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ULTRA STRUCTURAL DELETERIOUS IN THE COTTON LEAF WORM, *Spodoptera littoralis* (BOISD.) LARVAE TREATED WITH CERTAIN INSECTICIDES

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ABSTRACT

Cypermethrin, esfenvalerate, chlorpyrifos and *Bacillus thuringiensis*, Kurs. were tested against the cotton leaf worm, *Spodoptera littoralis* (Boisd.) treated as 4th instar larvae. In addition, LC_{50}'s of tested compounds were applied on *S. littoralis* larvae to investigate the ultra structural changes in the integument, muscle, fat body and mid gut of alive and dead larvae. Meanwhile, the nerve cord sections were investigated for alive larvae only. All investigations were done by light and electronic microscopes.

Cypermethrin was the most potent compounds against *S. littoralis* larvae, followed by esfenvalerate, chlorpyrifos and then *B. thuringiensis* that had the least toxicity on the cotton leaf worm compared to other tested compounds.

Ultra structural investigations showed that cypermethrin caused thickening of outer cuticle fibrous layer in the integument of *S. littoralis* larvae. Also, hypodermis layer had swelling at the same treatment and necrosis in other treatments. In addition, all the treatments caused appearance of fissure and breaking down of muscles into small parts. While, all tested compounds except *B. thuringiensis* caused swelling in the integuments of dead larvae compared to control. On the other hand, *B. thuringiensis* caused drastically necrosis in the integument and hypodermis layers of dead larvae. All the compounds caused a noticeable destruction on the fat body cells as well as vacuolization and destruction the fat body membranous sheath. Many deleterious effects in the mid gut of *S. littoralis* as destruction of columnar or hyperphesia cells lining mid gut, losses of brush border with increase of goblet cells. Mid gut of died larvae had the highly destruction as affected by cypermethrin treatment. Meanwhile, other treatments caused shrinking in mid gut parts and necrosis in another parts. Neurosecretory cells of *S. littoralis* larval nerve cord had shrunk and dwarfed in cypermethrin, chlorpyrifos and *B. thuringiensis,* while; it had swelling in esfenvalerate treatment. Also, nucleus and nerve cells were disappeared partly in the most treatment compared to control.

Keywords: *S. littoralis*, toxicity, ultra structural, integument, muscle, fat body, mid gut, nerve cord, cypermethrin, esfenvalerate, chlorpyrifos, *B. thuringiensis*.

INTRODUCTION

Cotton leaf worm, *Spodoptera littoralis* (Boisd.), is a major cotton pest having a high reproductive capacity that averages 1000 eggs/female. In Egypt, It has three generations during cotton season (Abul-Nasr and El-Sherif; 1973) and is considered a limiting factor affecting crop and vegetable production. Despite of using insecticides eventually created many problems as resistance, and adverse effects on non-target organisms, but insecticides still represent a secure valve in controlling most of pests, among the Integrated Pest Management Programs. The bacterium *Bacillus thuringiensis*, proved to be a highly successful weapon for fighting some agricultural pests and offering many advantages over chemical insecticides. Using such agent as a microbial bio-insecticide increased during the past decade (Dulmage and Co-operators, 1981). Abd-Elwahed, *et al.* (2011) stated the potency of *B. thuringiensis* var. kurstaki against *S. littoralis* treated as 4th instar larvae with LC_{50} of tested compound caused aberrations in the mid gut layers. Also, El-Sheikh (2012) evaluated the insecticidal and histological effects of diple-2X (*B. thuringiensis*) and one pyrethroid compound (cypermethrin) on 4th instar larvae of *S. littoralis* based on the LC_{50} values cypermethrin is more toxic to *S. littoralis* than other compound, different abnormal histological structures of ovary were noticed. Osman and Abou-zeid (2015) conducted that organophosphorus insecticide, Profenofos (selecron) was sprayed on cotton leaves for controlling 4th instar larvae of cotton leaf worm under semi field circumstances, follow up histological changes happened in destruction of cell walls body, mid gut and cuticle layers of treated insect.

Thus, the purpose of the current work was to study the effect of cypermethrin (synthetic pyrethroid), esfenvalerate (synthetic pyrethroids), chlorpyrifos (organophosphorus) and *B. thuringiensis* on the cotton leaf worm, *S. littoralis* treated as 4th instars larvae with LC_{50}'s of the mentioned compounds to be investigating the ultra structural of the larval integument, muscle, fat body and mid gut tissues of alive and dead larvae. Also, nerve cord section of *S. littoralis* for alive larvae was examined by using light and electronic microscopes.

MATERIALS AND METHODS

1. Insect Rearing.

The culture of the cotton Leaf worm, *S. littoralis* was maintained in the laboratory of Cotton Leaf Worm Department, Plant Protection Research Institute, Agriculture Research Center. Larvae were fed on fresh castor oil plant leaves, *Ricinus communies* under laboratory conditions of 27 ± 2 °C and 65% R.H. (El-Defrawi *et al*. (1964).

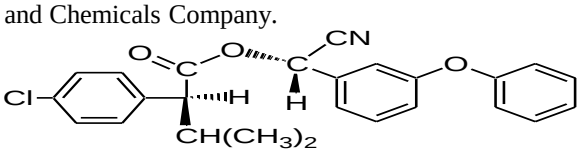
2. Tested compounds.

a. Synthetic pyrethroid compounds

1. Esfenvalerate; (Sumi-alpha, 5%EC)

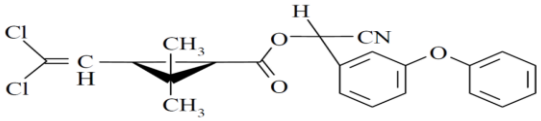
IUPAC Name: (*S*)-α-cyano-3-henoxybenzyl(*S*) -2-(4- -3-cloro- phenyl) methylbutyrate. The rate dose is 400

ml/faddan. It is the product of Kafr El-Ziat for Pesticide and Chemicals Company.



2.Cypermethrin: (Sparkel, 25% EC)

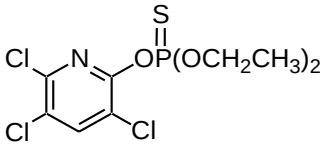
IUPAC Name: (RS)-α-cyano-3 phenoxybenzyl-(1RS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate (4 isomer pairs: cis-1, cis-2, trans-3, trans-4). It used at rate dose of 250 ml/faddan. It is the product of El-Helb for Pesticide and Chemicals Company.



b. Organophosphorus compound

1.Chlorpyrifos: (Dursban, 48% EC)

IUPAC Name: O, O-diethyl O - 3, 5, 6-trichloro -2-pyridyl phosphorothioate. The rate dose is 1L/faddan. It is the product of National Company for Agriculture Chemicals Production.



c. Biopesticides

1. Dipel 2X: 6.4%WP

Dipel 2X is one of the commercial microbial products in which the active ingredient based on the bacterium, *Bacillius. thuringiensis* var. *kurstaki*, a product of Vailent Bioscience Corporation, USA was obtained from May trade company, Giza, Egypt, containing 32.000 international unites potency per mg (IU /mg). It was used as wettable powder and at recommended at rate 200 gm/faddan.

3. Toxicity of tested compounds on *S. littoralis* larvae.

Dipping technique was used at the present work. The castor oil leaves dipping in five tested compound concentrations of cypermethrin, esfenvalerate, chlorpyrifos and *B. thuringiensis* (Dipel 2X). Four replicates for each concentration of the tested compound for 20 sec. and left the castor leaves until water evaporated, then put in glass jars (11x22 cm). Each jar was prepared by 25 fourth instar larvae of cotton leaf worm after larvae starving about 4 hours and maintained under 26±1°C. Then the numbers of alive and dead larvae were counted two days after treatment.

LC_{50} values were assessed according to Finney (1971) by using Ldp line software (www.Ehabbakr software/Ldp line).

4. Light microscope.

Fourth instars larvae of *S. littoralis* treated with LC_{50}'s of the tested compounds (cypermethrin, esfenvalerate, chlorpyrifos and *B. thuringiensis*). Larvae at 6- day after treatment were maintained in formalin 10% until histology.

The specimens from *S. littoralis* larvae samples were collected and fixed in 10% buffered neutral formalin solution. Paraffin sections of 5 microns thickness were prepared and stained with haematoxylin and eosin (H & E) according to Bancroft, *et al.* (1990) and examined microscopically. The alive and dead larval integument, muscle and fat bodies were investigated microscopically (X 200), While, mid gut (x 400).

All sections of *S. littoralis* larvae were done at Animal Health Research Institute, Agriculture Research Center.

Alive larval nerve cord sections were done at Cairo University Research Park (CURP) as the method of Bozzola and Russell (1999). Slice tissue was processed for TEM by fixation in glutaraldehyde and osmium tetroxide, dehydrated in alcohol and embedded in an epoxy resin. Microtome sections prepared at approximately 500-1000 µm thickness with a Leica Ultracut UCT ultra microtome. Thin sections were stained with tolodin blue (1X) then sections were examined by camera Lica ICC50 HD.

5. Electronic microscope.

The work was done in TEM lab FA-CURP, Fac. of Agric., Cairo University Research Park by using method of Bozzola and Russell (1999). Slice tissue samples into ~ 1 mm slices. Slice tissue was processed for TEM by fixation in glutaraldehyde and osmium tetroxide, dehydrated in alcohol and embedded in an epoxy resin. Microtome sections prepared at approximately 75-90 µm thickness and were stained with uranyl acetate and lead citrate, then examined by transmission electronic microscope JEOL (JEM-1400 TEM) at the candidate magnification. Images were captured by CCD camera model AMT, optronics camera with 1632 x 1632 pixel format as side amount configuration. This camera uses a 1394 fire wire board for acquision. The alive larval integument, muscle, fat body and nerve cord were investigated by electronic microscope.

RESULTS AND DISCUSSION

1. Efficacy of the tested compounds on *S. littoralis*.

The cotton leaf worm, *S. littoralis* treated as 4th instar larvae by synthetic pyrethroid compounds of cypermethrin and esfenvalerate; also, organophosphorus compound (chlorpyrifos) and Bactericide (*B. thuringiensis*). Table (1) showed that cypermethrin was the most potent compound against *S. littoralis* larvae (LC_{50}: 0.0387 ppm), followed by esfenvalerate (0.2546 ppm), chlorpyrifos (1.0884 ppm) and *B. thuringiensis* (7.7523 ppm) after 3- day from treatment.

El- Sheikh (2012) evaluated Diple 2x (*B. thuringiensis*) and cypermethrin on *S. littoralis* 4th instar larvae. Based on the LC_{50} values; cypermethrin is the more toxic to *S. littoralis* than another compound. Also, Bhatti, *et al.* (2013) presently two pyrethroids and three new chemistry insecticides in mixtures at their lethal concentrations against 2nd instar larvae of *Spodoptera litura*. LC_{50} of deltamethrin and bifenthrin were 619 & 100 and 0.06 & 73.4 ul/ml, respectively after 48 and 72 hour exposure. In addition, Massoud, *et al.* (2014) and

Osman (2014) showed that chlorpyrifos-methyl was effective against cotton leafworm. Moreover, Ibrahim, *et al.* (2014) indicated that mortality percentage increased with increasing Dipel 2X concentration. The highest mortality (90%) was obtained by using 4 g/L of Dipel 2X (5 days post treatment).

Table (1). Toxicity of tested compounds on 4th instar larvae of S. littoralis.

Compounds	LC_{50} (ppm) 95%Confidence limits	LC_{90} (ppm) 95%Confidence limits	Slope± SE	Toxicity index	
				LC_{50}	LC_{90}
Cypermethrin	0.0387 0.0261±0.0568	0.8082 0.4331±1.993	0.9708± 0.1050	100	100
Esfenvalerate	0.2546 0.163 ± 0.3964	9.0785 4.1673±29.841	0.8257± 0.0986	15.20	8.90
Chlorpyrifos	1.0884 0.7212 ± 1.6965	26.817 12.318±91.543	0.9209± 0.1162	3.55	3.01
B. thuringiensis (IU/L)	7.7523 5.1557 ± 12.456 ($24x10^6$) ($16x10^6$±$39x10^6$)	195.91 88.054±660.65 ($6x10^8$) ($2.8x10^8$±$21x10^8$)	0.9137± 0.1056	0.49	0.41

2. Ultra structural studies.

A. Integuments.

Normal cuticle of *S. littoralis* larvae composed of an outermost distinct layer, the epicuticle and the inner layer called procuticle. Procuticle parts are hardened to form the exocuticle; while, other parts remain flexible and colorless to form endocuticle. Finally there is commonly a distinct layer called hypodermal layer which composed of columnar or cuboidal cells (Figs. 1&2). Ultra structural examinations of *S. littoralis* 6th instar larvae treated as 4th instar larvae with LC50 of cypermethrin revealed a swelling and separation of the hypodermal cells from the endocuticle and the hypodermal cells showed several mitotic divisions and some fissure. Distortion in the endocuticle was also quite visible. This distortion revealed blockage of its formation. Es-fenvalerate caused abnormalities in the shape of exocuticle and hypodermal cells separated from the endocuticle (Figs. 1&2). As shown in (Figs. 1&2) the thickness of the cuticle decreased, some hypodermal cells became separated from the endocuticle. These results accorded with the demonstrated by El-Sheikh *et al*. (2005). Meanwhile, other tested compounds caused slurping from hypodermis layer. All the compounds caused elicited a lack of differentiation between outer cuticle and endo cuticle, destruction of the basement membrane and appearance of vacuoles between cuticle and hypodermis in the most treatments. Sampsont and Gooday (1998) mentioned that *B. thuringiensis* subsp. *israelensis* and subsp. *Aizawai* secreted exochitinase activity when grown in a medium containing chitin. The involvement of these chitinolytic activities during pathogenesis in insects has been investigated against larvae of *S. littoralis.*

B. Muscles.

The larval muscles are composed of striated fibers. Each fiber consists of parallel fibrillate numbers or sacrostyles, occupying the whole cross section of the fiber and laid down in plasma or sacroplasm. The nuclei of the sacroplasm are disposed immediately beneath the sarcolemma. All the treatments had appearance of fissure and breaking down of muscles into small parts are attributed to the destruction of the sarcolemma (Figs.1&2).

C. Fat bodies.

Ultra structural of the normal fat bodies of larvae indicated that they are composed of two layers. An outer or partial layer formed of ribbons beneath the body wall and an inner or visceral layer surrounding the various organs. The ribbon consists of many irregular cells. Their cells surrounded by sheath (Figs.1&2). The histological changes by different treatments used, showed a noticeable destruction on the fat body cells, as vacuolization of fat cells and destruction of membranous sheath (Figs. 1&2).

All the treatments had drastically destructions in integument, hypodermal layer; muscle and fat bodies of died larvae. Cypermethrin, esfenvalerate and chlorpyrifos caused noticeable swelling and necrosis; meanwhile, *B. thuringiensis* caused clearly shrinking and destruction in the most investigated tissues compared to normal dead larvae as showed in Fig. (3). El-Metwally, *et al.* (2007) evaluated the histopathological effects of four compounds; fenpropathrin, diflubenzuron, methomyl and Deenate on the larval integument of *Pectinophora gossypiella* (Saund.) and *Earias insulana* (Boisd.) treated as 10- day old larvae. The tested compounds destroyed the larval integument of the treated larvae in both insects. Also, separation between epidermal and hypodemal layers, reduction in the epidermal layer and reduction in muscles were shown by these compounds in addition to duplex structure between old and new cuticle of the spiny bollworm. Also, Osman and Abou-Zaid (2015) found that destruction of some cells in mid gut and cuticle layers of *S. littoralis* that treated with Profenofos. Amer, *et al.* (2015) mentioned that *B. thuringiensis* caused thickening of outer cuticle fibrous layer in the integument of *S. littoralis* larvae. Also, hypodermis layer had swelling and necrosis in *S. littoralis* larvae. Also, it caused appearance of fissure and breaking down of muscles into small parts and noticeable destruction on the fat body cells as vacuolization and destruction of the fat body membranous sheath.

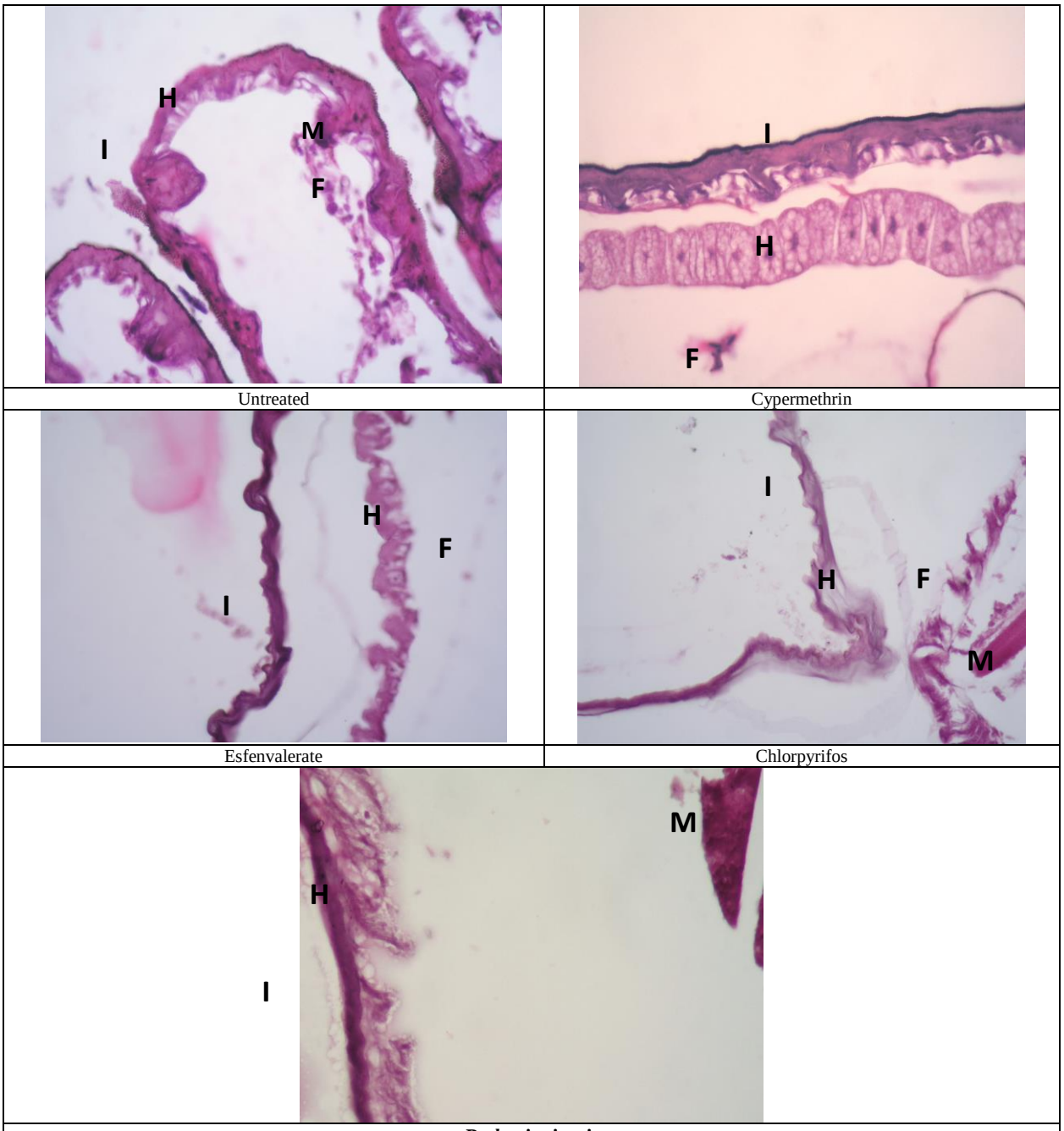


Figure (1): Longitudinal sections by light microscope in alive larval cuticle of *S. littoralis* treated as 4th instar larvae by tested compounds (X 200).

D. Mid guts:

Normal mid gut of *S. littoralis* is the main site for digestion and absorption of the digestion products and is a very metabolically active tissue. It consists of single layer of epithelium placed on a basement membrane. The epithelium is made up of columnar cells, secretory cells. These epithelial cells are relatively high; also, form a regular and compact wall. An oval nucleus is located in the central part of each columnar cell.

The apical surface of each columnar cell bordering with the gut lumen is covered with microvillae which create the tight structure called the brush border. The regenerative cells are another type of cells within the epithelium. These tiny cells from the regenerative cells that is regularly located at the base of the columnar cells (Figs. 4&5). Goblet cells are interspersed among the columnar cells. The cytoplasm of these pear-shaped cells is reduced, and the apical border of the cell surface invigilates to form a deep cavity. In this cavity there are numerous cytoplasm extensions. Flat nucleus of the goblet cell is located basally, below its cavity. The epithelium rests on well-developed basement membrane that is surrounded by a layer of circular muscles and an outer longitudinal muscle coat.

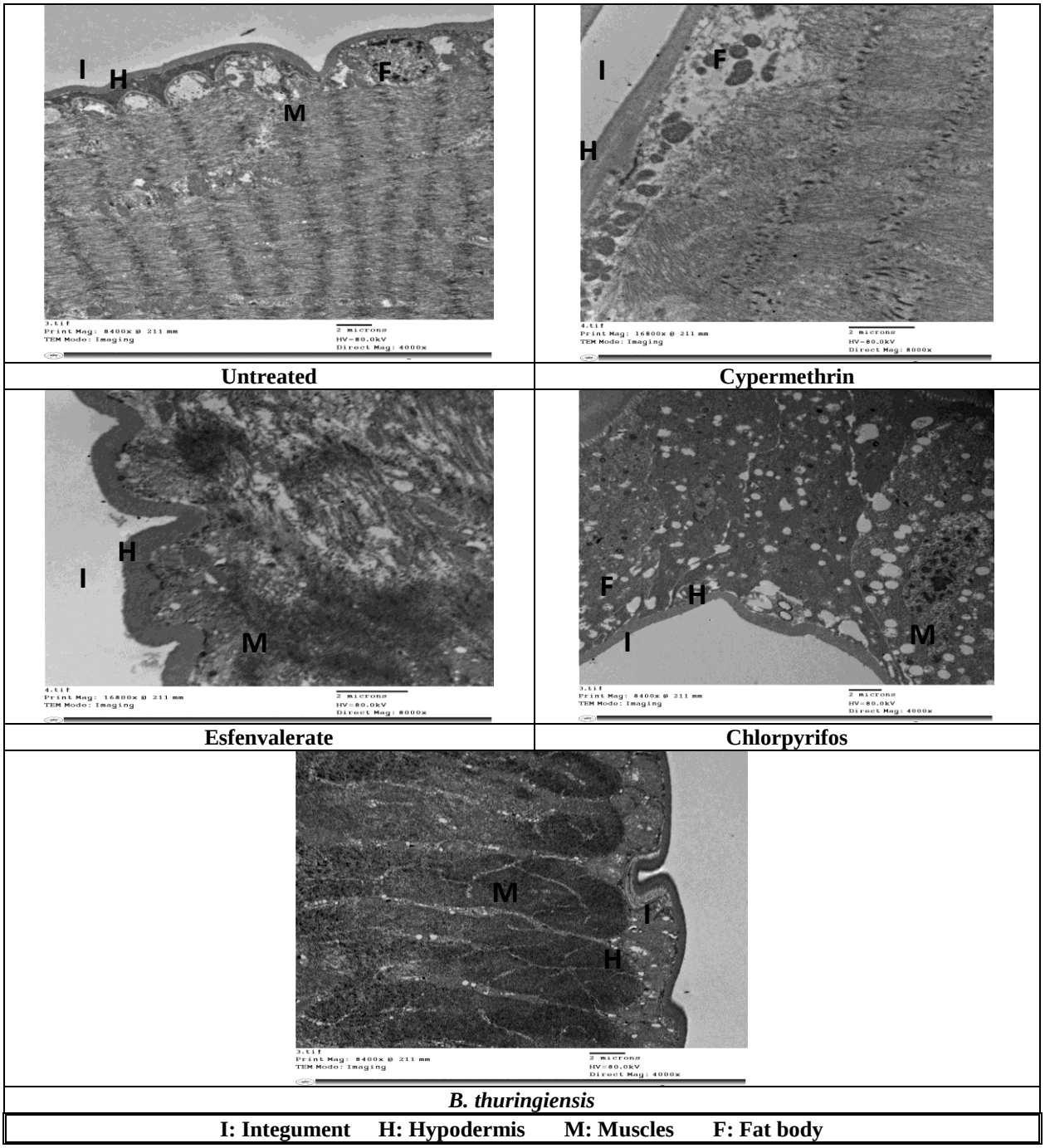


Figure (2): Longitudinal sections by electronic microscope in alive larval cuticle of *S. littoralis* treated as 4th instar larvae by tested compounds (X 4000).

The columnar, goblet and regenerative cells were dwarfing and shrinking noticeable, especially in esfenvalerate treatment, followed by chlorpyrifos and cypermethrin. While, had remarkable damage in all cells of mid gut was happened as a result of *B. thuringiensis* treatment as in Figs. (4&5). In addition, all tested compounds caused many deleterious effects in the mid gut of *S. littoralis* larvae as losses of brush border and epithelial cells that were vacuolated. Their nuclei were distinctly enlarged. Brush border was seen on a large surface of epithelial cells, but it disappeared on the most affected cells. Cytoplasm extensions of the goblet cells were degenerated and their fragments were often noted in the cavity of goblet cells. All regenerative cells were lost and the regular structure of the epithelium was disturbed (Figs. 4&5). Undifferentiated cells of the mid gut formed the regenerative cells of *S. littoralis* larvae that were most sensitive to tested compounds used. Damage to them resulted in the total disruption of the epithelium by preventing the replacement of the secretory cells exhausted by secretory activity. Degree of damage to the regenerative cells seems to be dependent on tested

compound (Figs. 4&5). The mid gut of dead larvae had noticeable necrosis among cells of columnar, goblet and regenerative cells. Also, destruction was happened between longitudal muscle layer and columnar cells, especially in cypermethrin treatment as showed in Fig. (6).

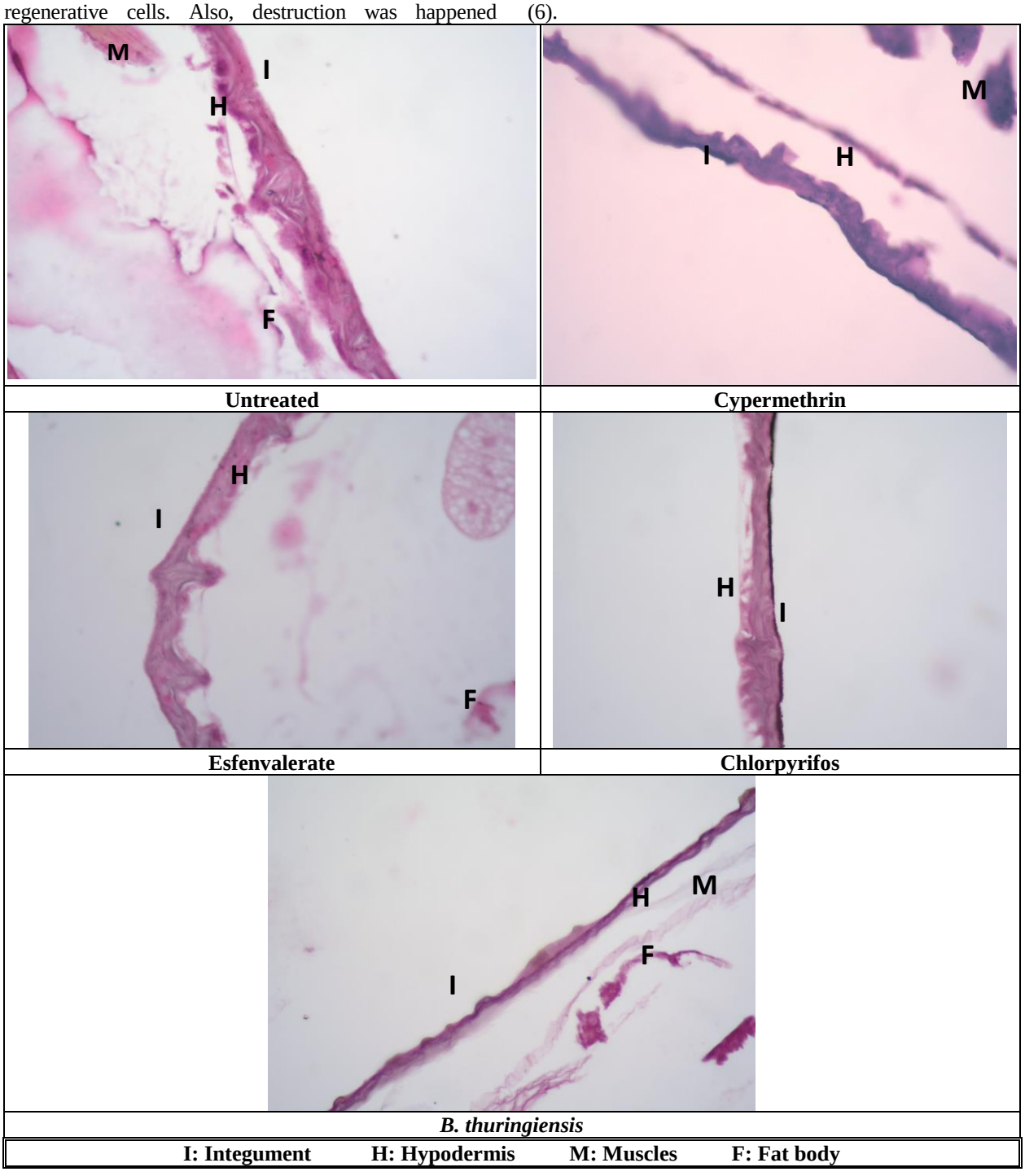
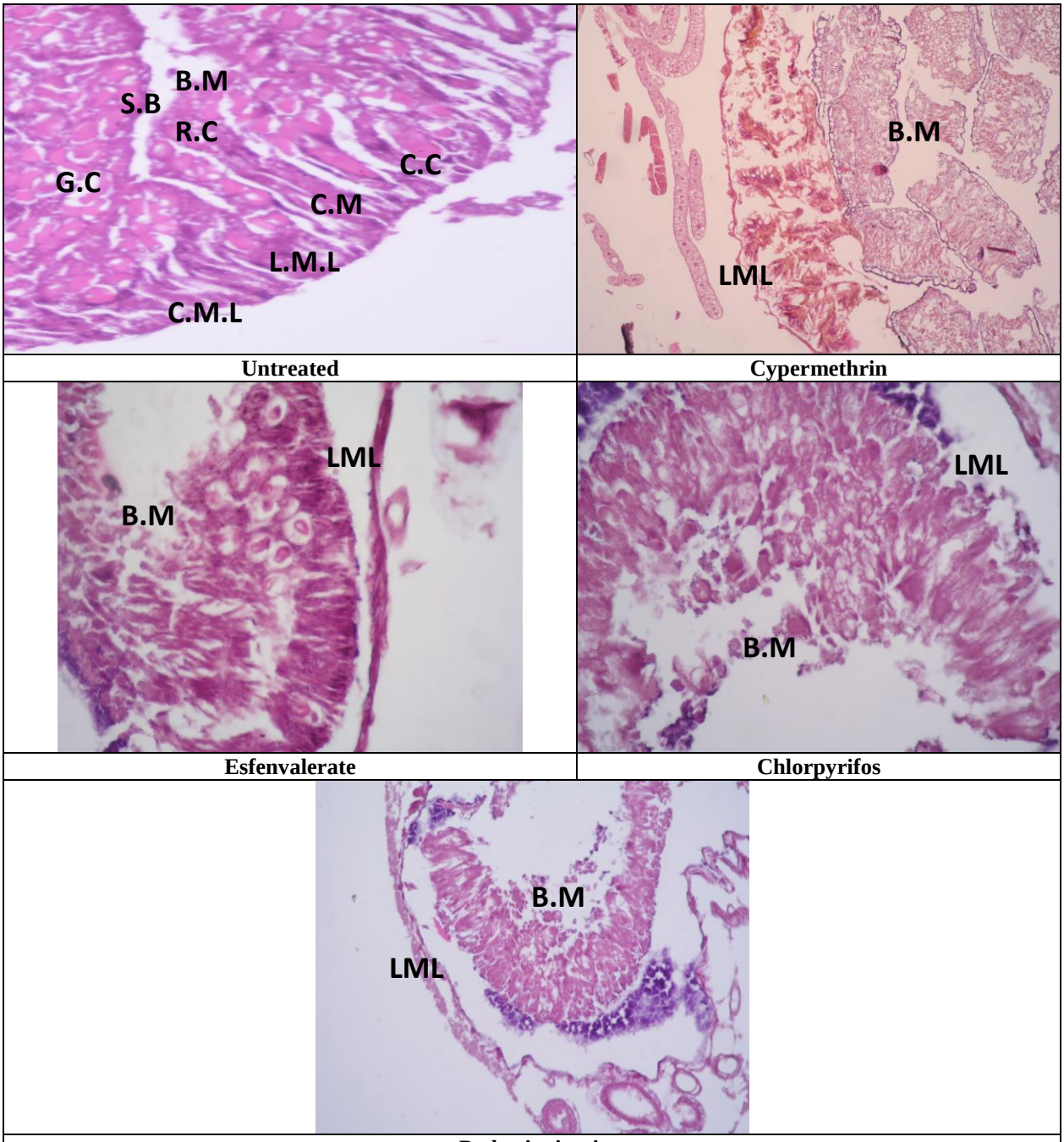
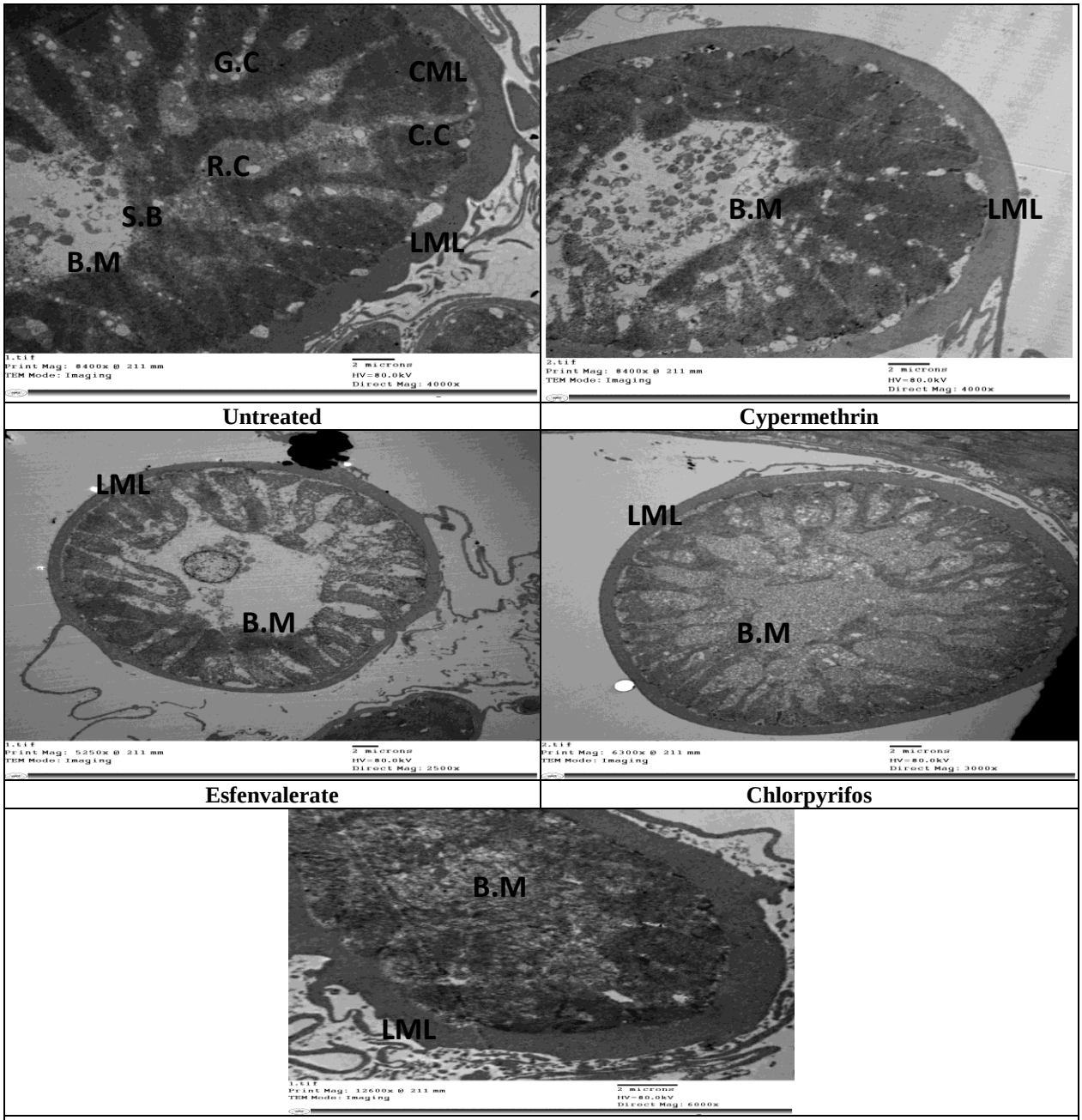


Figure (3): Longitudinal sections by light microscope in dead larval cuticle of *S. littoralis* treated as 4th instar larvae by tested compounds (X 200).



B.M: Basement membrane S.B: striated border C.C: Columunar cells G.C: Goblet cells R.C: Regenerative cells L.M.L: Longitudal muscle layer C.M.L. circular muscle layer

Figure (4): Longitudinal sections by light microscope in alive larval mid gut of *S. littoralis* treated as 4th instar larvae by tested compounds (X 400).

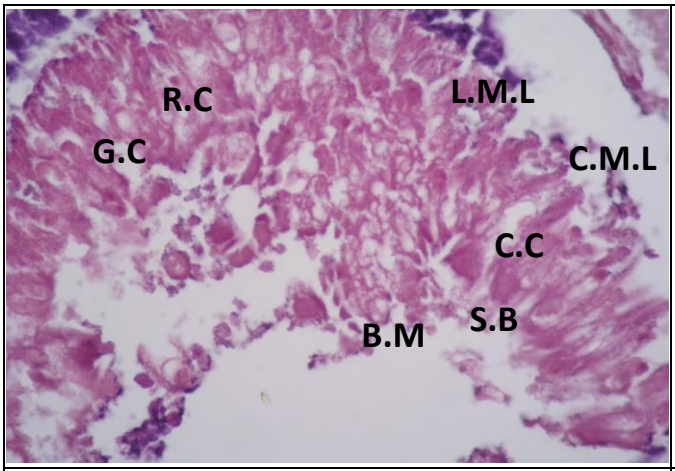


Untreated	Cypermethrin
Esfenvalerate	Chlorpyrifos

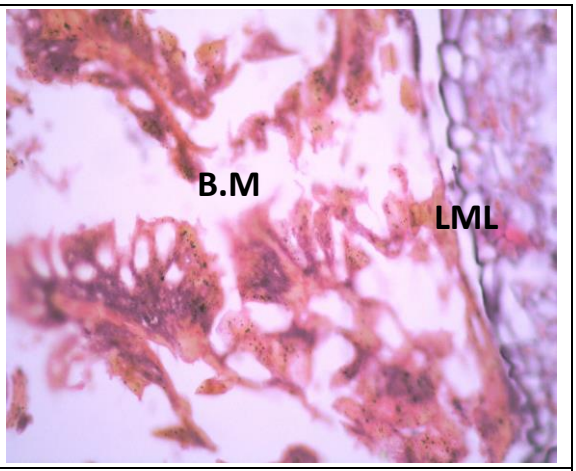
B. thuringiensis

B.M: Basement membrane S.B: striated border C.C: Columnar cells G.C: Goblet cells
R.C: Regenerative cells L.M.L: Longitudal muscle layer C.M.L. circular muscle layer

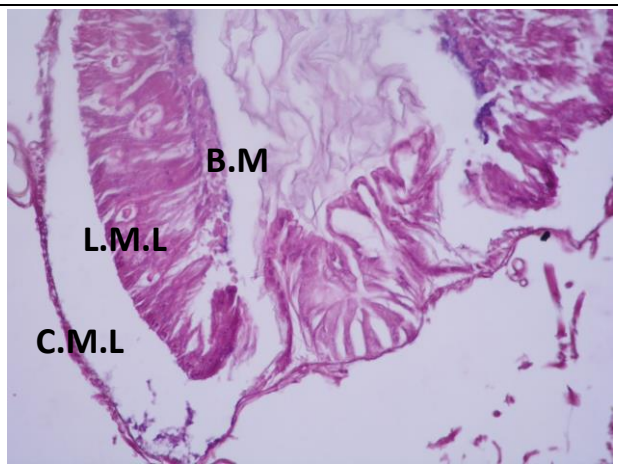
Figure (5): Longitudinal sections by electronic microscope in alive larval mid gut of *S. littoralis* treated as 4th instar larvae by tested compounds (X 4000).



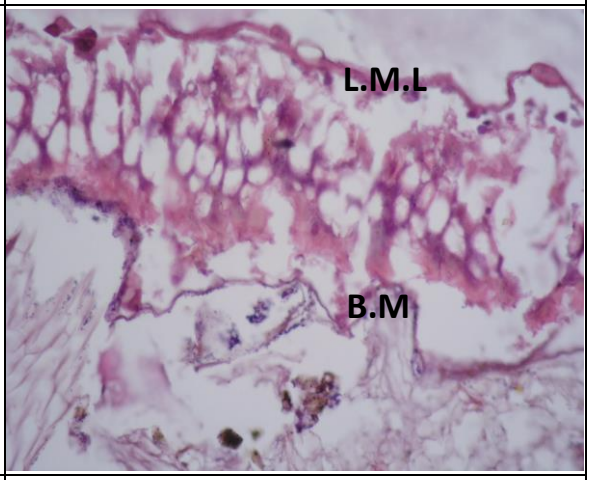
Untreated



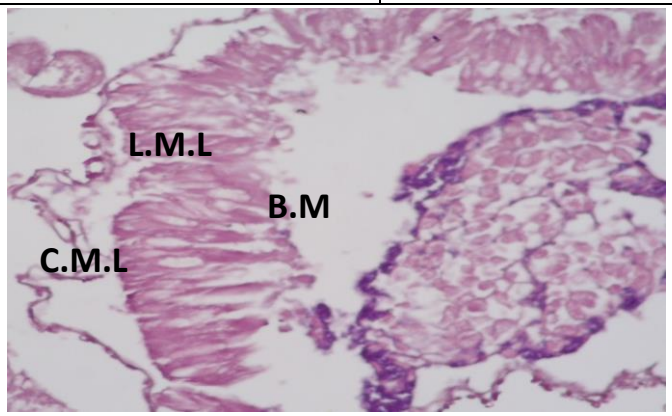
Cypermethrin



Esfenvalerate



Chlorpyrifos



B. thuringiensis

B.M: Basement membrane S.B: striated border C.C: Columnar cells
G.C: Goblet cells R.C: Regenerative cells L.M.L: Longitudal muscle layer

Figure (6): Longitudinal sections by light microscope in dead larval mid gut of *S. littoralis* treated as 4th instar larvae by tested compounds (X 400).

Similar results were gained by Abd-El Wahed, *et al.* (2011) who stated the mid gut histological sections of *S. littoralis* 6th instar larvae treated as 4th instar with LC_{50} of protecto, *B. thuringiensis* had aberrations in the mid gut layers. Also, Abd El-Mohsen, *et al.* (2013) showed the effect of *B. thuringiensis* on 2nd and 4th instar larvae of PBW. These effects are completed destruction of mid gut compared with untreated larvae. In addition, Osman and Abou-Zaid (2015) found that destruction of *S. littoralis* mid gut treated with profenofos. Moreover, Amer, *et al*. (2015) showed that *B. thuringiensis* caused many deleterious effects in the mid gut of *S. littoralis* as destruction of columnar or hyperphesia cells lining mid gut, losses of brush border with increase of goblet cells.

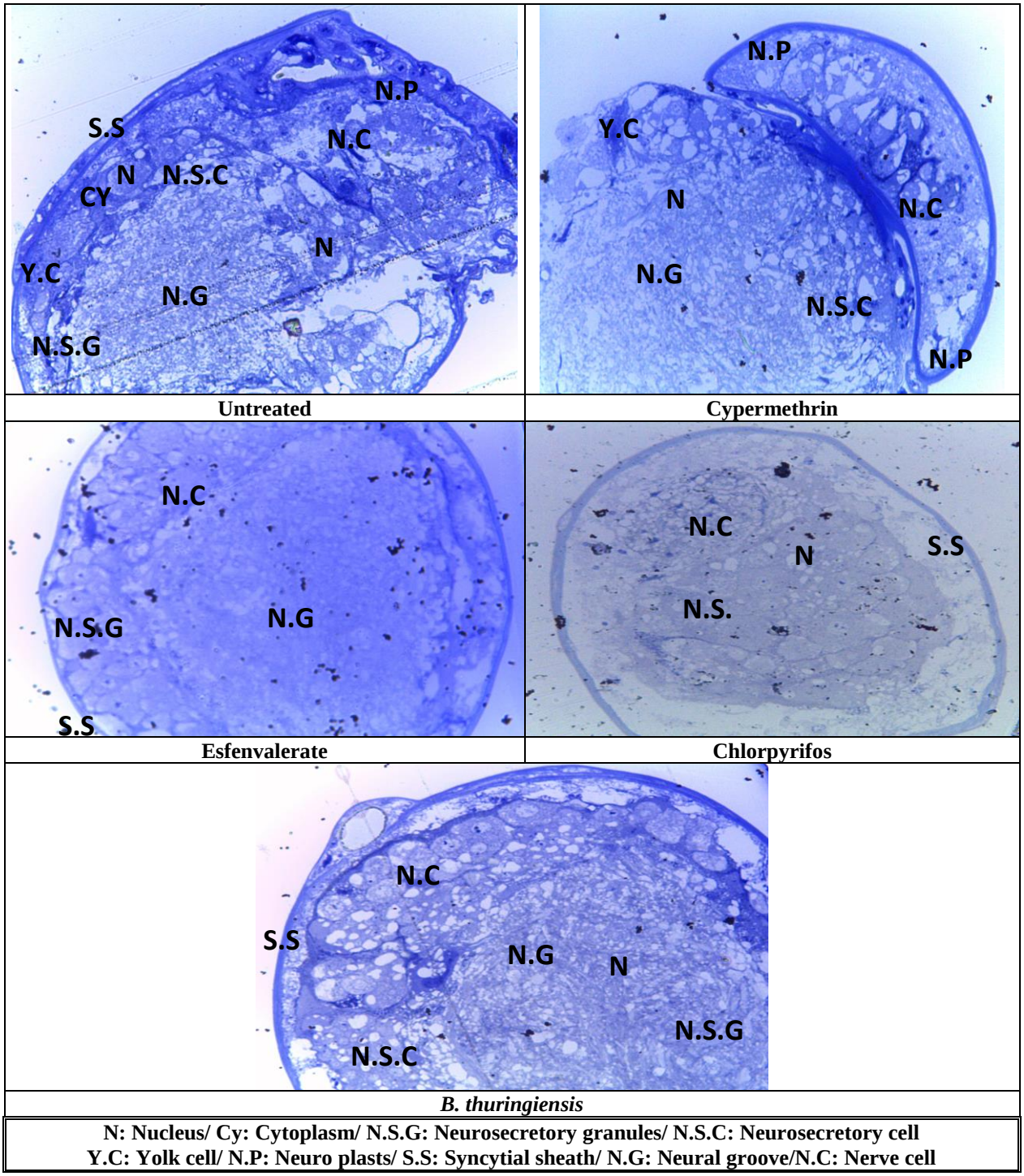
E- Nerve cord section.

The whole nerve cord of untreated *S. littoralis* larvae is limited by a syncytial sheath (Figs.7&8). Neurosecretory (NSCs) and nerve cells (NCs) of nerve cord are localized in the cortical as well as in the internal regions of the nerve cord (Figs.7&8). The NSCs showed different shapes and intensities of the neurosecretory material (NSM). At the anterior portion of the nerve cord, there was a cluster of round cells with round nuclei having plenty of chromatin. Some of these cells have prominent nucleoli at their nuclear membrane. Others have small round nucleus having scanty chromatin. The cytoplasm (CY) of all these cells appeared devoid of neurosecretory granules. On the other hand, the posterior portion of nerve cord exhibited different shapes of NSCs (round, irregular, triangle and oval). The neurosecretory granules (NSG) of all the previously mentioned cells were distributed in the entire perikarya of the cells and in triangle-shaped cells; they could be traced up to certain distance in the axon. These NSCs could be considered in the synthesis phase of cell activity according to Tripathi and Arif (2005) who observed four phases of cell activity in Indian silk worm, *Antheraea mylitta*, including synthesis, coalescence, release and resting phases. The synthesis phase is characterized by synthesis of neurosecretory granules that were scattered in the entire perikaryon. Also, the nerve cord section had yolk cells (Y.C) and neural groove (N.G) that has plenty cytoplasm as showed in figs. (7&8).

Hamouda and Dahi (2008) evaluated the neurotoxic effect of spinetoram on *S. littoralis* larvae. Spinetoram is neurotoxicity was manifested as evident histopathological changes in the structure of neurosecretory (NSCs) and ordinary nerve cells (NCs) of suboesophageal ganglion (SOG) of this pest after treatment with LC_{50} of this compound. It showed aggregation of neurosecretory granules in the oval, triangle and irregular shaped neurosecretory cells but not in the round shaped cells. Also, the SOG of treated larvae showed an apparent vacuolization and increase in the size of cytoplasm, abundance and aggregation of mitochondria in nerve cells and all kinds of NSCs (round, irregular, triangle and oval).

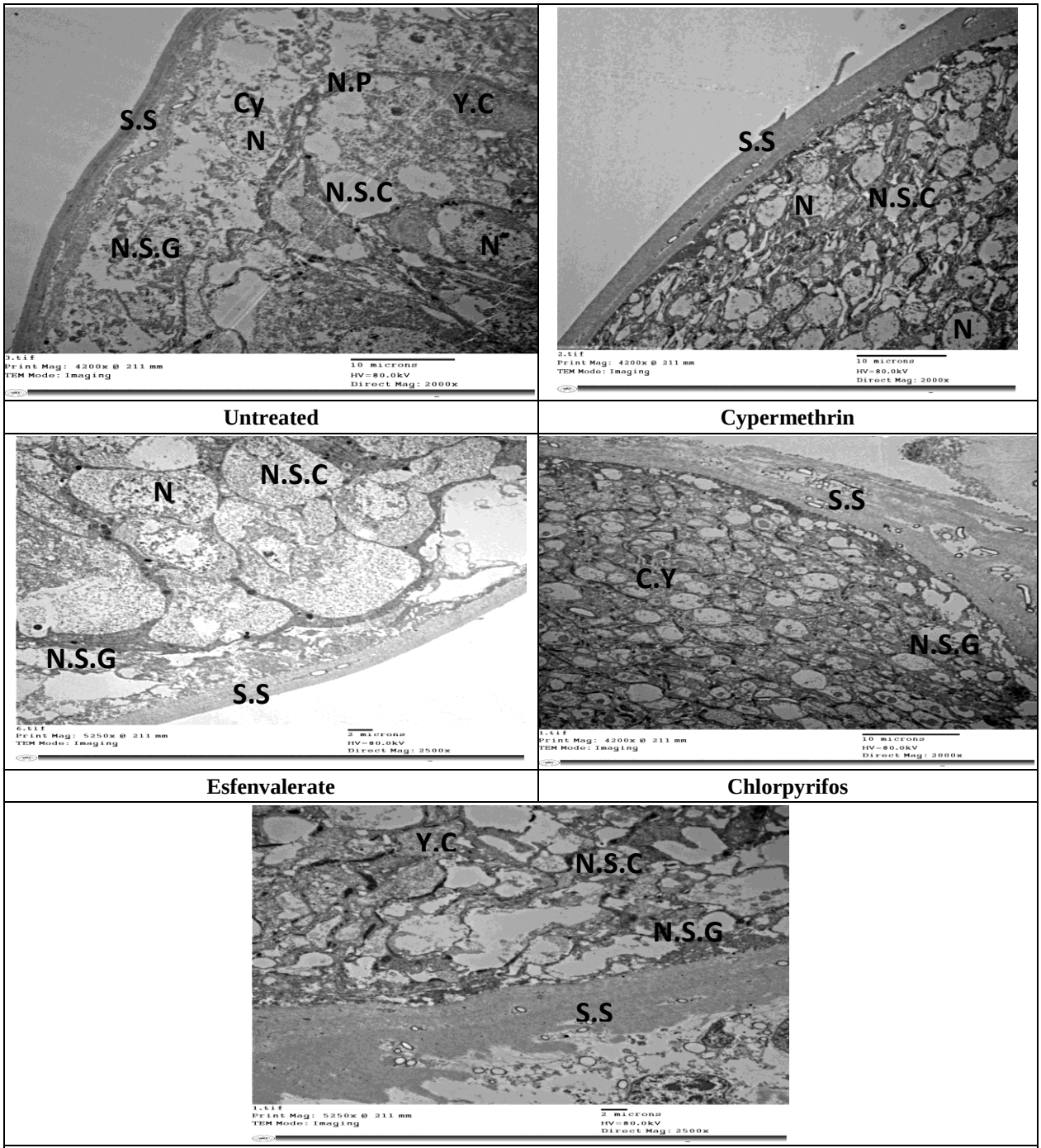
Generally, toxicity and ultra-structural studies cleared that tested compound of cypermethrin, esfenvalerate, chlorpyrifos and *B. thuringiensis* had destructive effects on alive and dead larvae of *S. littoralis* in integument, muscle, fat body and mid gut treated as 4th instars larvae compared to control. Also, cypermethrin is considered the best efficient compound, followed by esfenvalerate, chlorpyrifos and *B. thuringiensis*. Meanwhile, chlorpyrifos was the most neurotoxicity agent than other tested compounds.

In the present study, chlorpyrifos was the most compound had impact factors on the nerve cord, it caused shrinking in the nerve cord section; in addition, the same compound caused necrosis in neuro plasts, neurosecretory cells, nuleus and yolk cells as showed in Figs. (7&8). The different neurosecretory cells had clustered; also, necrosis in the most of nucleus, yolk cells and syncytial sheath as affected by cypermethrin treatment. Esfenvalerate had necrosis in neuro plasts and most of nerve cord content (Fig. 7,8). Meanwhile, *B. thuringiensis* had swelling in syncytial sheath and necrosis in both neuro plasts and neuleus (Figs. 7&8). According to Tripathi and Arif (2005) the NSCs in the treated *S.littoralis* larvae could be considered in synthesis phase of cell activity, while oval, irregular and triangle shaped cells were in releasing phase or in coinciding synthesis and releasing phases. These findings suggested that production of NSG might have been going on at higher rate than their release from most shapes of NSCs in *S.littoralis* larvae treated with LC_{50} of cypermethrin, esfenvalerate and chlorpyrifos might have interfered with normal release of NSG leading to their accumulation in oval, triangle and irregular shaped neurosecretory cells (Figs. 7&8). However, the scarcity of the NSGs in the round NSCs of the untreated and treated larvae may suggest slow production and / or high rate of NSG release than in other shapes of NSCs. Moreover; multivesicular bodies were observed in the cytoplasm of the NSCs of nerve cord the treated larvae, especially in *B. thuringiensis* in the present study.



N: Nucleus/ Cy: Cytoplasm/ N.S.G: Neurosecretory granules/ N.S.C: Neurosecretory cell
Y.C: Yolk cell/ N.P: Neuro plasts/ S.S: Syncytial sheath/ N.G: Neural groove/N.C: Nerve cell

Figure (7): Longitudinal sections by light microscope in nerve cord of *S. littoralis* treated as 4th instar larvae by tested compounds (X 400).



Untreated | **Cypermethrin**

Esfenvalerate | **Chlorpyrifos**

B. thuringiensis

N: Nucleus Cy: Cytoplasm N.S.G: Neurosecretory granules N.S.C: Neurosecretory cell Y.C: Yolk cell N.P: Neuro plasts S.S: Syncytial sheath

Figure (8): Longitudinal sections by electronic microscope in larval ganglion of *S. littoralis* treated as 4th instars larvae by tested compounds (X 2500).

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التغيرات التشريحية فى يرقات دودة ورق القطن المعامله ببعض المبيدات

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اختبرت مركبات السيبرمثرين - الإس. فينفاليرات - الكلوربيريفوس وبكتيريا الباسيلس ثورينجينسيز على دودة ورق القطن التى عوملت فى العمر اليرقى الرابع بالتركيز النصفى المميت للمركبات المختبرة وذلك بهدف دراسة التغيرات التشريحية على طبقة الكيوتيكل والعضلات والاجسام الدهنية والمعى الأوسط لليرقات الحية والميتة الناتجة من المعاملات المختلفة. بينما تم فحص قطاعات الحبل العصبى لليرقات الحية فقط. وتم فحص العينات بالميكروسكوب الضوئى والإلكترونى.

أوضحت النتائج أن مركب السيبرمثرين كان أفضل المركبات المستخدمة سمية على يرقات دودة ورق القطن ويليه فى ذلك مركب الإس. فينفاليرات و الكلوربيريفوس ثم المركب البكتيرى باسيلس ثورينجينسيزاللذى أظهر أقل سمية على يرقات دودة ورق القطن مقارنة بالمركبات المختبرة السابقة.

أوضح الفحص التشريحى أن المعاملة بمركب السيبرمثرين أدى إلى زيادة سمك الطبقة الخارجية لكيوتيكل يرقات دودة ورق القطن وإلى حدوث إنتفاخ لطبقة الهيبودرمس. كما حدث تآكل لطبقة الكيوتيكل والهيبودرمس فى اليرقات المعاملة بباقى المركبات الأخرى. بالإضافة إلى ظهور تدمير فى طبقة العضلات إلى أجزاء صغيرة فى المعاملات المختلفة. بينما أدت كل المركبات المختبرة ما عدا بكتيريا الباسيلس ثورينجينسيز إلى حدوث تورم فى كيوتيكل اليرقات الميتة مقارنة بالكونترول. ومن جهة أخرى سبب المركب البكتيرى تآكلا لطبقات الكيوتيكل والهيبودرمس فى اليرقات الميتة. كما سببت جميع المركبات تحطما ملحوظا فى التجويف الغشائى للأجسام الدهنية.

كما أدت المركبات المختبرة إلى تأثيرات عديدة على المعى الأوسط لليرقات والذى تمثل فى تحطم الخلايا العمادية المحاذية للمعى الأوسط مع فقدان فى أطراف الخلايا الهدبية وظهر ذلك خاصة فى المعاملة بالسيبرمثرين. كما ظهر إنكماش وتآكل فى مناطق عدة للمعى الوسطى فى اليرقات الميتة الناتجة من باقى المعاملات.

أدت مركبات السيبرمثرين- الكلوربيريفوس- بكتيريا الباسيلس ثورينجينسيز إلى إنكماش وتقزم للخلايا العصبية الإفرازية فى قطاع الحبل العصبى ليرقات دودة ورق القطن. كما حدث إنتفاخ لهذه الخلايا فى معاملة الإس.فينفاليرات. كما حدث إختفاء لمعظم الأنوية والخلايا العصبية لليرقات الناتجة من المعاملة بمعظم المركبات المختلفة وذلك مقارنة بالعقدة العصبية غير المعاملة (الكونترول).