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CROSS RESISTANCE OF DIPEL 2x RESISTANT STRAIN OF PINK BOLLWORM *PECTINOPHORA GOSSYPIELLA* (SAUND.) TO SOME CONVENTIONAL AND BIOINSECTICIDES

[53]

El-Zemaity, M.S.[1]; A.E. Bayoumi[1]; Mona F. Rofail[2] and Hemat Z. Moustafa[2]

ABSTRACT

A field strain of pink bollworm *Pectinophora gossypiella* (Saund.) collected from Sharquia Governorate, Egypt was exposed to the selection pressure of the formulation of *Bacillus thuringiensis* subsp. *Kurstaki,* Dipel 2x under laboratory conditions. Resistance ratio attained 16-fold based on the susceptible strain after 14 generations of selection. Study the response of Dipel 2x resistant strain to some insecticides indicated that there is no cross resistance to the conventional insecticides, esfenvalerate, chlorpyriphos and thiodicarb or the bioinsecticides Ecotech and Agerin. These data may be emphasizing the possibility of rotation the Dipel 2x with these insecticides in pest control program of pink bollworm to manage resistance to *Bt* products.

Keywords: Pink bollworm, Selection, Cross resistance, *Bacillus thuringiensis*, Dipel 2x

INTRODUCTION

Pink bollworm *Pectinophora gossypiella* (Saund.) is one of the most serious pest attacking cotton crop in Egypt as well as the most cotton producing countries which cause a great damage in the quality and quantity of cotton yield **(El-Naggar, 2003).** Since several decades, the extensive usage of the conventional pesticides to control such insect pest, caused the development of resistance against different compounds which belongs to various chemical groups. To solve this problem, it was substituted such traditional pesticides with the biopesticides based on microorganisms such as bacterial species which producing a specific toxins capable to kill the insect pests. Actually, *Bacillus thuringiensis (Bt)* as a biopesticide is a valuable source of insecticidal proteins for use in conventional sprayable formulations, in transgenic crops and it is the most promising alternative to synthetic insecticides **(Ferré and Van Rie, 2002)**. The benefits of using *B. thuringiensis* on cotton crops include reduced environmental and worker exposure to conventional insecticides, reduced selection for resistance to con-

1- Plant Protection Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt.

2- Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

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ventional insecticides and improved conservation of natural enemies. However Lepidopteran insect resistance to *Bt.,* has been known since 1985 but only in a few taxonomic families. Nonetheless, these insects were susceptible to other *Bt* toxins. Resistance to *B. thuringiensis* has documented for several insect species **(Tabashnik, 1994)**. Pink bollworm and more than a dozen other pests have been selected in the laboratory for resistance to *B. thuringiensis* toxin **(Frutos *et al* 1999).** Since the resistance of such pests is expected, the aim of the present work is to investigate the development of resistance of *P. gossypiella* to a formulation of *B. thuringiensis* Dipel 2x. Cross-resistance of the resistant strain to conventional insecticides (esfenvalerate, chlorpyrifos & thiodicarb) and bioinsecticides (ecotech & agerin) was also studied.

MATERIAL AND METHODS

Bioassay and selection pressure procedures for resistance

Newly hatched larvae of a susceptible strain of *P. gossypiella* (Saund.) were obtained from the Bollworm Research Division, Plant Protection Research Institute, ARC, Dokki, Giza, Egypt. Larvae were reared on semi-artificial diet under laboratory conditions for several generations away from exposure to any insecticidal pressure according to the method described by **Rashad and Ammar (1984).** Field strain (parents) was collected from Ebrahemia region, Sharquia Governorate, Egypt during 2002-2003 cotton season and reared under laboratory conditions as the same with susceptible strain.

Response of the newly hatched larvae of the two strains to Dipel 2x were studied. Serial dilutions of the agent ranged from 10 to 1000 ppm were prepared in distilled water. Each concentration was mixed with 50 g of the artificial diet with the exclusive of antibacterial ingredient. This treated diet was divided into four petri dishes (9 cm diameter). Ten newly hatched larvae were transferred to the surface of diet on each petri dish. Control dishes were mixed only with distilled water. All dishes were incubated at 27 ± 0.5 ^{o}C and 70-85% R.H. Two days later, dead larvae were counted and removed. The alive larvae were transferred individually to glass tubes (2 x 7.5 cm) containing untreated diet. Selection for resistance was carried out using concentration corresponding to the estimated LC_{50} value every generation by treated the newly hatched larvae.

According to the response of the treated larvae to selection, higher concentrations of Dipel 2x were used in subsequent generation. Mortality was recorded 5, 7, 10 days post treatment. Accumulative percentage mortalities were calculated and corrected according to the formula described by **Abbott (1925).** From the corrected mortality percent and the concentrations used, it was plotted the toxicity regression lines of the tested compound and represented in Log/probit relation according to the method of **Finney, (1972)** using the computer program, Sigma Plot for Windows, Version 2.0. LC_{50} and slope values were estimated for S-strain, parent (Field strain), 1^{st}, 3^{rd}, 7^{th}, 10^{th}, 12^{th} and 14^{th} generation. Development of resistance ratio as well as relative resistibility for each generation were calculated as follows:

Resistant ratio = LC_{50} of the selected strain / LC_{50} of susceptible strain.

Relative resistibility = LC_{50} of selected generation / LC_{50} of anterior generation.

Cross resistance of Dipel 2x resistant strain to the tested insecticides

The resistant strain as well as the susceptible strain were exposed to three chemical insecticides representing the major groups of insecticides, i.e. esfenvalerate (Sumialfa) as a pyrethroid compound, chlorpyriphos (Dursban) as organophosphorous and thiodicarb (Larvin) as carbamate and two bioinsecticides Ecotech and Agerin. Newly hatched larvae of each strain was exposed to serial dilutions of the tested insecticides as mentioned before. The corrected mortality percentages were estimated and the LC_{50} values were evaluated according to **Finney, (1972)**. The resistance ratio of the tested insecticides (LC_{50} value of R-strain / LC_{50} value of S-strain) was calculated and the differences of 5-fold or more were considered as indicating positive correlation while those between 1: 4-fold were considered as indicating to no correlation. Differences less than 1-fold represented a probable negative correlation.

Statistical Analysis

All of the toxicity values (LC_{50}'s) which estimated from the plotted toxicity regression lines and their corresponding slope values were calculated using the Probit Analysis Program designed by Dr. Nabil AM. Abd EL-Salam, Plant Protection Institute, Dokki, Giza, Egypt. The significant differences between the mentioned values were statistically analyzed using the Computer program Statitica for Windows, version 4.5.

RESULTS AND DICUSSION

Developed of resistance of pink bollworm to biopesticide Dipel 2x

LC_{50} values of Dipel 2x (*B. thuringiensis* subspecies *Kurstaki*) to the different strains of *P. gossypiella* (Saund.) during 14 generations of selection pressure were presented in **Table (1)** while the plotted toxicity regression lines were illustrated in **Figure (1)**. The estimated LC_{50} values clearly indicate a gradual increase during the tested generations from 200 ppm in 1^{st} generation to 1280 ppm in 14^{th} generation. In this respect, **Simmons *et al* (1998)** reported that the field populations of pink bollworm *P. gossypiella* were more susceptible to the endotoxin CryIAc than the susceptible laboratory strain. Regarding the resistance ratio data show a level of 2.5 and 2.63-fold in G1 and G2, respectively. Then reached a level of tolerance during the 3^{rd} generation (4.13-fold). With further selection, the resistance ratio show resistance level during G7 (5.63-fold). The resistance ratios increased again to 7.25, 11.25, 12.37 and 16-fold during generations 10, 11, 12 and 14, respectively. The slope values were nearly close to each other and remained nearly similar till the end of selection.

Similar findings were also indicated by **Tabashnik *et al* (1990 & 1995)** when they studied the resistance phenomenon in field population of diamondback moth *Plutella xylostella* (L.). In their studies, they showed that repeated selection with high concentrations of commercial formulation of *B. thuringiensis* subsp. *Kurstaki* caused development of resistance.

Table 1. Development of resistance rates to Dipel 2x in *P. gossypiella* (Saund.) during selection for 14 generations.

Selection generations	LC_{50} (ppm) (5%fiducial limits)	Slope	Resistance Ratio (Fold)	Relative Resis-tibility
Susceptible	80.0 (50.0-100.0)	1.38	-	-
Parent	70.0 (40.0-90.0)	1.20	0.88	-
G1	200.0 (150.0-260.0)	1.64	2.50	2.85
G2	200.0 (150.0-270.0)	1.59	2.50	1.00
G3	330.0* (220.0-460.0)	1.17	4.13	1.65
G7	450.0* (330.0-620.0)	1.31	5.63	1.36
G10	580.0** (430.0-790.0)	1.42	7.25	1.29
G11	900.0** (680.0-1190.0)	1.74	11.25	1.55
G12	990.0** (690.0-1410.0)	1.13	12.37	1.71
G14	1280.0** (840.0-1950.0)	0.98	16	1.29

Comparing to the parent generation, (***) highly significant $p \leq 0.001$, (**) moderately significant $p \leq 0.01$
and (*) significant $p \leq 0.05$ (student *t*-test).

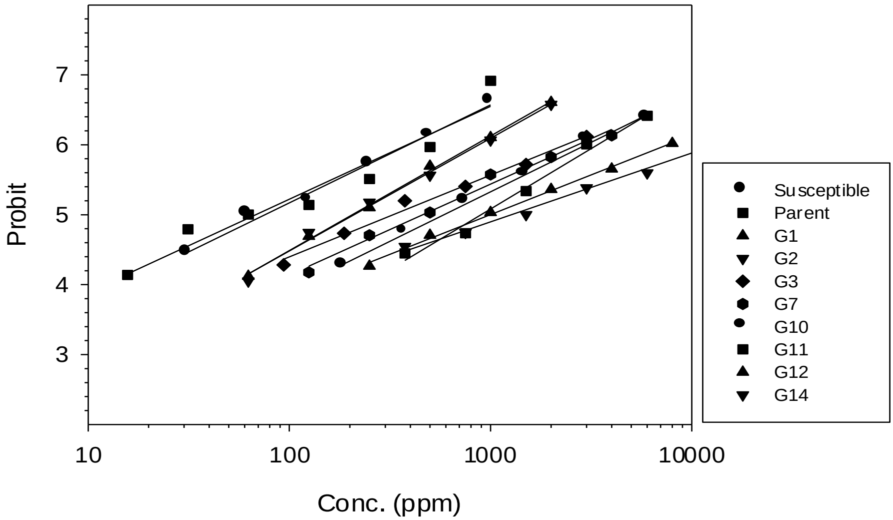

Fig. 1. Toxicity regression lines of Dipel-2x against the tested generations of *P. gossypiella*.

Also, **Gould *et al* (1995)** reported that selection of field collected strain of *Heliothis virescens* on artificial diet containing CryIA(c) developed only moderate resistance (7 to 8- fold) after 12 episodes of selection.

However, it is well documented that the further selection lead to higher levels of resistance. In this respect, **Bolin *et al* (1999),** and **Huang *et al* (1999)** reported that European corn borer *Ostrinia nubilalis* developed resistance to *B. thuringiensis* after 8 generations of selection. Recently, **Hussein (2002) and Sabry (2002)** stated that selection with Dipel 2x produced 16.32-fold after 10 generations of selection in *Spodoptera littoralis* and 14.8-fold after 11 generations of selection of *P. gossypiella.* Also, it was reported that laboratory selection increased resistance of pink bollworm *P. gossypiella* to the *B. thuringiensis* in artificial diet from a low level to >100-fold relative to a susceptible strain (**Patin *et al* 1999; Liu *et al* 2001 and Tabashnik *et al* 2002).** More recently, it was found that the obtained results are in agreement with that obtained by **(El-Zemaity *et al* 2003 & 2004)** which were studied the development of resistance and the response of *Bt* resistant strain of *Spodoptera littoralis* to certain conventional insecticides and bioinsecticides.

Cross resistance to certain insecticides

The estimated LC_{50} values of the tested insecticides on susceptible and Dipel 2x resistant strain of *P. gossypiella* are listed in **Table (2)** while the corresponding toxicity regression lines are illustrated in **Figures (2-6)**. According to such values, it was found that the resistance ratios reached to 1.71, 1.4 and 1.29-fold to esfenvalerate, chlorpyrifos and thiodicarb, respectively. These results indicate no cross resistance between these conventional insecticides and Dipel 2x resistant strain. The same finding were recorded with Ecotech and Agerin, where resistance ratios revealed 1.24 and 1.51-fold, respectively **(Figs.5-6)**. These data may be emphasize the possibility of rotation Dipel 2x with these insecticides in pest control program of cotton bollworm to manage resistance to *Bt* products.

Due to the different mode of action between *B. thuringiensis* and the conventional insecticides, **Whalon *et al* (1993)** reported that no cross resistance could be observed between organophosphate, carbamate, or pyrethroid resistance and *B.thuringiensis* resistant strain of Colorado potato beetle *Leptinotarsa decemlineata* (Say). Also, **Hussein (2002)** showed that no cross resistance could be recorded in *Spodoptera littoralis* between tested bioagents and the chemical insecticides. In the same way, **Wu and Guo (2004)** observed no positive cross resistance between Cry1Ac toxin and conventional insecticides lambda, cyhalothrin, phoxim and endosulfan and the level of the resistance to these insecticides gradually came down to a level similar to that of the susceptible strain of *Helicoverpa armigera.* Considering cross resistance between bioinsecticides, **Gould *et al* (1995) and Akhurst *et al* (2003)** found that a strain of *Helicoverpa armigera* and *Heliothis virescens* selected with CryIAc was not resistant to the commercial *Bt* spray formulations Dipel 2x and Xentari. In the contrary, it was found that the selected colonies of European corn borer *Ostrinia nubilalis* (Hubner) with *B.thuringiensis* CryIAc toxin was

Table 2. Response of Dipel 2x resistant strain of *P. gossypiella* (Saund.) to the tested compounds.

Tested insecticides	Susceptible strain		Dipel 2x resistance strain		
	LC_{50} (ppm) (Fiducial limits)	Slope	LC_{50} (ppm) (Fiducial limits)	Slope	Resistance ratio
Esfenvalerate	0.14 (0.09-0.20)	1.19	0.24(0.16-0.37)	1.14	1.71
Chlorpyrifos	0.03 (0.04-0.07)	1.47	0.07 (0.05-0.11)	1.05	1.4
Thiodicarb	70.80 (53.22-94.18)	1.88	91.36 (63.31-131.86)	1.30	1.29
Ecotech	380 (280-500)	1.41	470 (350-630)	1.53	1.24
Agerin	350 (240-510)	1.12	530 (390-730)	1.45	1.51

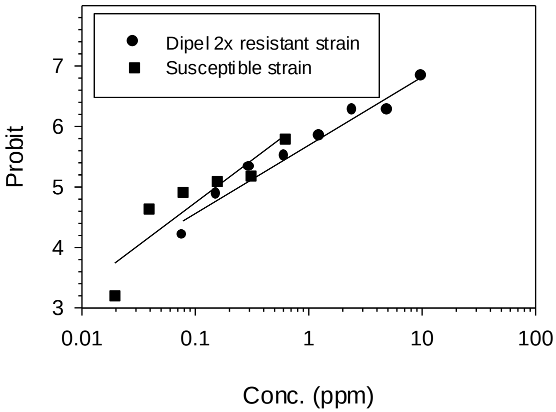

Fig. 2. Toxicity regression lines of esfenvalerate against susceptible and Dipel 2x resistant strain of *P. gossypiella.*

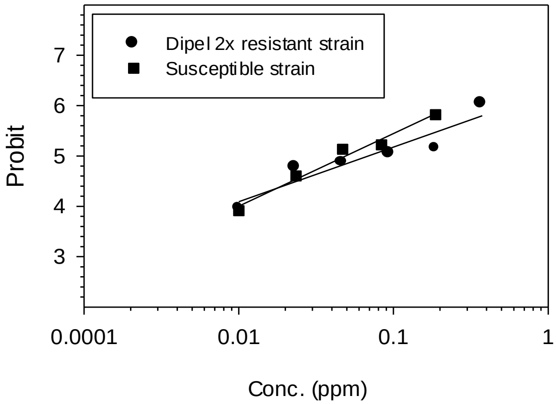

Fig. 3. Toxicity regression lines of chlorpyrifos against susceptible and Dipel 2x resistant strain of *P. gossypiella*.

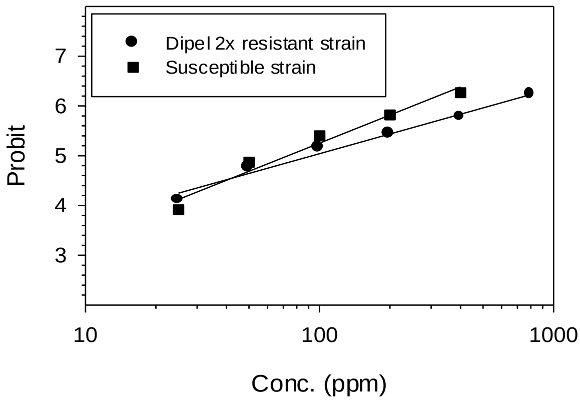

Fig. 4. Toxicity regression lines of thiocarb against susceptible and Dipel 2x resistant strain of *P. gossypiella*.

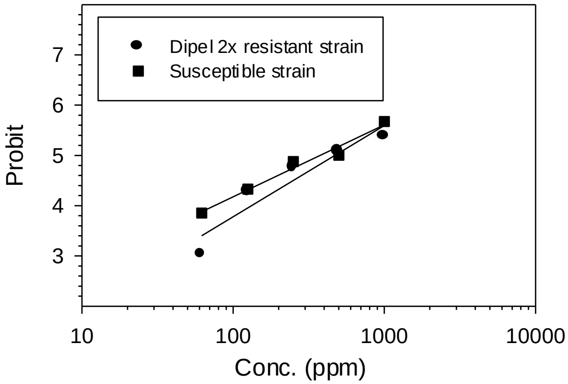

Fig. 5. Toxicity regression lines of Ecotech against susceptible and Dipel 2x resistant strain of *P. gossypiella.*

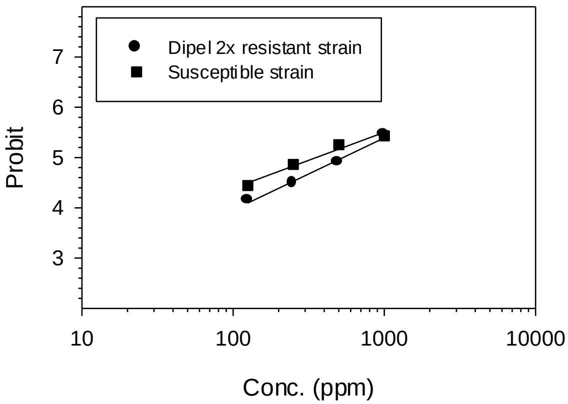

Fig. 6. Toxicity regression lines of Agerin against susceptible and Dipel 2x resistant strain of *P. gossypiella.*

marginally cross resistant to CryIab **(Bolin *et al* 1999).**

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عبور صفة مقاومة سلالة دودة اللوز القرنفلية المقاومة للدايبل لبعض المبيدات الحشرية المعتادة والحيوية

[53]

محمد السعيد صالح الزميتي[1]- علاء الدين بيومي[1]- مونا فكرى روفائيل[2]- همت زكريا محمد مصطفى[2]

1 - قسم وقاية النبات، كلية الزراعة، جامعة عين شمس، شبرا الخيمة، القاهرة، مصر.

2- معهد بحوث وقاية النباتات، مركز البحوث الزراعية، الدقي، الجيزة، مصر.

تم تعريض سلالة حقلية من دودة اللوز القرنفلية جمعت من محافظة الشرقية للضغط الإنتخابي لمستحضر بكتيريا باسيلس ثورنجينسيس (الدايبل) تحت ظروف معملية. وصل مستوي المقاومة 16 ضعفا بعد 14 جيلا من الضغط الانتخابي مقارنة بالسلالة الحساسة. وأظهرت دراسة إستجابة سلالة دودة اللوز القرنفلية المقاومة للدايبل أنه لا يوجد عبور لصفة المقاومة بين المبيدات الحشرية المعتادة إس فنفاليرات، كلوربيريفوس، ثيوديكارب أو المبيدات الحيوية إكوتيك و أجرين.

وتؤكد هذه النتائج علي إمكانية مناوبة الدايبل بهذه المبيدات في برنامج مكافحة دودة اللوز القرنفلية لإدارة مقاومة مستحضرات الباسيلس ثورنجينسيس.

تحكيم: ا.د محمد إبراهيم حسين

ا.د عادل عبدالحميد الفيشاوى