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HISTOPATHOLOGICAL CHANGES IN FENITROTHION AND AZINPHOS-METHYL RESISTANT STRAINS OF THE EGYPTIAN COTTON LEAFWORM, SPODOPTERA LITTORALIS

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ABSTRACT

Histopathological studies were carried out in the third instar larvae of the Egyptian cotton leafworm, Spodoptera littoralis (Boisd.) of two organophosphate resistant strains, azinphos-methyl and fenitrothion with resistance ratios of 14.1 and 17.03 fold respectively. A laboratory strain was also studied as a reference one. The tissues studied were cuticle , epethelium cells & muscles surrounding them, fat bodies and the nerve ganglion. The cuticle thickness and the epethelium cells were more sifnificantly elongated in the two resistant strains than in the susceptible one: Muscles were slightly thicker in the two resistant strains, but the difrences were not significant. The number of fat bodies was increased significantly in the susceptible strain than in the two resistant strains. The dimensions (length & width) of the nerve ganglion were more elongated significantly in azinphos methyl resistant strain. It could be suggested that resistance property to organophosphates is correlated with the thickness of some tissues which may decrease either penetration or absorbtion of the insecticides and hence, reduce the amount of the actual toxicant reaching the target.

INTRODUCTION

Several investigators studied the effects of insecticides on histopathological parameters in S. littoralis and other species. El bery (1964) concluded that azinphos methyl and carbaryl caused vacuolization, degradation and shrinkage of both nuclei and cytoplasm with little damage of membranous sheath of fat body of spiny bollworm, Earias insulana and pink bollworm Pectinophora gossypiella. Salama (1955) concluded that Endrin, DDT,carbaryl and parathion caused cytological changes in mid-gut epithelia of Prodenia littura (F.).Hassanein et al (1968) mentioned that DDT, trichlorfon, carbaryl and lebaycid caused pronounced destructive effects on the cuticle mid-gut malpighian tubules, fat body and muscles of the larvae of the spiny bollworm Earias insulana. Toppozada, et al (1968) mentioned that contact or oral uptake of carbaryl, parathion,

DDT and erdrin caused considerable cytopathological changes in the mid-gut equithelia of cotton leafworm Spodoptera littoralis (Boisd.). No quantitative difference occured between the two routes of application or different insecticides. Hassanein and Khalil (1969) studied the effect of DDT , toxaphene, dieldrin, endrin, lindane, parathion and azinphos methyl, chlorthion, demton , trichlofon and carbaryl on cuticle muscles, fat , body , malpighian tubules, and mid-gut of Coccinella undecimpunctata(Reiche). The compounds caused destruction to tissues of cuticle, muscles fat , body , malpighian tubules and mid gut. Moustafa (1969) reported that Nuvacron caused to all tissues of cotton leafworm in the mid gut. The epithelium was badly destroyed and showed vaculization and most cell boundaries disappeared. The cytoplasm became granulated and craked. Radwan (1970) found histopathological effect on various body tissues of the cotton leafworm when the third larval instar fed on leaves sprayed with Du-ter . The material caused pronouced destruction mainly to tissues of mid-gut and muscles while partial damage occured to fat bodies and malpighian tubes. Mohaimen (1971) concluded that dicrotophos, aldrin, ethion, mexacarbate and heptachlor caused distructive effect on the mid-gut, the nerve ganglion, malpighian tubules and the fat body of the 3rd instar of the Egyptian cotton leafworm Spodoptera littoralis. Abdallah and Salama (1977) reported that Dursban and cyolane caused histological changes in the med gut epithelia of Heliothis armigera . Saad et al (1985) concluded that mephospholane caused elongation in epithelial cells, detachment of pretrophic membrane and vaculization of boundary lines. Decamethrin caused also great damage to the epithelial layer, detachment of preitrophic membrane of 4th instar larvae of P. gossypiella . The aim of this study is to study the histological character of resistant strains after the frequent exposure to the organophosphates. This concept is of concern in the explanation of resistance mechanism.

MATRIALS AND METHODS

Insect strains used.

Reference (susceptible strain): A laboratory strain of the Egyptian cotton leafworm Spodoptera littoralis was reared in the laboratory on castor bean leaves.

Resistant strains: two resistant strains to fenitrothion (R_f) and to Azinphos-methyl (R_a) with 17.03 and 14.1 fold resistance respectively were developed by topical application technique as described by kassem et al (1982).

Histological technique: The third instar larvae were fixed in alcoholic Bouin's solution for at least 24 hours, and to ensure complete fixation of various tissues, the larvae were put under vacuum.The fixed larvae were dehydrated by transferring to several ascending grades of absolute alcohols (50,.60, 70, 80, 90, and 100%) for about 12 hours each. After dehydration the larvae were treated for clearing the tissues by placing it in solution of amyl acetate for minimum period of 3,4, days at room temperature. For embeding, the larvae were impregnated in hot paraffin wax for about two hours under vacuum. The wax block was solidified by putting it in cold water. The block was then stuck to the holder of the rotary microtome by melted wax and serial sections were obtained at a thick hot plate and then they were ready for slaining.The slides were dipped in xylol for 20-30 minutes to remove the wax. The slides were then dipped in a series of different alcohol solution each for a period of 5 min starting with absolute ethanol 90, 80 and 70% then the slides were rinsed in distilled water. They were dipped in the solution of the Ehrlich stain for 2 minutes. To wach aff the excess stain the slides were washed in distilled water, then in 70% acid alcohol- until the sections were reddish. The slides were then treated with alkaline alcohol 70% until the section turned blue the slides were then dipped in 95% alcohol for 3 minutes then in Eosin for few seconds. Excess Eosin was removed with 95% alcholol.

RESULTS AND DISCUSSION

Histopathological studies were carried out in two organophosphate resistant strains besides a susceptible one. The tow resistant strains were azinphos methyl resistant strain and fenitrothion resistant one with resistance ratios of 14.1 and 17,03 fold respecrively. Third larval instar of the cotton leafworm was used in this study.

Histopathological characteristice of azinphosmethyl and fenitorthion resistant strains:

1- The cuticle:

It is clearly obvious that the cuticle of azinphos-methyl and fenitrothion resistant larvae was relatively thicker . The thickness of the cuticle of fenitrothion and azinphos-methyl resistant strains was 18.70 µ and 16.42 µ respectively compared with 11.22 µ of the susceptible strain. Statistical analysis showed significant differences in cuticle thickness between the resistant strains and the susceptible one, Table(1) and Fig (1)

Table (1): Average characteristics of some histopathological parameters of larvae in two resistant strain and a susceptible one of *S. littoralis*

Strrins / Histological patameters	Cuticle	Epithelium	Muscles	Nerve ganglion		Fat body
	Thickness	Length (u)	thickness (u)	Length Thickness (u)	Width Thickness (u)	Number
Azinphos methyl	15.42	121.42	11.68	124.25	80.78	30.58
Fenitrothion	18.70	133.55	11.21	94.27	64.41	32.45
Susceptible	11.22	88.77	11.19	75.88	65.67	37.86
L.S.D	0.1	0.37	N.S	4.38	3.48	1.89

Fig 1(a)

Fig 1(b)

Fig(1): C.S. of the cuticle showing the thickness.
a- Susceptible strain.
b- azinphosmethyl resistant strain
c- Fenitrothion resistant strain (X) 800.

Fig 1(c)

2- The fat body:

The number of fat body was estimated in susceptible strain and the two fenitrothion and azinphos-methyl resistant strains. The average number of fat body was 37.86, 32.45 and 30.58 respectively. There were significant differences between the three strains, Table (1) Fig.(2).

3- The nerve ganglion:

The dimensions of nerve ganglion in each of the azinphos-methyl, fenitrothion resistant and susceptible strains were measured. The nerve ganglion dimensions of susceptible strain were 79.08 u in length and 65.07 u in width, while in the two resistant strains, azinphos-methyl and fenitrothion were (124.25 in length, 80.78 in width and 94.67 in length 64.41 in width) respectively. The statistical analysis showed significanct differences between azinphos-methyl resistant strain and the susceptible one while the variations between fenitrothion resistant strain and the susceptible one were insignificant. In azinphos-methyl resistant strain the neuropile mass appeared as one mass and the ganglion neurolemma appeared to be thicker as an outer imaginal nerve cells. Table (1) and Fig (3)

4- The muscles surrounding the epithelium cells (Musculosa):

The studies revealed that there were no significant differences between the two resistant strains and the susceptible one Table (1)

5- The mid-gut:

The epithelium cells of fenitrothion and azinphos-methyl resistant larvre were elongated and also few vaculizations were observed in some cells. The nuclei became obviously elongated. In some parts the peritrofic membrane was detached from the epithelial cells. Chromatin lysis was observed in some parts. Obvious space between the surrounding muscles layer and epithelial cells were noticed. The statistical analysis emphasized that there were significant differences between the two resistant strains the susceptible one. Table (1) and Fig (4)

In this study the histopathological results revealed that, the cuticle of fenitrothion resistant strain was thicker than azinphos-methyl resistant strain. The thickness of the cuticle of both the two resistant strains was obviously noticed than the susceptible one. Hassanein et al

Fig 2(a)

Fig 2(b)

Fig 2: C.S. Showing the number of fat body

a- Susceptible strain

b- fenitrothion resistant strain

c- azinphos-methyl resistant strain X (126)

Fig 2(c

Fig 3 (a)

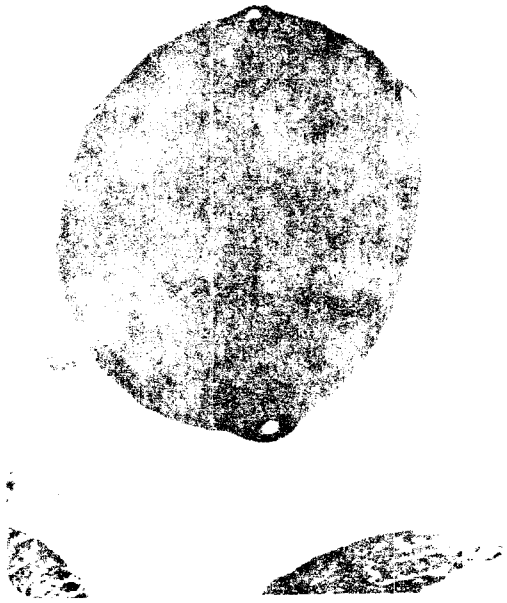

Fig 3 (b)

Fig (3): C.S. of nerve ganglion

a- Susceptible strain

b- fenitrothion resistant strain

c- azinphos-methyl resistant strain X (320).

Fig 3 (c)

Fig 4 (a)

Fig 4 (b)

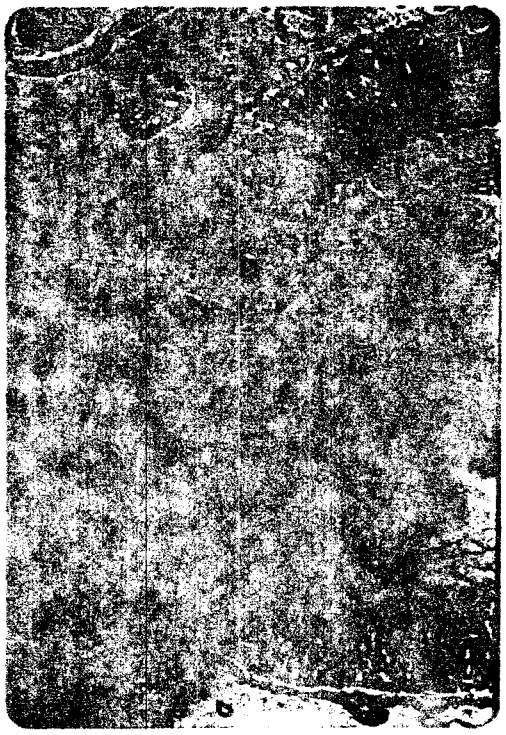

Fig 4 (c)

Fig 4: T.S. of mid-gut showing the elongation of epithelial cells

a - Susceptible strain

b - fenitrothion, resistant strain.

c - azinphos-metnyl resistant strain X (126).

(1968) studied the effect of some insecticides on the cuticle, the mid-gut malpighian tubules, fat body, and muscles of the larvae of spiny bollworm Earias insulana (Boisd.)

Concerning the fat body there were also significant differences in the average number of the fat body between the two resistant strains and the susceptible one. The average number of the fat body in the susceptible strain was much more than the average number of those in both azinphos-methyl and fenitrothion resistant strains but there were no significant differences between the two resistant strains. This result lead to the conclusion that the storage of organophosphprous in fat body as a resistance mechanism which could refer to the species spesificity of the fat body rather than to its number.

Ruswan (1970) found that destructive damage appeared mainly on the mid-gut and muscles while partial damage occured to the fat body when third instar larvae of cotton leafworm were fed on leaves sprayed with Du-ter.

Concerning the nerve ganglion there were signifrcant differences between azinphos-methyl resistant and both fenitrothion resistant strain and susceptible one. There were no changes through the nerve ganglion in fenitrothion resistant strain and the susceptible one. This property may cause the resistance in fenitrothion resistant strain. Mohaimen(1971) showed that heptachlor did not show any effect on neurolemma, the imaginal nerve cells, and the larval nerve cells of third instar of S. littoralis, the results revealed that hte neuropile mass in azinphos-methyl resistant strain appeared as one mass, and the ganglion neurolemma was larger as an outer imaginal nerve cells were noticed. In this case the resistance property is probably because of other reasons such as the thickness of the cuticle or the elongation of the epithelial cells. Soliman and Soliman (1958) in their work also on S. littoralis found that degeneration of the nuclei of the nervous cells was caused by toxaphene.

According to histopathological effects on the muscles, the data obtained demonstrated that there were no obvious changes in the larval muscles of the two resistant strains compared with the susceptible one. These results agreed with those found by Mohaimen (1971). He found that very little changes were caused by heptachlor on the third larval instar of S. littoralis. The muscular bundles were not damaged, and the sarcole-

mma was not affected. Contrary he also found that in some larval stages sarcolemma of muscles was destroyed and the muscular bundles were broken into several parts by dicrotophos, endrin, ethion and meixacarbate.

For the mid-gut azinphos-mehtyl and fenitrothion resistant strains showed some symptoms on the mid-gut. The epithelial cells were elongated in both resistant strains but in fenitrothion resistant strain it was longer than the epithelial cells in azinphos-methyl resistant one. The nuclei also elongated, little vaculization took place. The pretrophic membrane was detached from the epithelial cells in some parts but it was not affected. The epithelial cells were detached from the surrounding sarcolemma. Soliman and Soliman (1958) in their work on S. littoralis found parathion and DDT caused vaculization of fat body, and vaculization of mid-gut epithallur from its basment membrane. El Deeb and Zeid (1961) demonstrated that carbaryl caused elongation in the epithelial cells of the pink bollworm. Salama (1963) also studied that carbaryl, parathion DDT and endrin caused cytological changes in the mid-gut epithelial cells of Prodenia litura .

In conclusion it could be suggested that the resistance property in both resistant strains under study were correlated to the thickness of the cuticle which prevents some what the penetration of the insecticides to the interral tissues and hence, reduces the amount of the actual toxic principal reaching the target.

REFERNCES

Abdallah, S.A. and A.E. Salama (1977). Toxicological studies of Dursban and cyolane against Heliothis armigera.The second Arab pesticide conference, Fac. of Agric., Tanta Univ., September 26-29, part I, 97 - 106.

El-Sery, A.H.H. (1964). Effect of some insecticides on the tissues of both spiny and. Pink bollworms during the course of their control M.SC. Thesis, Fac. of Agric., Univ: of Alex.

El-Deeb, A. A. and M.I. Zeid (1961). Histopathological studies of the effect of some insecticides on various tissues of the larvae of pink bollworm Pectinophora gossypiella (sound. Alex. Jour Agric Res., 9: 55-91.

Hassanein, M.H. and F.M. Kalil (1969). Histopathological effect of various insecticides on the larval stage of *Coccinella undecimpunctata* Reidhe. Bull. Ent. Soc. Egypt. Econ. Ser., 3: 29-42.

Hassanein, M.H., F.M. Zaki and A.A.M. Kamel. (1968). Histopathological Studies on the effect of certain insecticides on various tissues on the larvae of *Earias insulana* (Boisd.) Bull. Ent. Sec. Egypt. Econ., Ser., 2: 181-191.

Kasser [illegible]sh, M.I.; A.A. Mahran; Fathia I. Moustafa and A. Nomeir (1982). Development of resistance and cross resistance for two organophosphorus insecticides and their mixture in the Egyptian cotton leafworm, Alex. Sci. Exch. [illegible]

[illegible] M.H. (1971). The effect of some pesticides on some insect tissues. M. Sc. Thesis, Fac. of Agric., Univ. of Alexandria.

Moustafa, [illegible] H. (1969). Control of the most important economic pests by [illegible] pesticides and the effect of these pesticides on tissues of some of these insects. Ph. D. Thesis, Fac. of Agric., Univ. of Alex.

Radwan, H.S.A. (1970). Various measurements for the control of cotton leafworm, *Spodoptera littoralis* (Boisd.). Ph. D Thesis, Fac. of Agric., Ein shams Univ.

Saad, A.S.A.; [illegible] Hosny; A. Awad and A.E. Salama (1976). Histopathological effect of organotin compounds (Du-Ter and Plictran) on Egyptian cotton leafworm *Spodoptera littoralis* (Boisd.). Res. Bull. No 26 Fac. of Agric. Zagazig University

Salama, A.E A. (1966). Contact versus stomach effect of various insecticides on the Egyptian cotton leafworm *Prodenia litura*. MSc. Thesis, Fac. of Agric., Univ. of Alex.

Soliman, S.A. and A.A. Sultan [illegible]. Histopathological destruction caused to the cotton leafworm *Prodenia litura* F. by some insecticides. Bull. Soc. Ent. [illegible], 4[illegible]: 1[illegible]

Sorour[illegible], A., A.E. Salama; [illegible] and [illegible] (1968). Histopathological effects of insecticides on the mid-gut of Egyptian cotton leafworm *Spodoptera littoralis*. Annals of Entomol. Soc. of America; 61: 1326-1333.

الملخص العربي

تغيرات هستوباثولوجية في سلالات مقاومة للفنتروثيون والازينفوس ميثايل من دودة ورق القطن المصرية

أجريت الدراسات الهستوباثولوجية في يرقات العمر الثالث من دودة ورق القطن المصرية المقاومة لمبيدين من مجموعة مركبات الفوسفور العضوية هما الازينفوس ميثايل والفنتروثيون والتي لها درجة مقاومة ١٫٤١ ، ٣٠٫١ مثل على التوالي الى جانب دراسة السلالة الحساسة وذلك للمقارنة . والانسجة المستهدفة هي الكيوتكل والخلايا الطلائية والعضلات المحيطة بها والاجسام الدهنية والعقدة العصبية . ولقد أظهرت السلالات المقاومة زيادة في سمك الكيوتكل واستطالة في الخلايا الطلائية وكانت الزيادة معنوية بالمقارنة بالسلالات الحساسة اما العضلات المحيطة بالخلايا الطلائية فانها زادت في السمك ايضا عنها في السلالة الحساسة ولكن الفروق كانت غير معنوية . واظهرت السلالة الحساسة زيادة في عدد الاجسام الدهنية عن السلالات المقاومة وكانت الفروق معنوية . اما العقدة العصبية فان ابعادها (الطول والعرض) زادت زيادة معنوية في السلالة المقاومة وعلى الاخص لمبيد الازينفوس ميثايل عنها في السلالة الحساسة . ويمكن اقتراح أن صفة المقاومة للمبيدات الفوسفورية العضوية التي درست لها علاقة بسمك بعض الانسجة التي درست والتي تقلل اما نفاذ او امتصاص المبيدات وبالتالي تقلل من الكمية الحقيقية السامة التي تصل في النهاية الى الهدف .