

HISTOPATHOLOGICAL EFFECTS OF CERTAIN INSECTICIDES ON THE PINK AND SPINY BOLLWORMS

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Abstract

The present work aimed to evaluate the histopathological effects of four compounds, namely fenpropathrin, diflubenzuron, methomyl and Deenate on the larval integument and female ovaries of the pink bollworm, *Pectinophora gossypiella* (Saund.) and the spiny bollworm, *Earias insulana* (Boisd.) treated as 10- day old larvae.

The results showed that tested compounds destroyed the larval integument of the treated larvae in both insects by the compounds of fenpropathrin, diflubenzuron and methomyl. Also, separation between epidermal and cuticle layers, reduction in the epidermal layer and reduction in the larval muscles were shown by these compounds. Weak and elongation in the larval muscles in the Deenate treatment of the pink bollworm were recorded in addition to duplex structure between old and new cuticle of the spiny bollworm.

The tested compounds caused destructive effects in the adult females by absence of the nurse cells in the four treatments except diflubenzuron treatment to the pink bollworm. Reduction in the oocytes diameter with fenpropathrin treatment to the pink bollworm and diflubenzuron treatment to both insects were noticed. Fenpropathrin, methomyl and Deenate lead to hypertrophid the diameter of oocytes in the spiny bollworm.. Fenpropathrin treatment induced shrinking oocyte to the spiny bollworm and Deenate treatment to both insects were observed. Clustered the follicular epithelium especially, fenpropathrin, diflubenzuron and Deenate treatments of the spiny bollworm was observed. Absence the follicular epithelial nucleus in the most treatments especially in Deenate and fenpropathrin treatments to the pink and spiny bollworms, in addition, diflubenzuron and methomyl treatments to the spiny bollworm were recorded. Moreover, Vacuoles in fenpropathrin treatment to the pink bollworm was found.

INTRODUCTION

Cotton is consider one of the most economic crop in Egypt. The pink bollworm, *Pectinophora gossypiella* (Saund.) and the spiny bollworm, *Earias insulana* (Boisd.) are the most destructive pests infest cotton plants in the country.

There are many ways of the integrated pests management for controlling the bollworms but, the pesticides still used for this economic crop. In Egypt, growers used in average 3-4 insecticidal applications on cotton per season starting from mid-June at

which insect growth regulator, benzoyl phenyl ureas as diflubenzuron which had been used alone or in mixtures with insecticides. This treatment intended to check the initial infestation for as long as possible shown that standard control measures were often inadequate once previously used threshold levels had been reached (Sawicki and Denholm, 1987). Only pyrethroids are used for the second treatment which applied in late July. The third spray was implemented in mid or late August and applied to prevent the bollworms attack. The fourth spray was done in the late season to control the bollworms. However, some field populations of cotton bollworms are resistant to several insecticides (Ayad, *et al.* 1994) and (Rofail, *et al.* 1995).

The present work was planned to evaluate the histopathological effects of sublethal concentrations (LC_{25} 's) of fenpropathrin, diflubenzuron, methomyl and Deenate on the integument (epidermis and cuticle) and developed ovaries of both pink and spiny bollworms.

MATERIALS AND METHODS

Tested compounds:

Synthetic pyrethroid compound:

Fenpropathrin (Danitol 30% EC.): alpha-cyano- 3 – phenoxy phenyl methyl 2,2,3,3-tetra methyl cyclopropane carboxylate.

Carbamate compound:

Methomyl (Lannate 90% SP.): S-methyl N-(methyl carbamoyloxy) thioacetimidate.

Insect growth regulator (IGR):

Diflubenzuron (Dimilin 4% SP.): 1-(4-chlorophenyl)-3-(2,6 difluorobenzoyl) urea.

Carbamate/ IGR combination:

Methomyl 27% / Diflubenzuron 4% mixture (Deenate 31% FL.).

A laboratory strain of the larvae and eggs of the pink and spiny bollworms were reared in the Bollworms Department, Plant Protection Research Institute, Agricultural Research Center on semi artificial diet for the pink bollworm as described by Abdel-Hafez *et al.*, (1982) and the diet for the spiny bollworm described by Rashad and Ammar (1985). Rearing conditions were controlled at $27\pm 1^\circ\text{C}$ and 65-75% RH with complete darkness all day time.

For this purpose, four compounds fenpropathrin, diflubenzuron, methomyl and Deenate were selected for histopathology study. Ten day old larvae were treated by a contact method with the LC_{25} 's of these four compounds against the pink and spiny bollworms. At 5-day old after treatment for larvae specimens and 8-day old after emerging for female moths which resulted from 10- day old larvae treatment of both insects were obtained.

For histological investigation, several sections of the cuticle or ovariole of the pink and spiny bollworms were done following the method of Gad (1951). The procedure is summarized as follows:

A-The cuticle sections:

- 1- The whole larvae fixed in alcoholic Bouin's solution for 24 hours, washed in 70% alcohol, transferred to 90% alcohol followed by absolute alcohol for 4 hours.
- 2- Specimens were then transferred to a mixture of equal parts of absolute alcohol & ether and left overnight, thereafter to a mixture of 4 parts of 0.5% celloidin to one part of cedar wood oil for 24 hours to ensure complete infiltration.
- 3- The specimens were then placed in a drop of celloidin on a cover glass which has been previously dipped in melted paraffin to get a smooth surface on which the celloidin stands up.
- 4- The cover glass was quickly inverted and floated on the surface of a dish of chloroform at 1/2 hour followed by fresh quantity of chloroform at 2 hours. The embedded material fall away the cover glass and was transferred to a mixture of equal parts of chloroform and paraffin and left overnight on a hot plate. Finally it was transferred to melted paraffin in the oven for 3 hours (2 changes), embedded and sectioned as usual (8 μ thick).
- 5- The sectioned were stained with Heidenlein's iron haematoxylin or Erlisch's haematoxylin and counter stained with eosin.

B- The Ovariole Sections:

The ovarioles were went out from the females putted in fixed solution (water Bouin's solution) at 24 hours and washed in alcohol 70, 80, 90, 96 and 100% concentrations. The ovarioles were cleared in xylene, embedded in wax, made blocks, cutted the ovarioles and covered the glass cover. Finally, the sections were stained with haematoxylin and eosin.

A parallel control of non-treated larvae and female moths were also carried out. The general state of the ovariole was examined, and the oocyte diameter was also measured.

All measurements of the histological sections were taken by an object and ocular micrometer and were based on at least 20 sections.

All drawings were made by the camera lucida.

RESULTS AND DISCUSSION

Histopathological effects of certain insecticides on the pink and spiny bollworms:

The present work was planned to evaluate the histopathological effects of sublethal concentrations (LC_{25} 's) of fenprothrin, diflubenzuron, methomyl and

Deenate on the integument (epidermis & cuticle) and developed ovaries of both the pink and spiny bollworms.

A- Histopathological effects on the larval integument:

The integuments of the pink and spiny bollworms were obtained at the sixth day after treating the 10- day old larvae. A light microscope was used in the observation.

1- The untreated check (Control):

As shown in Figure (1) the epidermis is a single layer beneath the cuticle and extensive of the larval muscles appeared clearly in both pink and spiny bollworms, respectively.

2- The treated larvae:

The larval integument of the pink and spiny bollworms showed a separation between the epidermal layer and the cuticle of the treated larvae with fenprothrin, diflubenzuron and methomyl as shown in Figure (1) for the pink bollworm and Figure (2) for the spiny bollworm. In addition, reduction of the epidermal layer and the muscles of the larval integument was caused by the insecticides treatments were observed. All of those observations lead to failure the larvae in eclosion and death. Moreover, the Deenate had elongated the muscles layer towards the center and reduced their size compared with the control in the pink bollworm as shown in Figure (1) . On the other hand, the same compound caused duplex structure from old and new cuticle in the integument of the spiny bollworm as shown in Figure (2). This duplex structure lead to blackened colour which observed on the larvae before death.

Many authors obtained the same conclusion as El-Badawy, *et al.* (1979) stated that Dimilin caused abnormal endocuticular deposition in *S. littoralis*. Also, Abol- Ela *et al.* (1986) showed that diflubenzuron caused an abnormal reduction in the epidermal layer in the treated larvae of *S. littoralis* . In addition clear separation of the cuticle from the epidermis was observed with triflumuron of the same insect. On the other hand, the section of the spiny bollworm of 5- day old larvae obtained from IKI-7899 treatment showed that separation of the epidermal cells from the cuticle occurred in some sites and did not occurred in another (Shalaby, 1996). Generally the size of sections much reduced and the epidermal layer appeared very weak compared with the untreated check.

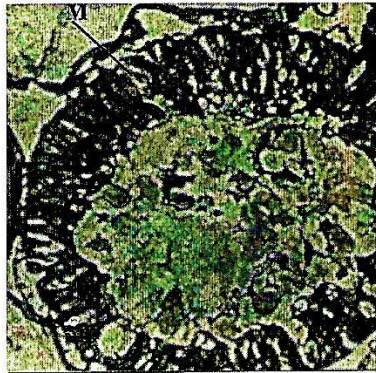
B- Histopathological effects on the adult female ovaries:

The ovaries of the pink and spiny bollworms were obtained from 8- day old females after emergence of the treated larvae at 10- day old and the light microscope was used in the observation.

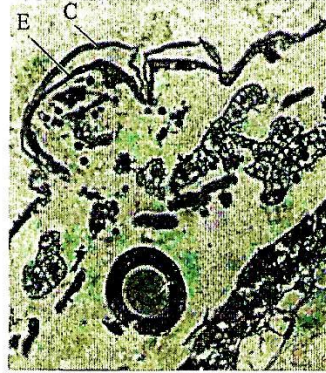
1- The untreated check (control):

The normal females of the pink and spiny bollworms had well developed ovaries with four polytrophic ovarioles consists of developing ova.

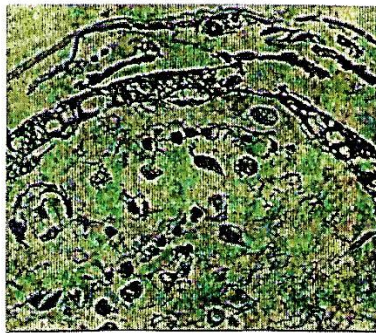
Fig. 1. Across sections in the larval integument of the pink bollworm treated as 10-day old larvae by certain insecticides.



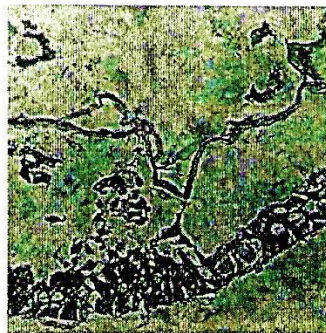
Untreated larvae (Check)



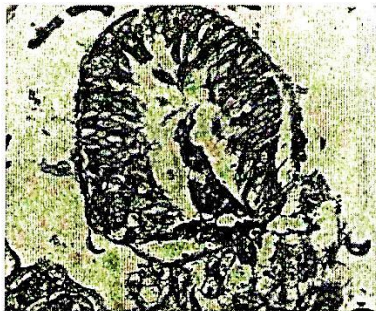
fenpropathrin treatment



Diflubenzuron treatment



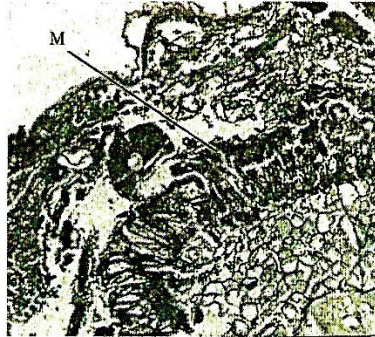
Methomyl treatment



Deenate treatment

C = Cuticle layer
E = Epidermal layer
M = Muscles
100x

Fig. 2. Across sections in the larval integument of the spiny bollworm treated as 10-day old larvae by certain insecticides.



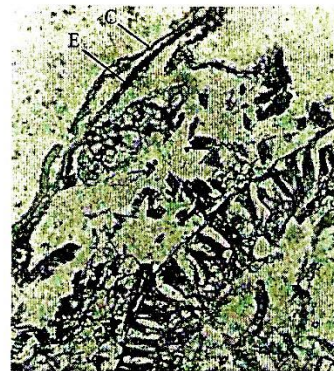
Untreated larvae (Check)



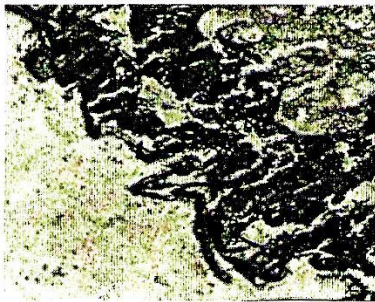
fenprothrin treatment



Diflubenzuron treatment



Methomyl treatment



Deenate treatment

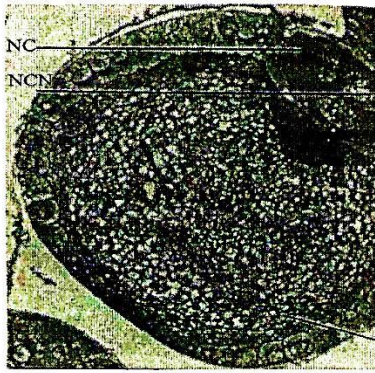
C = Cuticle layer

E = Epidermal layer

M = Muscles

100x

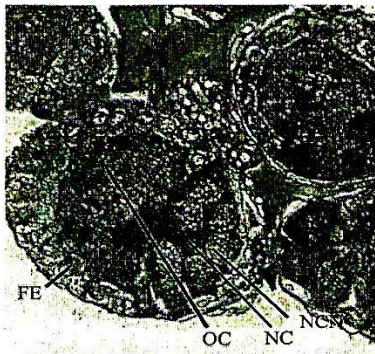
Fig. 3. Across sections in the oocyte of the adult female moth of the pink bollworm treated as 10-day old larvae by certain insecticides.



Untreated larvae (Check)



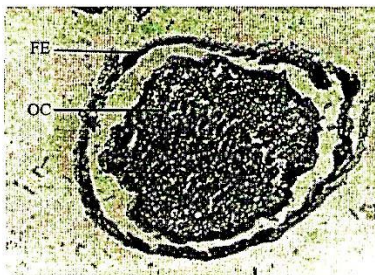
fenpropathrin treatment



Diflubenzuron treatment



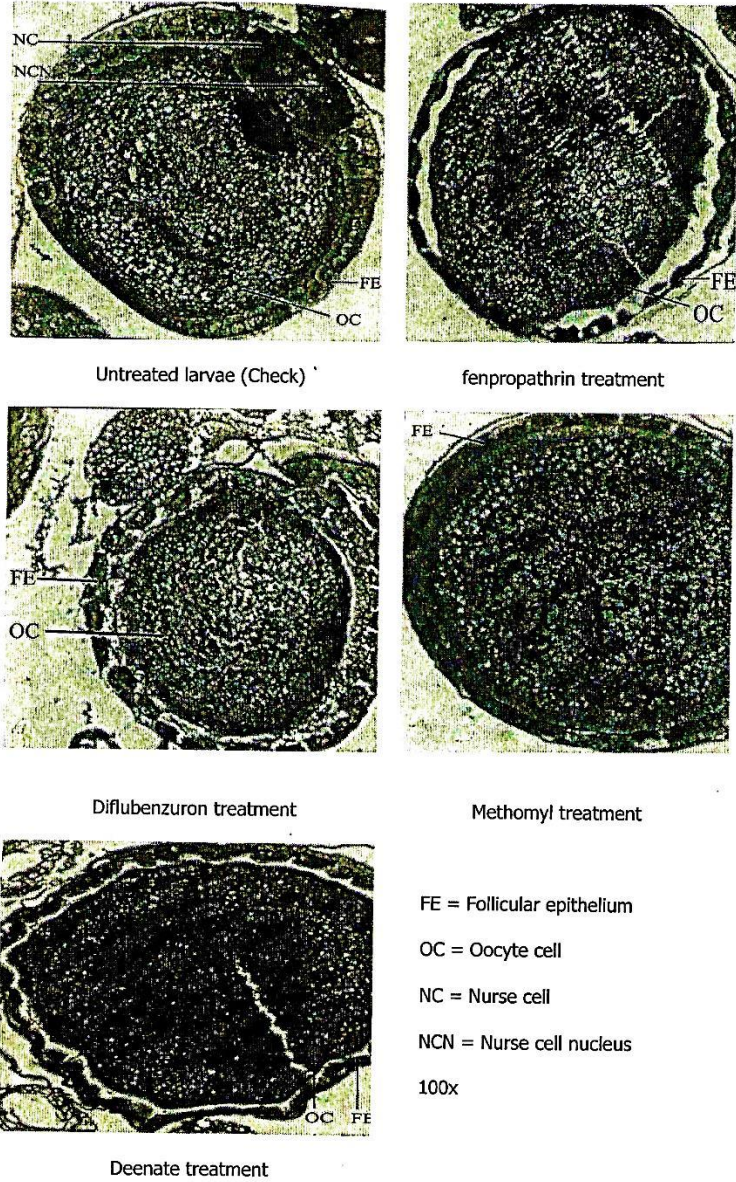
Methomyl treatment



Deenate treatment

FE = Follicular epithelium
OC = Oocyte cell
NC = Nurse cell
NCN = Nurse cell nucleus
V = Vacuoles
100x

Figure 4. Across sections in the oocyte of the adult female moth of the spiny bollworm treated as 10- day old larvae by certain insecticides.



Histologically, as shown in figures (3), each growing oocyte is accompanied anteriorly by a few number of nurse cells. The oocyte is surrounded by some follicular epithelial cell and its nucleus clearly, measuring about 0.318 mm of the pink bollworm and about 0.418 of the spiny bollworm in diameter as shown in Table (1).

2- Treated insects:

Fenpropathrin treatment: As shown in figure (3), the oocytes of the pink bollworm were reduced than in the untreated check measuring by about 0.251 mm in diameter, absence the nurse cells, disappearance of the nucleus of the most follicular epithelial cells were noticed. Another the same cells appeared but unclear in addition to the appearance of the vacuoles in the oocyte cell. Also, the tested chemical caused more destruction appeared acts in the shrink of the oocyte cell and the nucleus of the follicular epithelial cells which were absent. Moreover, the same cells were clustered as shown in figure (4).

Diflubenzuron treatment: The diameters of the oocytes were more reduced in both insects than the control, especially in the pink bollworm (0.243 mm) and the nurse cells were appeared as shown in Figure (3). On the other hand, in the spiny bollworm, the nurse cells disappeared in most oocytes in addition to absence of the most nucleus of the follicular epithelial cells and the same cells appeared clustered as shown in figure (4).

Methomyl treatment: As for the effect of methomyl on the pink bollworm appeared in figure (3), the follicular epithelial and nurse cells were mixed that is very difficulty to be differentiated between them, while in the spiny bollworm as shown in figure (4), the nurse cells were absent and most oocytes hypertrophid, the diameter was 0.42 1 mm. In addition, the follicular epithelial cells were greatly damaged in many parts in the spiny bollworm and appeared the vacuoles among them in the pink bollworm.

Deenate treatment: In the pink bollworm, Deenate had more destructive acts in shrunk of the oocyte cell, scattered the follicular epithelial cells and didn't make distinguished shape. In addition to the absence of the nucleus of the follicular epithelial and nurse cells as shown in figure (3). Moreover, the diameter of the oocytes was reduced to about 0.293 mm. in the same insect. While, in the spiny bollworm, the diameter of the oocyte was about 0.419 mm, caused hypertrophid than the control. The same compound caused shrunk the oocyte cell away the follicular epithelial cells. The absence of the nurse cells and most nucleus of the follicular epithelial cells and the same cells clustered in some sites and didn't make distinguished shape in another ones in the spiny bollworm as shown in figure (4).

Table 1. Effect of sub-lethal concentrations (LC₂₅) of certain insecticides on the diameter oocytes of the pink and spiny bollworms treated as 10 - day old larvae.

Tested insecticides	Pink bollworm		Spiny bollworm	
	LC ₂₅ (ppm)	Oocyte diameter (mm)	LC ₂₅ (ppm)	Oocyte diameter (mm)
Fenprothrin	1.90	0.251	1.80	0.420
Diflubenzuron	1.30	0.243	1.30	0.265
Methomyl	1.25	0.297	1.20	0.421
Deenate	1.10	0.293	0.98	0.419
Check (Control)	-	0.318	-	0.418

All of these destructives act in shrunk the oocyte cell, clustered the follicular epithelial cells, absence the nurse cells and the nucleus of the follicular epithelial cells in addition to reduction in the oocyte diameter especially in fenprothrin and Deenate treatments. Moreover, diflubenzuron and methomyl treatments caused the reduction in the number of egg chambers/ovariole and ovariole length which lead to laying few number of eggs or deposited immature eggs which caused reduction in the hatchability percentages

The previous data are in harmony with results obtained by Sammour and Abdalla (1988). They showed that the larvae of *Heliothis armigera* which reared in the laboratory and treated with teflubenzuron and coumarin by 20&10% reduced the number of egg chambers, ovariole and ovariole length. While, El- Kady *et al.* (1990), observed ovarian deformities in *Agrotis ipsilon* by using the hormone medroxy progesterone acetate (MPA) and the insect growth inhibitor (XRD). Moreover, Abdel-Aal (1996) showed that both IKI- 7899 and flufenoxuron significantly reduced the ovariole length of the *Agrotis ipsilon* surviving treatment of the 4th instar larvae with LC₅₀ of these two IGR's.

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التأثيرات الهستوباثولوجية لبعض المبيدات على دودتى اللوز القرنفلية و الشوكية

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تهدف هذه الدراسة إلى تقييم التركيز تحت المميت LC₂₅ لأربعة مركبات (فينبروباثرين fenpropathrin - دينيت Deenate و مخلوطه المكون من الميثومايل methomyl و الدايفلوبنزورون diflubenzuron) على يرقات عمر ١٠ أيام لدودة اللوز القرنفلية *Pectinophora gossypiella* (Saund.) و دودة اللوز الشوكية (*Earias insulana* (Boisd.)). و دراسة التأثيرات الهستوباثولوجية للمركبات السابقة على جدار جسم اليرقة و مبايض الإناث للأفتين السابقين. و قد أشارت النتائج إلى:

أولاً: التأثير على جدار جسم اليرقة:

أظهرت المركبات تأثيراً ضاراً بكيوتيكل دودتى اللوز القرنفلية و الشوكية تمثل فى حدوث إنفصال بين طبقة البشرة و الكيوتيكل - اختزال فى طبقات البشرة الداخلية - حدوث اختزال لعضلات اليرقات وذلك فى معاملات الفينبروباثرين و الدايفلوبنزورون و الميثومايل - ضعف العضلات و إسئطلتها فى معاملة الدينيت لدودة اللوز القرنفلية بالإضافة إلى عدم إنفصال طبقة الكيوتيكل القديم عن طبقة الكيوتيكل الحديث لدودة اللوز الشوكية فى المعاملة بنفس المركب.

ثانياً: التأثير على مبايض الإناث:

سببت المركبات تأثيراً ضاراً على بويضات الإناث البالغة تمثل فى غياب الخلايا المغذية فى الأربع معاملات ما عدا معاملة الدايفلوبنزورون لدودة اللوز القرنفلية - صغر قطر البويضات فى معاملة الفينبروباثرين لدودتى اللوز القرنفلية و الشوكية كما أدت معاملات الفينبروباثرين و الميثومايل و الدينيت إلى كبر قطر البويضات لدودة اللوز الشوكية - حدوث إنكماش للبويضات فى معاملة الفينبروباثرين لدودة اللوز الشوكية و معاملة الدينيت لدودتى اللوز القرنفلية و الشوكية - ظهور النسيج الطلائى فى صورة عنقايد خاصة فى معاملات الفينبروباثرين و الدايفلوبنزورون و السدينيت لدودة اللوز الشوكية - إختفاء أنوية النسيج الطلائى فى معظم المعاملات خاصة فى معاملات الدينيت و الفينبروباثرين لدودتى اللوز القرنفلية و الشوكية بالإضافة إلى معاملات الدايفلوبنزورون و الميثومايل لدودة اللوز الشوكية - وجود فجوات فى معاملة الفينبروباثرين لدودة اللوز القرنفلية.