Sci. J. Fac. Sci. Mohoufia Univ. Vol. V (1991) 115 - 128 .

HISTOLOGICAL AND HISTOCHEMICAL EFFECTS OF FENVALERATE ON SPODOPTERA LITTORALIS LARVAE

Taha M. A., Sakr S. A. and Eid T. M. A.

Faculty of Science, Menoufia University

ABSTRACT

The histological and histochemical effects induced in the 3rd larvae instars of S. littoralis by the pyrethroid fenvalerate were studied. Fenvalerate induced histopathological destruction in the tissues of mid gut epithelium, Malpighian tubules, nerve ganglion and muscles. A slight decrease in glycogen content as well as total proteins was observed in most of the tissues. RND content was decreased in the majority of epithelial cells of the mid gut and Malpighian tubules. DNA content in the cells of most tissues was apparently unchanged as compared with that of control larvae.

INTRODUCTION

The cotton leaf worm *Spodoptera littoralis* is considered as the most pest which causes serious damage to cotton plant and many other crops in Egypt.

Some investigators studied the effect of pyrethroids on the cotton leaf worm (Cantu *et al.*, 1970; Hosny *et al.*, 1977; El-Okda *et al.*, 1978; Taha, 1981 and Hosny *et al.*, 1985).

The effect of pyrethroids and different insecticides on the histology and histochemistry of insects attracted the attention of some authors (Hartzell, 1934; Hartzell and Scudder, 1942; Merrill *et al.*, 1946; Soliman and Soliman, 1958; El-Defrawi *et al.*, 1964; Ramade, 1973; Hamed *et al.*, 1974; Sambought *et al.*,

1974; Rawash *et al.*, 1977 and Metwally *et al.*, 1978).

The mode of action of pyrethroids in different insects is not well understood, therefore the present work was initiated to investigate the histological and histochemical changes induced by fenvalerate in the tissues of S. *littoralis* larvae.

MATERIAL AND METHODS

a - Insects :

Egg masses of Egyptian cotton leaf worm *S. littoralis* were reared in the laboratory under constant conditions. (temperature 25 ± 2°C and 70 ± 5% relative humidity). The produced larvae were kept in separate jars, while the 3rd instars were selected in the present work.

b - Insecticide :

The pyrethroid insecticide, fenvalerate (sumicidin) was used. The LC_{50} of this insecticide was determined in the laboratory and found to be 0.24 µg/g body weight for the 3rd larval instars (Taha, 1981). To obtain the desired concentrations, fenvalerate was dissolved in acetone and then applied on the dorsal region of the prothorax of each larva.

c- Histological and Histochemical Techniques :

. Third larval instars were treated with LC_{50} of fenvalerate for 24 hours. For histological studies, control and treated larvae were fixed in Bouin's fluid and serial sections were stained with haematoxylin and eosin. For histochemical studies, larvae were fixed in Carnoy's fixative. Glycogen was detected in the larval tissues by

Periodic Acid Schiff's technique (PAS) of Hotchkiss (1948), total proteins were demonstrated by the mercuric bromophenol blue method of Mazia *et al.* (1953), and nucleic acids (DNA and RNA) were demonstrated by feulgen-methylene blue method of Garvin *et al.* (1979).

RESULTS

Cross sections of the different regions of the 3rd larval instar showed that most of the tissues were affected by the insecticide, e.g., mid gut, Malpighian tubules, nerve ganglia and muscles.

1- Histological Effect of Fenvalerate on the 3rd Larval Instar :

a - Control Larvae

The histological structure of the 3rd larval instar is shown in figures 1 and 2. The mid gut wall is made up of the musculosa to the outside. it is composed of two layers of muscle fibers, outer longitudinal fibers and inner circular fibers. The epithelium consists of cylindrical columnar cells, goblet cells and small regenerative cells. The contents of the mid gut are enclosed in a thick peritrophic membrane. There are three Malphighian tubules running along each side of the mid gut. Malpighian tubule epithelium is composed of a single layer of cells which contains large nuclei and is invested by connective tissue. The cytoplasm of the cells is filled up with pigmented droplets. The epithelium possesses a striated border. The ventral nerve cord ganglion is an oval mass of nerve tissue as common for other generalized insects.

b - Treated Larvae

Histological examination of the 3rd larval instar treated with fenvalerate revealed many pathological changes (Fig. 3). The epithelium of the mid gut is exfoliated from its basement membrane. The cytoplasm of the epithelial cells became vacuolated and the nuclei appeared to be of different sizes and some nuclei were pyknotic (Fig. 4). Parts of the peritrophic membrane was found to be destroyed. The epithelium of Malpighian tubules showed considerable vacuolization, degeneration or disintegration with some of the degenerated parts falling in the lumen. The nerve fibrous mass showed vacuolization and appearance of certain granules among the nerve cells particularly among those at the dorsal part of the ganglion of the ventral nerve cord was appeared. The sarcolemma of the muscles of treated larvae was completely destroyed and the muscle fibers were broken into several parts.

2- Histochemical Effects of Fenvalerate on the 3rd larval instar :

a - Glycogen

Cross section in the control larvae indicated that the mid gut, Malphighian tubules, nervous tissue and the muscles gave a positive reaction with PAS characteristic for carbohydrate. The epithelial cells of the mid gut stained with various degrees of intensity (Fig. 5). The cytoplasm of Malpighian tubules epithelium stained with bright red granules. In case of nerve ganglion a positive reaction is shown in the form of very fine granules scattered in the homogenous cytoplasm. Muscles gave purple stain with PAS reaction. In case of larvae treated

with fenvalerate (Fig. 6), the results showed that there was a slight decrease in the intensity of glycogen in all tissues.

b - Total Proteins

In the mid gut of control larvae (Fig. 7), the total protein appears in the form of deep blue granules scattered in both the cytoplasm and nuclei of the epithelial cells. in the epithelium of Malpighian tubules, the nuclei displayed blue granules less than those of the cytoplasm. The muscle cells and nerve ganglion tissue gave positive reaction. In case of treated larvae with fenvalerate (fig. 8), the staining affinity of the gut epithelial cells and other tissues was slightly reduced and the protein granules in most cells were relatively decreased.

c - Nucleic acids

The RNA appeared as numerous granules distributed throughout the cytoplasm. The nuclei were also stained indicating the presence of RNA. The tissues of mid gut, Malpighian tubules and nervous tissue of control larvae contain a great amount of RNA in their cytoplasm which appeared as deep bluish colour (Fig. 9). DNA gave a purple colour in the nucleus of epithelial cells of the mid gut and Malpighian tubules. The chromatin particles in the nerve cells are strongly stained. In case of treated larvae with fenvalerate, the results showed that a decrease occurred in the RNA content in the majority of epithelial cells of the mid gut and Malpighian tubules. The muscle and nerve tissues react positively with RNA, but with lower magnitude (Fig. 10). DNA content in the cells of most tissues was apparently unchanged as compared with that of control larvae.

DISCUSSION

Application of pyrethroid as insecticides has proved to be highly effective for the control of *S. littoralis* (Hosny *et al.*, 1977, El-Okda *et al.*, 1978 and Taha, 1981). In the present study, fenvalerate resulted in tissue destruction of the mid gut, Malpighian tubules, nervous tissue and muscles of the 3rd larval instar of S. littoralis.

The histological changes brought about to the larval tissue in the present work were somewhat similar to changes reported by many investigators in other insects treated with different insecticides (Hartzell, 1934; Hartzell and Scudder, 1942; Soliman and Soliman, 1958). Metwally *et al.* (1978) found that the tissues most affected by organophosphorus insecticides were found to be those of the mid gut, and nervous system. Hamed *et al.* (1974) reported that dieldrin caused a serious effects in the gut epithelium, salivary glands and Malpighian tubules. They added that DDT was less effective compared with dieldrin.

Concerning the histochemical effects of fenvalerate, the results showed that there was a slight decrease in the concentration of glycogen in all the tissues of treated larvae.

Some reports declared the existence of carbohydrate metabolism impairment as a result of application of some insecticides other than pyrethroids. Merrill *et al.* (1946) found that the DDT-poisoned insects would have a decrease in their carbohydrate content 24 hours post treatment. Hamed *et al.* (1974) found that the common effect of DDT and dieldrin in mosquitoes larvae was the disappearance of glycogen from the myofibrils. Rawash *et al.* (1977) reported that both the 4th larva

instar and adult females of *Culex pipens* treated with LC_{50} of malathion has significant less carbohydrate content in comparison to control. It was reported that the inhibition in the activity of poisoned insects would accompany impairment in carbohydrate hydrolysis (El-Defrawi *et al.*, 1964). The decrease in glycogen content induced in the larvae by fenvalerate in the present work may be attributed to the reduction in the activity of the larvae.

Histochemical examination of the 3rd larval instar treated with fenvalerate showed that total protein was decreased in the tissues of mid gut, Malpighain tubules, muscles and nervous tissue. These results are similar to those of Ramada (1973) who found that the protein content was decreased in house fly poisoning with lindane. On the contrary, Rawash *et al.* (1977) recorded that crude protein of the body of the 4^{th} larval instar of *Culex pipens* tended to be higher with the increase of LC_{50} of DDT or malathion. Sambough *et al.* (1974) found no consistent patterns was observed in protein content between DDT-treated and control cockroaches.

The results also showed that fenvalerate induced no change in DNA content of larval tissues. The RNA content in the majority of epithelial cells of the mid gut and Malpighian tubules was decreased. This might indicate that fenvalerate greatly interfere with one or more of the metabolic activities controlling the RNA levels. Datta and Pramanik (1979) reported that dimethoate significantly decreased the amount of DNA, RNA and protein concentrations in *Peripleneta americana*. Mitlin *et al.* (1977) studied the effect of diflubenzuron (dimilin) on the RNA and DNA contents of boll weevile Anthonomus grandis. Their results indicated that the biosynthesis of DNA was inhibited, but neither RNA nor protein synthesis was affected. Hamed *et al.* (1974) mentioned that treatment with dieldrin did not affect the

RNA content in the gut cells of *Anopheles pharoensis* larvae. Comparing our results with the other reported data (Mittin *et al.*, 1977 and Datta and Pramanik, 1979), it would be reasonable to stress here that, the tissue or parameter affected depends mostly on the structure of the insecticide, and the nature, age of the insect under study.

It is evident from the results obtained in this work that tissues of the 3rd instar larvae of *S. littoralis* showed marked tissue destruction when being treated with fenvalerate insecticide. Moreover, the larval histochemical contents including glycogen, RNA and protein seemed to be heavily affected.

ACKNOWLEDGEMENT

The authors would like to thank Prof. Dr. A. A. El-Aaser, Chairman of Cancer Biology Department, National Cancer Institute, Cairo University, for his cooperation.

REFERENCES

Cantu, E. and Wolfenbarger D. A. (1970) : Toxicity of three pyrethroids to several insect pests of Cotton. J. Econom. Entomol. 63, (4) 1373 - 1374.

Datta, S. and Pramanik, H. (1979) : Effects of dimethoate and actinomycin-D on nucleic acids and cholesterol of *Periplaneta americana*. Indian J. Zool., 6(2) : 9 4- 98.

El-Defrawi, M. E.; Toppozada A.; Mansour N. and Zeind M. (1964) : Toxicological studies on the Egyptian cotton leaf-worm, Prodenia litura I. Susceptibility of different larval instars of *Prodenia* to insecticides. J. Econ. Entomol. 57 : 591 - 593.

El-Okda, M. M. K.; Youssef, K. E. H.; Elewa, M. A. and El Assar, M. R. (1978) : Toxicity and joint action of four synthetic pyrethroids combined with

certain insecticides against the cotton leaf worm, Alexandria strain proc. 4th Conf. Pest. Comrol, NRC, Cairo, 1 : 514 - 522.

Garvin A. J.; Hall, B. J.; Brissie, R. M. and Spicer, S. S. (1979) : Cytochemical differentiation of nucleic acids with a schiff-methylene blue sequence. J. Histochem. Cytochem. 24. 587 - 590.

Hamed, M. S.; Guneidy; A. M.; Riad, Z. M. and Soliman, A. A. (1974) : Histopathological and histochemical studies on *Anopheles pharoensis* larvae treated with insecticides. Bull Ent. Soc. Egypt, Econ. Ser. VIII : 91 - 98.

Hartzell, A. (1934) : Histopathology of insect nerve lesions caused by insecticides. Comtr. Boyce. Thompson. Inst. (USA). VI : 211 - 233.

Hartzell, A. and Scudder, H. I. (1942) : Histological effects of pyrethrum an activator on the central nervous system of house fly. J. Econ. Ent. 36 : 428 - 433.

Hosny, M. M.; Zidan, Z. H.; Abdel-Megeed, M. I. and Ishak, R. (1977) : Toxic effec of new synthetic pyrethroids on certain cotton pests in relation to their effect on cotton plants. Proc. 2nd Arab Pesticide Conf., Tanta Univ. 1 : 144 - 154.

Hosni, S.; Fahmy, M.; Kandil A. and Mansour M. R. (1985) : Toxicological studies on synthetic pyrethroids on the cotton leaf worm *Spodoptera littoralis* (Boisd) Bull. Ent. Soc. Egypt, Econ. Ser., pp. 79 - 86.

Hotchkiss, R. D. (1948) : A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. Arch. Biochem. 16 : 131.

Mazia, D.; Brewer, P. A., and After, M. (1953) : The cytochemical staining and measurement of protein with mercuric bromophenol blue. Biol. Bull. 104 : 57 - 67.

Merrill R. S.; Savit, J. and Tobis, J. M. (1946) : Certain biochemical changes in the DDT poisoned cockroach and their prevention by prolonged anesthesia. J. Cell. and Comp. Physiol. 28 (3) : 465 - 467.

Metwally E. M.; Allam S. and Gohar K. (1978) : Histological changes in the larvae of the cotton leaf worm caused by sublethal doses of three insecticides. Fac. of Agric., Zagazig Univ. 4th Conf. Pest Control NRC, Cairo.

Mitlin, M. W.; Glenn, H. and Jack, W. (1977) : Inhibition of DNA synthesis in boll weevlis (*Anthonomus grandis* Boheman) sterilized by Dimillin. Pestic. Biochem. Physiol. 7(6), 559 - 563.

Ramade, F. (1973) : Changes in the protein in the level of the haemolymph in the female of *Musca domestica* after poisoning with lindane. C. R. Acad. Sci. Paris. D. 276(5) : 985 - 988.

Rawash, L. A.; Gaaboub I. A. and Saleh M. S. (1977) : Relation between the total proteins, carbohydrates and lipids in the body of *Culex pipiens* L., its resistance to DDT and Malathion. Bull. Ent. Soc. Econ. Ser. 10 : 191 - 198.

Sambourgh, G. F.; Zellter, L. H. and Bylica, S. M. (1974) : Soluble amino oxidases in cockroach (Blatta) Insect. Biochem. 4(2) : 185 - 196.

Soliman, S. A. and Soliman A. A. (1958) : Histopathological destruction caused to cotton leaf worm, *Prodenia litura* F. by some newer insecticides. Bull. Soc. Entom. Egypt, XLII : 199.

Taha, M. A. (1981) : Physiological studies on the cotton leaf worm *Spodoptera littoralis* (Biosed) as affected by recent insecticides. Ph. D. Thesis, Faculty of Science, Menoufia University.

EXPLANATION OF FIGURES

PLATE I

Fig. 1. T. S. in the control 3rd larval instar of *S. littoralis* showing the arrangement of various body structure, (X80).

Fig. 2. Enlarged portion of the previous section showing the mid gut epithelial layer (X200), C: columnar cell, G: Goblet cell, L: lumen, T: Malpighian tubule, R: Regenerative cell, SM: striated muscle.

Fig. 3. T.S. in the 3rd larval instar treated with fenvalerate, (X80).

Fig. 4. Enlarged protein of the previous section showing destruction of mid gut epithelium, P: pyknotic nuclei, NG: nerve ganglion, T: Malpighian tubule, L: lumen, (X200).

PLATE II

Fig. 5. T. S. in the control 3rd larval instar showing distribution of PAS positive material, (X120).

Fig. 6. T. S. in the 3rd larval instar treated with fenvalerate showing reduction in PAS positive material, (X120).

Fig. 7. T. S. in control larva showing distribution of total protein, (X120).

Fig. 8. T. S. in treated larva showing a slight reduction in total protein, (X140).

Fig. 9. T. S. in control larva showing deeply stained RNA particles in mid gut epithelium, (X120).

Fig. 10. T. S. in treated larva showing distribution of RNA and DNA contents, (X120).

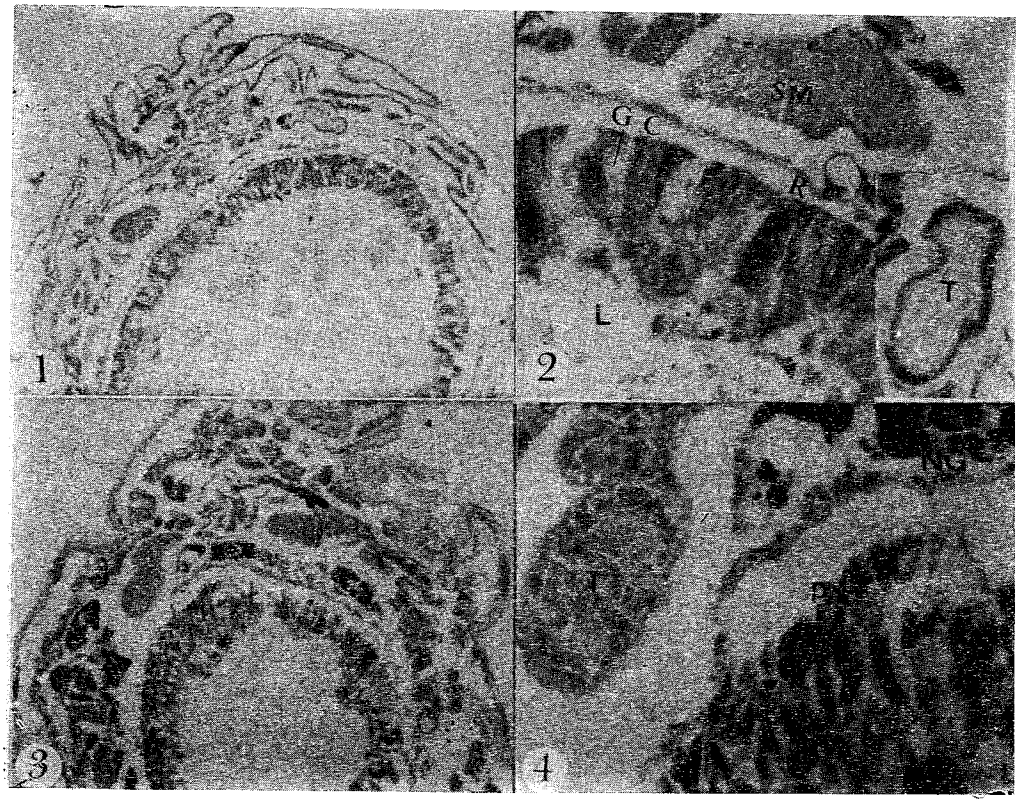

PLATE I

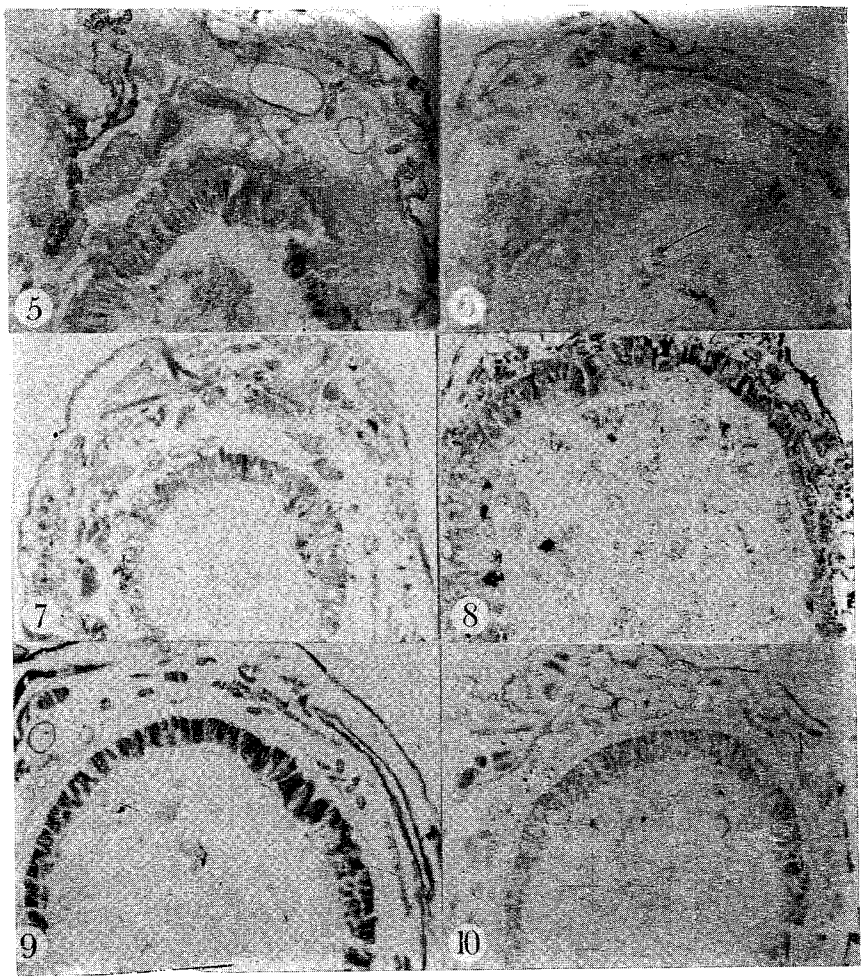

Plate II

التأثيرات الهستولوجيه والهستوكيميائيه لمبيد الفينفاليرات على يرقات دودة القطن

مصطفى أمين طه ، صابر عبد الرحمن صقر وتهانى محمود عبد الحليم عيد

كلية العلوم - جامعة المنوفية

إجريت الدراسه الحاليه لإظهار التأثير الهستولوجى والهستوكيميائى لمبيد الفينفاليرات على يرقات دودة ورق القطن فى العمر الثالث.

وقد ظهر من الفحص الهستولوجى أن المبيد تسبب فى ظهور فجوات فى الخلايا الطلائيه لكل من المعى المتوسط وأنابيب ملبيجى وحدث تهتك فى الألياف العضليه وظهور بعض الأضرار فى أنسجة الحبل العصبى.

لوحظ من الفحص الهستوكيميائى إنخفاض ضئيل فى محتوى الجليكوجين والبروتين فى خلايا المعى المتوسط وأنابيب ملبيجى وإنخفاض طفيف فى محتوى الجيكوجين فى الانسجه العصبيه والعضليه وأدت المعامله الى إنخفاض محتوى الحمض النووى و.ن١ فى معظم الانسجه ولم يتأثر حمض د.ن١ بالمبيد وقد نوقشت النتائج فى ضوء البحوث السابقة.