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BIOCHEMICAL AND HISTOPATHOLOGICAL ASPECTS OF RESISTANT DEVELOPMENT IN PINK BOLLWORM, *PECTINOPHORA GOSSYPIELLA* (SAUND.) TO DELTAMETHRIN AND DIPEL 2X

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

ABSTRACT

Study the resistance development of the 4th instar larvae of pink bollworm, *pectinophora gossypiella* (Saund.) against two insecticides, i.e. deltamethrin (as pyrethroid) and Dipel 2x (as bioinsecticide derived from *Bacillus thuringiensis* subsp. *kurstaki*) was carried out under laboratory conditions throughout the selection pressure until 14 generations. Mechanism of development of resistance was studied at biological, biochemical and histopathological levels. Biologically, the calculated midpoint toxicity values, (LC_{50}'s), indicated that the resistance to deltamethrin increased to 215.11-fold while the resistance to Dipel 2x, attained 16-fold based on the susceptible strain after14 generations of selection. At biochemical level, detoxication enzymes assay revealed that activity of glutathione *S*-transferase was higher in all selected generations than susceptible strain. Regarding phosphatases activity in Dipel 2x-resistant strain, acid phosphatase increased than susceptible strain but alkaline phosphatase decreased in all generations than the susceptible strain. Also, the activity of alkaline phosphatase was singnificantly decreased in parent, G3, G7 and G14 in deltamethrin resistant strain while the activity of acid phosphatase was not affected except at G10 when it was increased significanlty comparing with the control value. On the other hand, study of protein electrophoresis in midgut of resistant and susceptible larvae revealed that there are new bands appeared in the resistant strains of both compounds which not present in susceptible strain. Considering the histopathological effects, the obtained results showed a histological alterations, i.e. thickness of epithelium cells of the larval midgut of both deltamethrin and Dipel 2x resistant strains.

Keywords: Resistance, Pink bollworm, Deltamethrin, Dipel 2x, Biochemical and Histological Effects

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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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INTRODUCTION

Pink bollworm *Pectinophora gossypiella* (Saund.) is considered as one of the most serious insect pest attacking cotton crop in Egypt (**El-Naggar, 2003**). In the early 1980's, pyrethroid insecticides and at 1990's, the biopesticides based on *Bacillus thuringiensis* were rapidly substituted for organophosphorus and organochlorine insecticides for control of the pink bollworm to overcome the development of resistance in such insect pest against a wide range of compounds. Unfortunately, resistance by the bollworm to such insecticides became more and serious because of indiscriminate applications (**Wang, 1992**).

From another viewpoint, the determination of the biochemical factors of resistance to pesticides is considered as an urgent need because it may help to design a highly sensitive monitoring technique, which is one of the key factors in developing a successful resistance management program (**Scott, 1990**). Despite the importance to study the biochemical mechanisms in developing the resistance against insecticides, very few instance are known and documented in which resistance is clearly linked with the biochemical and histological role in resistant development (**Soderlund and Bloomquist, 1990**).

However, some investigators are found a significant alteration in the activity of various enzymes in the resistant strains of different insect species, i.e. esterases and glutathione *S*-transferase in the resistant strains of *Heliothis armigera* to deltamethrin and cypermethrin (**Martin *et al* 2002**), and changes in the protein profiles of the *Bt* resistant cotton leafworm strain (**El-Zemaity *et al* 2003**). At the histological level, a little studies were carried out to prove its role in the resistance mechanisms. For example, it was found that Dipel 2x (*Bt* biopesticide) exhibited magnitude to the midgut epithelium of the laboratory colony. Building up of resistance resulted in obvious thickness of the epithelium in *P. gossypiella*. (**Sabry, 2002**). According to the mentiones facts, the resistance of such pests are expected, hence the aim of the present work is to investigate the biochemical mechanism of resistance and the corresponding histopathological changes of the resistant strain of *P. gossypiella* to the pyrethroid deltamethrin as well as a formulation of *B. thuringiensis* (Dipel 2x).

MATERIALS AND METHODS

1- Insect rearing technique

Newly-hatched larvae of a susceptible strain of *Pectinophora gossypiella* (Saund.) were obtained from the Bollworm Research Division, Plant Protection Research Institute, Dokki, Giza, Egypt. The larvae were reared in the laboratory for several generations according to **Rashad and Ammar (1984)**. Field strains were collected from Ebrahemia region, Sharquia Governorate during 2001-2003 cotton season by collecting the green bolls during November. The collected bolls were exposed to the sun until dryness and kept in the laboratory until January. The Larvae were obtained from the dried bolls and kept in glass tubes (2 x 7.5 cm) closed with piece of cotton and reared in the laboratory until pupation and adult emergence.

2. Tested Insecticides

Two insecticides were tested in such study including a pyrethroid compound, deltamethrin [(S)-α-cyano-3-phenoxybenzyl (1R, 3R)-3-(2, 2-dibromovinyl)-2, 2-dimethylcyclopropanecarboxylate] (2.5% E.C) and Dipel 2x (commercial product of *B. thuringiensis*, subspecies *kurstaki*. At 32000 IU/mg) obtained from Abbots laboratory USA.

3- Bioassay and Selection Pressure Procedures for Resistance

Serial concentrations (0.1-500 ppm) of deltamethrin and a range of (0.03 to 1 g/L) of Dipel 2x were added to the artificial diet through spraying into petri dishes (9 cm diameter) using a hand atomizer in a three replicates for each concentration. The treated surfaces were left to dry. Thirty newly hatched larvae were transferred with a clean brush to each treated dish. The dishes were covered with toilet paper then further covered with their covers and kept in an incubator adjusted at 27 ± 2°C and 70-85% R.H. After an hour of exposure, the treated and untreated (check) insects were transferred individually on semi artificial diet poured into glass tubes (2 x 7.5 cm) covered with cotton piece and kept under the previous constant conditions.

The mortality percent was determined and corrected after 24 hour using the **Abbott's formula (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_{30} and LC_{50} values were calculated from the plotted toxicity lines. Selection for resistance was carried out on the newly-hatched larvae at the LC_{30} levels. Concentration of 0.078 ppm of deltamethrin or 32.14 ppm of Dipel 2x was applied on artificial diet in glass tubes of (2 x 7.5 cm), each tube was infested by neonatal larvae and capped with cotton piece. Concentrations of deltamethrin and/or Dipel 2x representing the LC_{30} were used in subsequent generations with the increase of resistance levels. The LC_{50} values were calculated for 1st, 3rd, 5th, 6th, 7th, 10th and 14th generations. 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.

4. Biochemical studies

4.1. Sample preparation

The whole body of treated and untreated larvae of *P. gossypiella* was extracted through homogenization in the presence of cold phosphate buffer 0.2 M (*pH.* 6.5) as source for glutathione S-transferase enzyme, cold citric acid-sodium citrate buffer, 0.1 M (pH 4.9) as source for acid phosphatase enzyme and/or cold carbonate buffer, 0.1 M (pH 10) as source for alkaline phosphatase enzyme. After then, the homogenized samples were centriguged at 8000 rpm for 25 min in a refrigerator centrifuge at 2°C and the supernatant was freezed at – 80°C tell the enzymatic determination.

4.2- Enzyme assay

The enzyme activity of glutathione *S*-transferase was determined spectrophotometrically at 340 nm according to the method of **Habig *et al* (1974)** whereas the activity of acid phosphatase (Ac-pase) and alkaline phosphatase (Alk-pase) were determined according to the method described by **Powell and Smith (1954).**

4.3- Determination of Total Protein

The determination was carried out according the method of **Gornal *et al* (1949).**

4.3- Protein Electrophoresis

Determination of molecular weight of different proteins by using SDS- polyacrylamide gel electrophoresis as described by **Laemmili (1970).**

5- Histological Study

The larvae of resistant strains after last selection of deltamethrin (215-fold) and Dipel 2x (16-fold) as well as susceptible strain were taken in the 4th instar larvae for histological studies.The larvae were dissected and the mid-guts were fixed in aqueous Bouin's solution, then dehydrated in ethanol solutions and cleared in xylene and then embedded in paraffin. Sections of 5 μ thick were cut and stained by the Ehrlich's haematoxylin for nuclei and eosin for cytoplasm and cell walls **(Lee, 1913).**

Statistical Analysis

The significant differences between the calculated midpoint toxicity values of the selected generation and the susceptible strain and between those related with the enzymatic activities were statistically analyzed following the student t-test using the computer program Statitica for Windows, version 4.5.

RESULTS AND DISCUSSION

1. Development of Resistance of Pink Bollworm to Deltamethrin

The calculated LC_{50} values of deltamethrin to the different strains of pink bollworm *P. gossypiella* (Saund.) during fourteen generations of selection are shown in **Table (1).** The results clearly indicate that LC_{50} values increased gradually during the first five generations. At G3 the resistance ratio attained a level of 11.49-fold then increased in generations 5 and 6 to be 20.82 and 69.69-fold, respectively. With further selection pressure, rapid increase in resistance ration was observed during generations 7 and 10 to be 99.80 and 165.84-fold and reached to 215.11-fold in generation fourteen.

In addition, the toxicity regression lines were characterized by slight fluctuations in the slope values, which were comparatively low and were nearly close to each other. In this respect Hoskins and **Gordon (1956)** pointed out that the development of true resistance was characterized by regression line becoming shallower as it moves to the right direction, finally it becomes steeper again as resistance genotypes comes to characterize the new population. Moreover, **Osman *et al* (1991)** reported that sixteen generations of larval selection of a field strain of pink bollworm *P. gossypiella* exposed to permethrin produced 9.7- fold resistance in

Table 1. Rate of development of resistance to deltamethrin in *P. gossypiella* (Saund.) during selection for 14 generations.

Selection generations	LC_{50} (ppm) (5%fiducial limits)	Slope	Resistance Ratio (Fold)	Relative Resistibility
Susceptible	0.55 (0.28-1.06)	0.69	-	-
Parent	1.03 (0.58-1.84)	0.63	1.87	-
G1	2.93 (1.82-4.71)	1.31	5.32	2.84
G3	6.32* (3.48-11.51)	0.73	11.49	2.16
G5	11.45** (7.98-16.42)	1.30	20.82	1.81
G6	38.33*** (23.46-62.62)	0.85	69.69	3.35
G7	54.90*** (31.66-95.20)	0.85	99.80	1.43
G10	91.21*** (61.60-135.06)	1.14	165.84	1.66
G14	118.31*** (82.89-168.87)	1.31	215.11	1.30

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).

adults. Higher slope values obtained in F12 through F16 generations indicated an increasing of homogeneity in later generation. On the other hand, the estimated slope values indicated that a population that was apparently heterogeneous at the beginning of the study tended to become relatively more homozygous as selection progressed. However, similar finding was obtained by **Aldosari *et al*** (1996).

2. Development of Resistance of Pink Bollworm to the Bioinsecticide Dipel 2x

The computed LC_{50} values of dipel 2x to the different strains of pink bollworm *P. gossypiella* (Saund.) during fourteen generations of selection are shown in **Table (2).** The parent field colony showed a level of LC_{50} less than the susceptible LC_{50}. The LC_{50} values indicate gradual increase during the tested generations from 200 ppm in 1st generation to 1280 ppm in 14th 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 showed a level of 2.50 - fold in G1, then reached a level of tolerance during the 3rd generation (4.13-fold). With further selection, the resistance ratio increased to be 5.63-fold at G7. The resistance ratios increased again to 7.25, 12.37 and 16-fold at generations 10, 12 and 14, respectively. The slope values were nearly close to each other and remained nearly similar till the end of selection

Table 2. Rate of development of resistance 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 Resistibility
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).

except the slope value of the toxicity lines of G14 which decreased to be 0.98. However, similar findings were also showed that *B. thuringiensis* subsp. *Kurstaki* caused development of resistance in field population of diamondback moth *P. xylostella* (L) (**Tabashnik *et al* 1995**), *Heliothis virescens* (**Gould *et al* 1995**), European corn borer *Ostrinia nubilalis* (**Bolin *et al* 1999**), *Spodoptera littoralis* (**El-Zemaity *et al* 2003**) and *P. gossypiella* (**Tabashnik *et al* 2002**).

3- Biochemical Studies on Resistance of *P. gossypiella*

3.1- Glutathione *S*-transferase Activity

The specific activity of GST for the susceptible, parent, deltamethrin and diple 2x resistant strains of *P. gossypiella* (Saund.) are shown in **Figure (1)**. In the deltamethrin resistant strain, a significant increase in GST activity was found from the 1st generation as compared with the susceptible strain. The levels of activity were (1.01 and 4.91 n mole/min/mg protein) for S-strain and G1, respectively. A slight decrease was observed in G7 (2.80 n mole/min/mg protein) then significant increase to 10.60 n mole/min/mg protein in G10 then decrease to 4.46 nmole/min/mg protein in G14. There is no correlation between specific activity of GST and resistance level to deltamethrin ($r = 0.47$, $P<0.05$).

In case of the Dipel 2x resistant strain, a significant increase in GST activity was found from the 1st generation as compared with the susceptible strain. The levels of activity were 1.01 and 10.35 nmole/min/mg protein for S-strain and

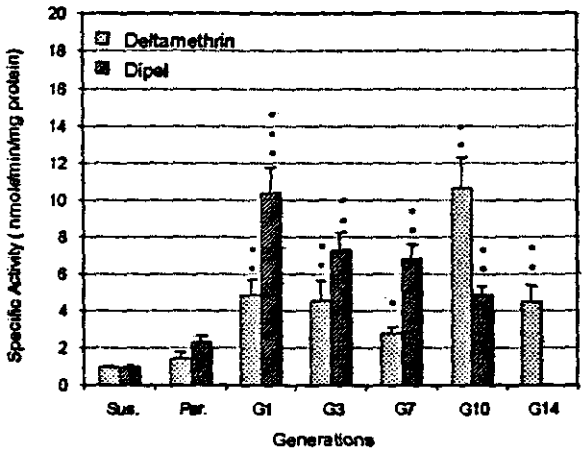

Fig. 1. GST activity of full grown larvae of *P. gossypiella* in susceptible, parent and different generations of deltamethrin and Dipel 2x resistant strains. Comparing to the susceptible strain, (***) highly significant $p \leq 0.001$, (**) moderately significant $p \leq 0.01$ and (*) significant $p \leq 0.05$ (student *t*-test).

G1, respectively. From G3 there is a significant decrease in GST activity, the levels of activity were 7.28, 6.80 and 4.87 nmole/min/mg protein for G3, G7 and G10, respectively. From the statistical analysis results it was suggested that there is no significant correlation between specific activity and resistance level to Dipel 2x ($r=0.08$, $P<0.05$).

Similar findings were recorded by **Ibrahim *et al* (1996)** which showed that GST activity was greater in pyrethroid resistant strain than in susceptible strain and than those of the parent of tobacco budworm *H. virescens*. There were no significant correlations between the enzyme activities and resistance to cypermethrin. **Yu and Nguyen (1996)** stated that there were no significant differences in GST detoxiction enzyme system between parental and permethrin selected strains in *P. zylostella*. **Martin *et al* (2002)** found that GST activity of the deltamethrin selected strain was significant (2.7-fold) higher than in the susceptible strain in the cotton bollworm *H. armigera*, but it did not found any correlation between GST activities and deltamethrin resistance. Similar finding was also reported by **Taha (2001) and Yu *et al* (2003)** which showed that no correlation however was indicated between GST activity and resistance to *B. thuringiensis* (Dipel2).

3.2-Phosphatases Activity

Acid and alkaline phosphatase activities in susceptible, parent, deltamethrin and Dipel 2x resistant strains of *P. gossypiella* (Saund.) are illustrated **Figures (2 & 3).** In the deltamethrin resistant strain, the data showed that alkaline phosphatase activity was decreased significantly in parent, G3, G7 and G14 and the decrease were 30.11, 31.62, 36.40 and 33.89%, respectively, compared with the susceptible strain. Significant increase

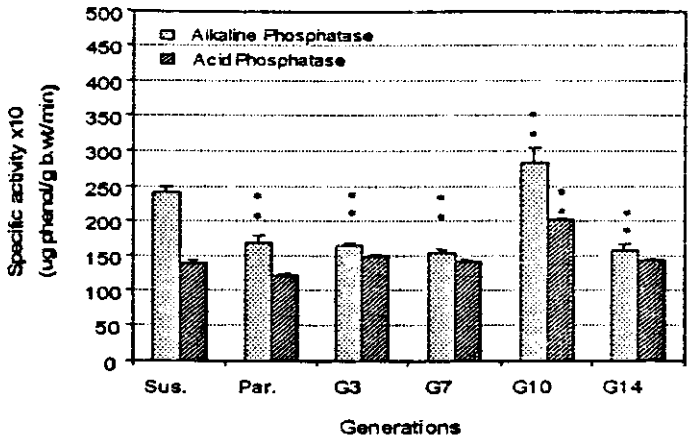

Fig. 2. Alkaline and acid phosphatases activity of full grown larvae of *P. gossypiella* in susceptible, parent and different generations of deltamethrin resistant strains. Comparing to the susceptible strain, (**) moderately significant $p \leq 0.01$ and (*) significant $p \leq 0.05$ (student *t*-test).

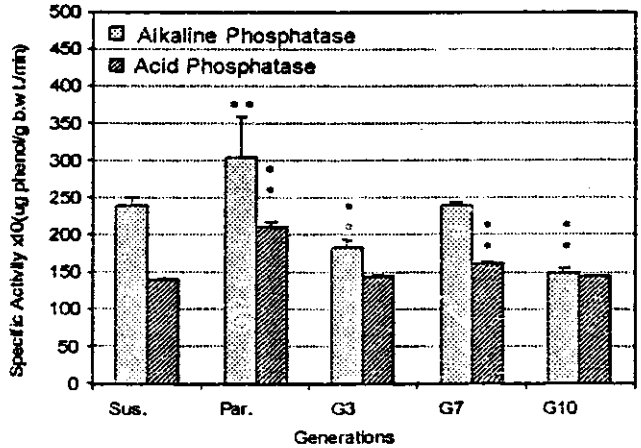

Fig. 3. Alkaline and acid phosphatases activity of full grown larvae of *P. gossypiella* in susceptible, parent and different generations of Dipel 2x resistant strains. Comparing to the susceptible strain, (***) highly significant $p \leq 0.001$, (**) moderately significant $p \leq 0.01$ and (*) significant $p \leq 0.05$ (student *t*-test).

was found in G10 which was 17.68% than the susceptible strain. There is no correlation between alkaline phosphatase activity and resistance level to deltamethrin ($r = 0.30$, $P<0.05$). Data showed that there is no significant increase in acid phosphatase activity in G7 and G14 compared with susceptible strain and the increase were 1.43 and 2.86% while a significant increase by 44.63% than susceptible strain in G10 was recorded. There is no correlation between acid phosphatase activity and resistance level to deltamethrin ($r = 0.48$, $P<0.05$).

In case of the Dipel resistant strain, the obtained results showed that alkaline phosphatase activity increased significantly only in the parent, while no significant decrease was found in G7. There were significant decreases in G3 and G10 which reached to 23.20 and 38.68%, respectively. There is a negative correlation between alkaline phosphatase activity and resistance level to Dipel 2x ($r=-0.95$, $P<0.05$). The obtained data showed that the increase in acid phosphatase activity of G3 and G10 were not significant compared with susceptible strain, while in parent and G7 there were significant increase reached to 51.55 and 16.22%, respectively, compared with the susceptible strain. There is a negative correlation between acid phosphatase activity and resistance level to Dipel 2x ($r =-0.84$, $P<0.05$).

In this respect, **Farag (1978)** found that activity of acid and alkaline phosphatase showed marked differences during the development of resistance to insecticides. The acid phosphatase activity increased with the development of resistance or tolerance. On the other hand the alkaline phosphatase showed slight decrease in resistance rather than in susceptible strain of *Spodoptera littoralis*. However, it was reported that correlation coefficient between deltamethrin tolerance and acid phosphatase and alkaline phosphatase was less than the critical value in *S. littoralis* **(Afifi 1988 and Amin 1992)**. Also, several investigators were showed the high correlation between the activity of acid and alkaline phosphatase and the development of resistance to methomyl, deltamethrin and chlorpyrifos in pink bollworm **(Al-Beltagy *et al* (1993)** and to *B. thuringiensis* in *Galleria mellonella* **(Shen and Qian, 1994)**.

4- Electrophoretic study

Data of SDS electrophoretic patterns of the soluble proteins fractions extracted and separated from gut of full grown larvae of susceptible, deltamethrin and dipel 2x resistant strain at G14 of the pink bollworm *P. gossypiella* (Saund.) are shown in **Table (3) and Figure (4)**. The comparison between midgut protein of susceptible and selected strains indicated that there were new different number of protein bands appeared in the midgut extracts of resistant strains compared with the susceptible strain. Number of protein bands in deltamethrin resistant strain 12 bands (MW. 239.41- 9.84 K Da) and 14 bands (MW. 327.98- 9.67 KDa) in Dipel 2x resistant strain as compared to susceptible strain 8 bands (MW. 142.74- 9.50 KDa). The mechanism by which an insect evolves resistance is due to changes in a midgut membrane receptor **(Van Rie *et al* 1990 and Ferre *et al* 1991)**. However, it was found that variations in toxin processing gut *pH* and gut protease activity may therefore provide the *Bt* selectivity observed in some insect species **(Milne *et***

Table 3. Comparison between total gut protein of susceptible strain, Dipel 2x and deltamethrin resistant strains of *p. gossypiella* (Saund.)as shown in the electrophoretic pattern.

Lane:	1	Amount	2	Amount	3	Amount	4	Amount
Bands	(MW*)	%	(MW*)	%	(MW*)	%	(MW*)	%
1	142.74	18.80	327.98	6.60	239.41	16.40	200.00	15.20
2	87.02	17.30	256.12	8.82	149.31	15.40	97.40	14.80
3	75.29	7.79	119.25	10.60	94.82	6.57	68.00	26.60
4	70.60	9.77	94.82	14.20	79.44	10.80	43.00	22.60
5	56.14	11.50	78.17	10.00	76.51	5.46	14.30	20.20
6	42.24	20.10	71.36	14.30	73.30	12.70		
7	10.95	12.90	53.40	5.78	53.84	6.68		
8	9.50	1.85	47.12	4.27	45.20	6.88		
9			39.34	8.74	40.05	5.75		
10			31.23	3.30	31.79	2.28		
11			25.69	2.22	26.15	5.53		
12			18.99	2.68	9.84	5.41		
13			11.35	5.91				
14			9.67	3.41				

(*) Molecular weight (Kda), 1. Susceptible strain, 2. Dipel 2x resistant strain, 3. Deltamethrin resistant strain and
4. Marker protein.

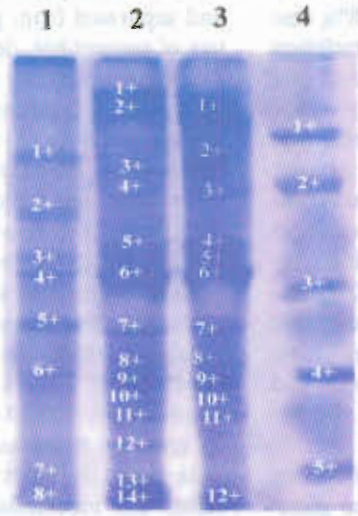

Fig. 4. Electrophoretic pattern carried out by SDS-PAGE gel of gut protein which extracted from full grown larvae of susceptible, G14 of Dipel 2x and deltamethrin resistant starin of *p. gossypiella*. 1: Susceptible strain, 2: G14 of dipel 2x resistant strain, 3: G14 of deltamethrin resistant strain and 4: marker protein.

al 1990). In Lepidoptera, one study by **Johnson *et al* (1990)** did not find differences in midgut proteases between susceptible and resistance strains of *P. interpunctella* to *B. thuringeinsis var.Kurstaki* HD-1) and in this case resistance might be explained by changes in a midgut membrane receptor (**Van Rie *et al* 1990**). It was found that an incomplete and slow processing of CryIA© protoxin by midgut extracts from another *Bt*-resistant strain of *P. Interpunctella* (selected using *Bt subsp entomocidus*), when compared to extracts from the susceptible parent strain and another resistant strain (selected with Dipel®) suggesting that some strains of *Bt* may elicit a proteinase mediated mechanism (**Oppert *et al* 1994**).

In this respect, it was demonstrated that there are different bands of proteins which could be interpret the susceptibility difference between the field resistance populations and laboratory strain, the bands varied in intensity and in molecular weight between laboratory and field strain of pink bollworm *P. gossypiella* when exposed to different compounds, methomyl, deltamethrin and chlorpyrifos (**Lee *et al* 1995 and Forcada *et al* 1996**).

5- Histopathological effects

The effect of the tested insecticides on the mid-gut tissues of susceptible, deltamethrin and Dipel 2x resistant strain of *P gossypilla* was studied through examining the histopathological changes occurred in the mentioned tissue. So that, the cross sections of the midgut of the of 4th instar larvae of the pink bollworm *P. gossypiella* (Saund) of the susceptible, deltamethrin and Dipel 2x strains are shown in **Figures (5-7)**.

According to (**Wigglesworth, 1972**) the midgut of normal 4th instar larvae consists of a single cellular layer with a large granular nucleus resting upon a basement membrane which is surrounded first by circular then by longitudinal muscles. The lumen is surrounded by a peritrophic membrane (**Fig. 5**). Histological changes were noted in **Figs. (6 and 7)**, i.e. two groups of larvae were fed on diet one of them mixed by LC_{50} of deltamethrin and the other by LC_{50} of Dipel 2x. The epithelium cells had elongated also slight vacuolization appeared. Similar observation took place with insecticides in larvae of *S. littoralis* (**Toppozada *et al* 1968**) and with *B. thuringiensis* in larvae of *P. interpunctella* (**Mohamed *et al* 1996**).

In this respect, **Kaushik and Kumar (1998)** found that larvae treated with carbaryl and aldrin induce severs lesions. These pesticides seem to be stronger midgut poisons than monocrotophos to *Paratelphusa masoniana*. The presently tested sublethal concentrations of both carbaryl and aldrin induced persistent hyperplasia and proliferation of mucosal epithelium. Also, **Zidan *et al* (1998)** found that cross section of the midgut of treated MVP larvae of *P. gossypiella* show that the arrangement of the peritrophic membrane was not clear also excessive cellular vacuolization of epithelial cells was observed. Moreover, in other studies, it was found that midgut cross section of Dipel 2x resistant larvae of *S. littoralis* showed disappear of microvilli vesiculation of endoplasmic reticulum in columnar cells and the volume of epithelium cells was increased more than the untreated control (**Martinez-Ramirez *et al* 1999 and Hussein, 2002**).

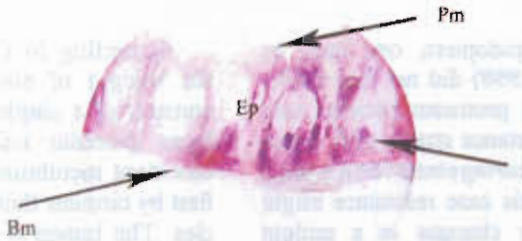

Fig. 5. Midgut epithelium cross section of susceptible 4th instar larvae of *P. gossypiella* (100x). Bm: Basement membrane, Ep: Epithelium cells, Re: Regenerative cells and Pm: Peritrophic membrane.

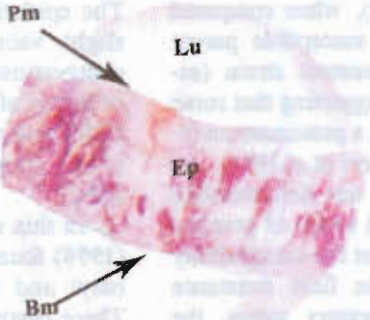

Fig. 6. Midgut epithelium cross section of resistant 4th instar larvae selected with Dipel 2x (100x). Bm: Basement membrane, Ep: Epithelium cells, Re: Regenerative cells and Pm: Peritrophic membrane.

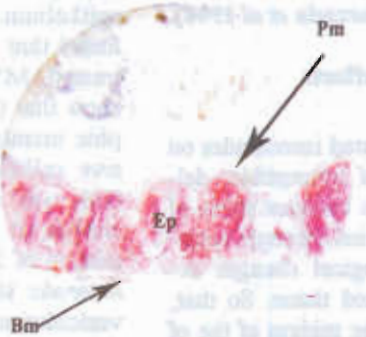

Fig. 7. Midgut epithelium cross section of resistant 4th instar larvae selected with deltamethrin (100x). Bm: Basement membrane, Ep: Epithelium cells, Re: Regenerative cells and Pm: Peritrophic membrane.

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المؤتمر العاشر لبحوث التنمية الزراعية، كلية الزراعة، جامعة عين شمس، القاهرة، مصر، ٢٠٠٦
مجلد خاص، حوليات العلوم الزراعية، عدد خاص، ١، ٢٧١-٢٨٧، ٢٠٠٦

المظاهر البيوكيميائية والنسيجية المرضية لتطور مقاومة دودة اللوز القرنفلية (*Pectinophora gossypiella*) للدلتامثرين والدايبل (2X)

[٢١]

علاء الدين بيومي[١]- محمد السعيد صالح الزميتي[١]- مونا فكري روفائيل[٢]- همت زكريا محمد مصطفي

١. قسم وقاية النبات- كلية الزراعة- جامعة عين شمس- شبرا الخيمة- القاهرة- مصر
٢. معهد بحوث وقاية النبات- مركز البحوث الزراعية- الدقي- الجيزة- مصر

أجريت دراسة تطور مقاومة العمر الرابع لديدان اللوز القرنفلية *Pectinophora gossypiella* تجاه مبيدين حشريين هما دلتامثرين (مبيد بيريثرويدى) و دايبل 2x (مبيد حيوى مشتق من بكتريا *Bacillus thuringiensis* subsp. *kurstaki*) تحت الظروف المعملية من خلال الضغط الإنتخابى حتى ١٤ جيلاً. وقد درست ميكانيكية تطور المقاومة على المستويات البيولوجية، البيوكيميائية، والنسيجية المرضية. وقد دلت النتائج الحيوية المتعلقة بقيم السمية النصفية (LC_{50}'s) على أن مقاومة الدلتامثرين قد تزايدت إلى ٢١٥,١١ مرة بعد ١٤ جيلاً من الإنتخاب بالمقارنة بالسلالة الحساسة، بينما تزايدت مقاومة الدايبل 2x بمقدار ١٦ مرة تحت نفس الظروف. وعلى المستوى البيوكيميائى، فإن التقييم الإنزيمى للسمية قد أظهر أن نشاط إنزيم جلوتاثيون الناقل (S-transferase) قد إرتفع فى كل الأجيال المنتخبة عن ما هو بالسلالة الحساسة. وبالنسبة لنشاط إنزيمات الفوسفاتيز، فقد تزايد نشاط إنزيم الفوسفاتيز الحامضى بكل الأجيال فى سلالة الديبل عن السلالة الحساسة،وعلى العكس من ذلك، فقد

تناقص نشاط الفوسفاتيز القلوى بهذه الأجيال عن ما هو فى السلالة الحساسة.

أما بالنسبة للسلالة المقاومة للدلتامثرين، فقد إنخفض نشاط إنزيم الفوسفاتيز القلوى بينما لم يتأثر إنزيم الفوسفاتيز الحامضى إلا فى الجيل العاشر من هذه السلالة. ومن ناحية أخرى، فإن دراسة التفريد الكهربى لحزم البروتين قد أوضحت ظهور حزم جديدة بالسلالة المقاومة والتى لم تكن موجودة بالسلالة الحساسة.

وفيما يتعلق بالتأثيرات النسيجية المرضية، فإن النتائج المتحصل عليها توضح حدوث تغيرات نسيجية مثل سمك طبقة الخلايا الطلائية للمعى الأوسط فى كلا السلالتين المقاومتين لكل من دلتامثرين ودايبل 2x.

تحكيم: ا.د أمجد محمد كامل صبيحة
ا.د محمد باسم علي مقبل عاشور