorus resistance fully disappears before that insecticide is reused. Therefore it is suggest that a method of integrated pest management needs to be developed in order to reduce the number of insecticidal applications, incorporating rotational application with other techniques.

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دور المبيدات الحيوية في زيادة حساسية السلالة المقاومة للبروفينوفوس من دودة ورق القطن لبعض المبيدات

عزيزة حسن محمدي

المعمل المركزى للمبيدات – مركز البحوث الزراعية – الدقى

أجريت هذة الدراسة لتقييم تأثير المبيد الحيوي باسيلس ثيورنجينسيس (الديبل ٢ اكس والزنتاري) في تقليل مقاومة دودة ورق القطن لبعض المبيدات ومدى تأثير هذة المعاملة على بعض الصفات البيولوجية.

أوضحت النتائج إلى أن معاملة العمر اليرقي الأول للسلالة المقاومة من دودة ورق القطن بتركيز ١٥٠، ٣٠٠ جم /للفدان أدى إلى نسب عالية من الموت في الطور اليرقي تراوحت بين ٨٣,٤٦ – ٩٣,٠٨ بينما كانت نسب الموت طفيفة في طوري العذراء والحشرة الكاملة حيث تراوحت بين ٠,٣٨ – ١,١٥ و ١,١٥ – ٣,٤٦ على التوالي. أدت المعاملة كذلك إلى انخفاض نسبة التعذير وخروج الحشرات الكاملة. أدت المعاملة بالديبل ٢ أكس الى ارتفاع نسبة الموت في اليرقات والعذارى وانخفاض عدد الفراشات الكاملة وبناء عليه فان مركب الديبل ٢أكس كان أكثر تأثيرا" من مركب الزنتاري.

أدت المعاملة بهذه المركبات الى نقص عدد البيض التي تضعه الإناث الناتجة من معاملة اليرقات وكذلك نسبة فقس البيض الموضوع وقصر عمر الفراشات بينما استطالت فترات أعمار اليرقات والعذارى. وعلى الجانب الأخر تسببت معاملة العمر اليرقي الأول بالديبل بمعدل ٣٠٠ جم /للفدان الى استطالة عمر الفراشات.

تسببت معاملة العمر اليرقي الأول للسلالة المقاومة بالديبل والزنتاري بتركزي ١٥٠و ٣٠٠ جم /للفدان لمدة جيل واحد إلى زيادة فاعلية المبيدات المختبرة على السلالة المقاومة عند مقارنتها بالتأثير الحادث لجيل الآباء وقد كان التأثير أكثر وضوحا عند استخدام مبيد البروفينوفوس وهو المبيد المستخدم في الانتخاب لتكوين السلالة المقاومة. كما أدت المعاملة إلى انخفاض معدل المقاومة المشتركة لبعض المبيدات بينما حدثت زيادة طفيفة في معدل المقاومة للمبيدات الأخرى. كما تشير النتائج أيضا إلى أن التركيز النصفي من هذه المركبات قد أظهر انخفاض في معدل المقاومة لبعض المبيدات مقارنة بالتركيز الأعلى.