HISTOLOGICL EFFECTS OF TWO CHITIN SYNTHESIS INHIBITORS ON THE LARVAL MIDGUT OF THE COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (BOISD.)(LEPIDOPTERA:NOCTUIDAE)

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Abstract

The histological effects of two chitin synthesis inhibitors (CISs) on the larvae of the cotton leafworm were investigated under the laboratory conditions. Restts obtained grown 4th instar larvae showed that tredtment of the 4th with CISs revealed many ultrastructural alterations in the midgut of the 6th instar larva. These alterations appeared clearly in the cytoplasm, the nuclei and cell organelles. The histology and ultrastructure of the alimentary canal, and the mid gut in particular have revealed various effects of tow tested CSIs. Obtained results indicated that CSIs could be good substitutes for traditional chemical insecticides for controlling *S. littoralis* due to their effect on midgut epithelial cells which may lead to malnutrition and eventually death.

Keywords: Chitin synthesis inhibitors, Atabron®, (Chlorfluazuron), Cascade®, (Flufenoxuron), cotton leafworm, ultrastructure, histopathology.

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisduval) is a major cotton pest in Egypt attacking economically important crops all year round. It is considered highly destructive due to its high reproductive rate. Chemical control of *S. littoralis* has been extensively used especially on cotton which has led to development of resistance to these compounds. The use of the insect growth regulators for the control of insects of economic importance have been widely acclaimed, either as juvenile or ecdysone hormone mimics, chitin synthesis inhibitor or other compounds (Smagghe *et al*., 1995). These compounds interfere with the normal growth or development of insects and their effect could extend to affect the insect's reproductive potential as well as other effects on the physiology of treated insects (Abdel-Aziz, 2007; Abdel-Wahed *et al*., 2011 and Abdel-Aziz, 2012). Compared with knowledge of the developmental, morphogenic and reproductive effects of CSIs, knowledge of their effects on tissue architecture and cell ultrastructure is somewhat poor. However, the histological and/or ultrastructure changes in some insect species had been investigated by many

investigators (Younes *et al*., 2002); studied the effect of CSIs on *Spodoptera exigua* (Hübner) triflumuron against *Tribolium castaneum* Herbst (Parween, 1997); Flufenoxuron and Lufenuron against *Schistocerca gregaria* Forsskål (Bakr *et al*., 2008); Triflumuron on *Agrotis ipsilon* Hufnagel (Abdel-Hakim *et al*., 2016). Though, scarce literatures are available on the ultrastructure modifications that may be obtained due to CSIs in *S. littoralis* larval midgut. In respect to this, the current investigation aimed to estimate the ultrastructure changes of the CSIs Chlorfluazuron and Flufenoxuron in larval midgut of the cotton leafworm, *Spodoptera littoralis*.

MATERIALS AND METHODS

1. Rearing Technique:

A laboratory susceptible strain of the cotton leafworm, *Spodoptera littoralis* (Boisduval) which reared in the laboratory for more than 10 generations, was obtained as egg masses from the cotton leafworm,Divison of Plant Protection Research Institute, Dokki, Giza, Egypt. Insects were reared in an incubator under controlled conditions at 26 ± 2°C and 65 ± 10% R. H., with 8:16 L:D photoperiod at the Plant Protection Research Institute. Larval instars were supplied daily with fresh castor leaves, *Ricinus communis* L., as a source of food.

2. The tested compounds:

Two chitin synthesis inhibitors namely; Atabron® 5% (Chlorfluazuron) and Cascade® 10% (Flufenoxuron) were obtained from Syngenta Agro S.A.E. and Sumitomo Chemical Co., Ltd., respectively.

3. Specimen treatment and preparation:

Larvae were treated as fourth instar with previously estimated LC_{50} of Atabron® and Cascade® (Faiz, 2014). Treated larvae were dissected in the late 6^{th} instar and prepared for Transmission Electron Michrograph. Preparation and ultrascan micrograph were carried out at the Military Medical Research Unit, Abassia, Cairo, Egypt. Midguts from the larvae were dissected and immediately fixed in 2.5% glutaraldehyde at 4°C, for three days. The midguts were then washed in 0.1M Nacl buffer, fixed in 2% osmium tetroxide in 0.2 M Nacl buffer solution for one hour then rinsed in 0.2 M Nacl buffer. The specimens were dehydrated by ethanol series dehydration. They were then added to Propylene oxide and transferred to eponate epoxy. Finally the specimens were embedded in labeled capsules with freshly prepared resin and polymerized at 60°C for 48hours. The pH was kept within the range 7.2 - 7.4.

4. Ultrathin section preparation:

Ultrathin sections of the resin embedded specimens were obtained using an ultra-cut E microtome. Sections for TEM analysis were collected on Formvar carbon films on Copper grids (Thin cast films of Formvar strengthened with the addition of a layer of evaporated carbon on copper grids), stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined in a SEM electron microscope equipped with a ProScan slow scan CCD camera.

RESULTS AND DISCUSSION

1. Normal midgut ultrastructure of untreated larvae of the cotton leafworm:

The midgut epithelial ultrastructure of untreated larvae of *S. littoralis* is presented in Figures (1-4). The normal lining epithelium of the midgut consists of columnar cells resting on a basement membrane with an oval centrally located nucleus bound by a well-defined nuclear envelope. The cytoplasm is clear. Patches of varying densities of nuclear chromatin are visible (Figure 1). Figure (2) shows the normal epithelial cells. The continuous integrated brush border characterizes the midgut epithelium of the normal larval midgut. The lumen and microvilli are observed. The presence of some secretory vesicles is also recognized. Microvilli project inwards from the luminal surface of the epithelial cells into the luminal cavity. The Golgi's appear as flattened curved sacs with cluster bodies at their edges (Figure 3). The outer surface of the cell rests on the basement membrane. Within the cytoplasm lie the more or less elongated or spherical shaped mitochondria. Lamellate rough and smooth endoplasmic reticula can be observed with Ribosomes bordering the outer surface of the rough endoplasmic reticulum (Figure 4).

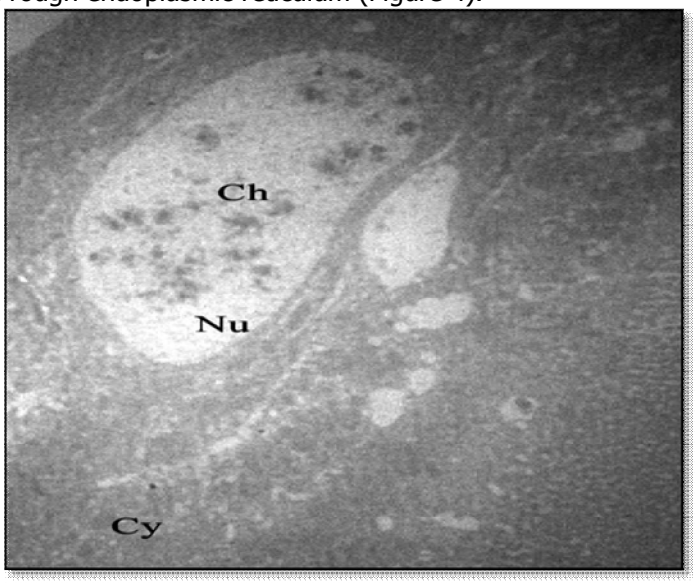

Figure 1. Transmission electron micrograph of the midgut of the late 6th instar larva of *Spodoptera littoralis* showing the normal epithelial cell. Cytoplasm (Cy), Nucleus (Nu), Chromatin material (Ch) (x1500)

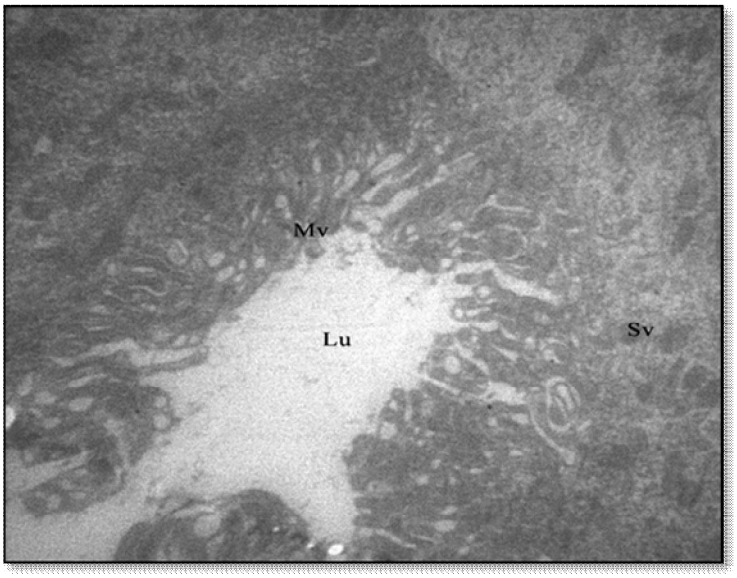

Figure 2. Transmission Electron micrograph of the midgut of the late 6th instar larva of *S. littoralis* showing the normal epithelial cell. Brush border (arrow). Lumen (Lu), microvilli (Mv), Secretory vesicles (Sv) (x3000)

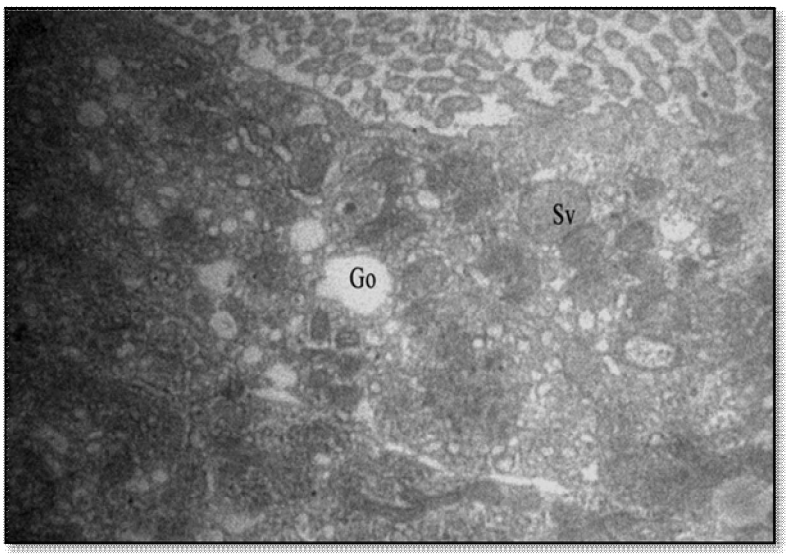

Figure 3. Transmission electron micrograph of normal epithelial cell of the midgut of the late 6th instar larva of *S. littoralis*. Secretory vesicles (Sv), Golgi apparatus (Go) (x6000)

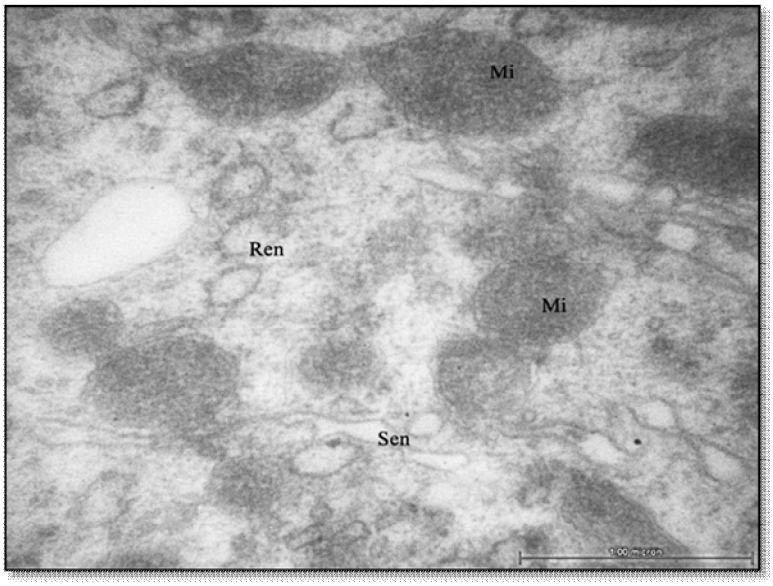

Figure 4. Transmission electron micrograph of normal midgut of the late 6th instar larva of *S. littoralis* showing the presence of many normal mitochondria (Mi). Rough endoplasmic reticulum (Ren) with ribosomes attached to its surface. Smooth endoplasmic reticulum (Sen). (x20,000)

2. Histopathological effect of Chlorfluazuron (Atabron®) on the larval midgut:

The histopathological effects of the LC_{50} of Chlorfluazuron (Atabron®), treatment on the epithelial cells of the midgut of late 6th instar larvae of the cotton leafworm treated as 4th instar larvae, are shown in Figures (5-8). The nucleus appeared to lose its oval shape and the nucleolus was not recognized (Figure 5). The nuclear membrane appeared to be sinuous (Figures 5 and 6). The chromatin materials appeared scattered and heavily condensed (Figure 5). Mitochondria were available in large number (Figures 5, 6 and 7). Some mitochondria appeared elongated and clumped to each other and the cristae appeared malformed and not uniform (Figures 6 and 7). Rough and Smooth endoplasmic reticulum recognized unarranged and scattered (Figures 6 and 7). Golgi's apparatus was observed unaffected (Figure 7). The microvilli were swollen and disintegrated into the gut lumen. The brush border was separated from the cell border (Figure 8).

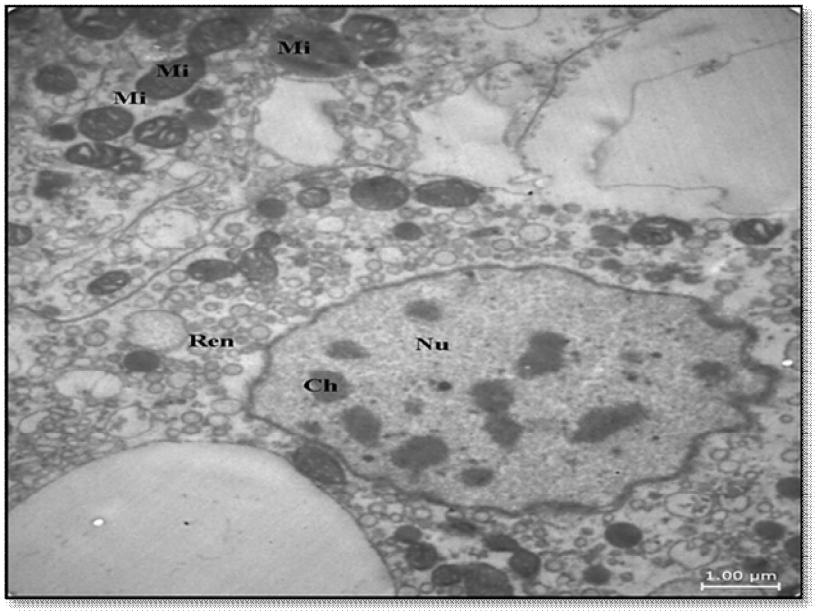

Figure 5. Transmission electron micrograph of the midgut of the late 6th instar larva treated with LC_{50} of Chlorfluazuron. The nucleus (Nu) lost its oval shape and the nuclear membrane appeared to be sinuous. The chromatin materials (Ch) appeared scattered and condensed. Mitochondria (Mi) are available in large number. Rough endoplasmic reticulum (Ren) is recognized unarranged (x10000)

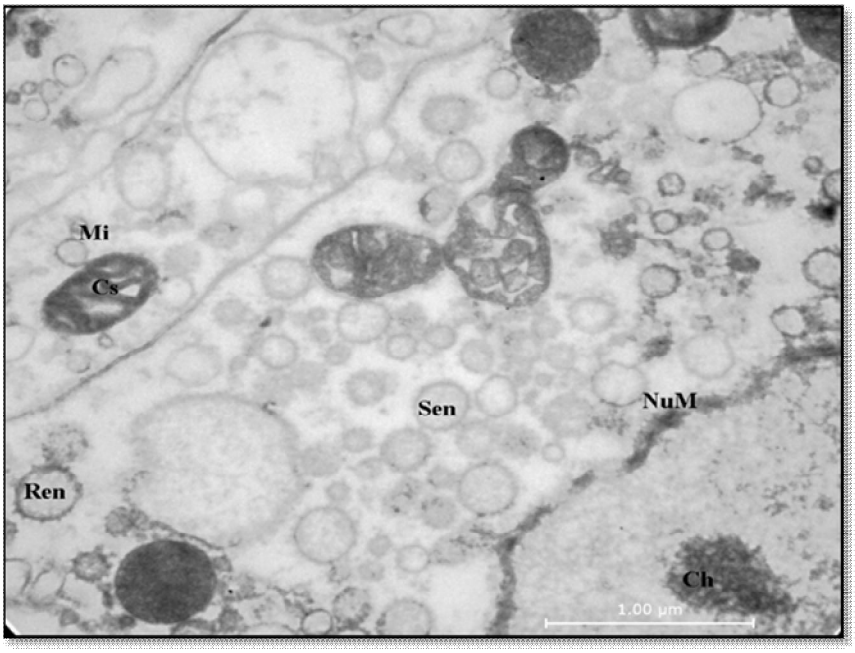

Figure 6. Transmission electron micrograph of the midgut of the late 6th instar larva treated with LC_{50} of Chlorfluazuron. The nuclear membrane (NuM) appeared to be sinuous. Some mitochondrial cristae (Cs) appeared malformed. Rough (Ren) and Smooth (Sen) endoplasmic reticulum unarranged (x30000).

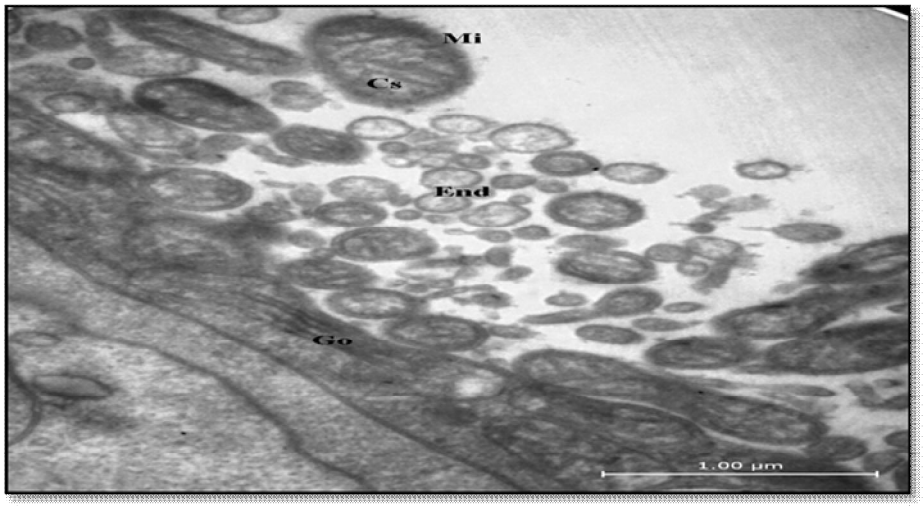

Figure 7. Transmission electron micrograph of the midgut of the late 6th instar larva treated with LC_{50} of Chlorfluazuron. Some mitochondrial cristae (Cs) appeared malformed. Some mitochondria (Mi) appeared elongated. Rough (Ren) and Smooth (Sen) endoplasmic reticulum were not arranged. No malformation in Golgi (Go) apparatus was recognized (x30000)

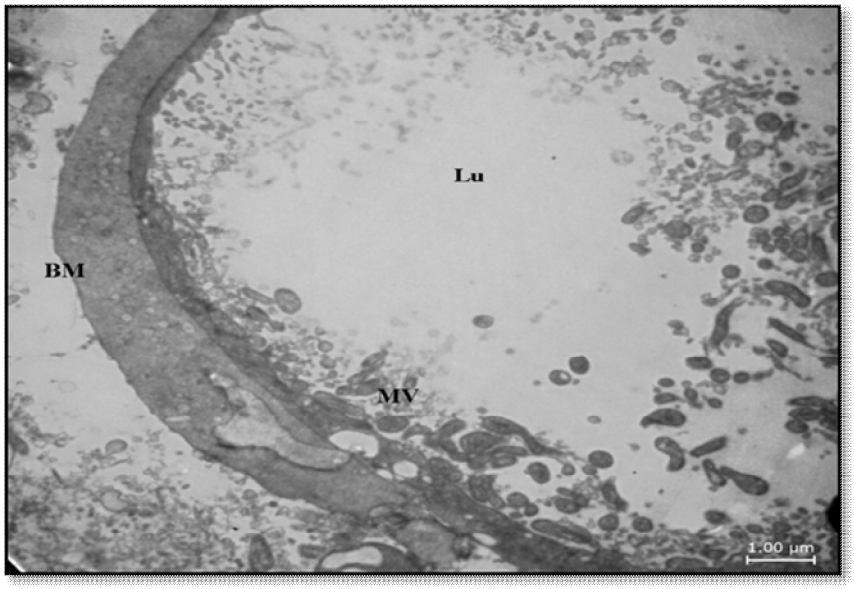

Figure 8. Transmission electron micrograph of the midgut of the late 6th instar larva treated with LC_{50} of Chlorfluazuron. The microvilli (MV) were swollen and disintegrated into the lumen (Lu). The brush border separated from the cell border (BM) (x6000).

3. Histopathological effect of Flufenoxuron (Cascade®) on the larval midgut

The histopathological effects of the LC_{50} of Flufenoxuron (Cascade®), treatment on the epithelial cells of the midgut of late 6th instar larvae of the cotton leafworm treated as 4th instar larvae, are shown in Figures (9-11). The nucleus was elongated and the chromatin materials heavily condensed and scattered (Figure 9). The nuclear membrane appeared sinuous (Figure 9). The nucleolus was not recognized. Many vacuoles were observed (Figure 9). Mitochondria were available in large number (Figures 9 and 10). Some mitochondria appeared elongated and clumped to each other and the cristae appeared malformed and not uniform (Figures 9 and 10).

Lacerations were observed in midgut tissue due to treatment (Figure 11). The continuous integrated arranged microvilli were observed (Figure 11).

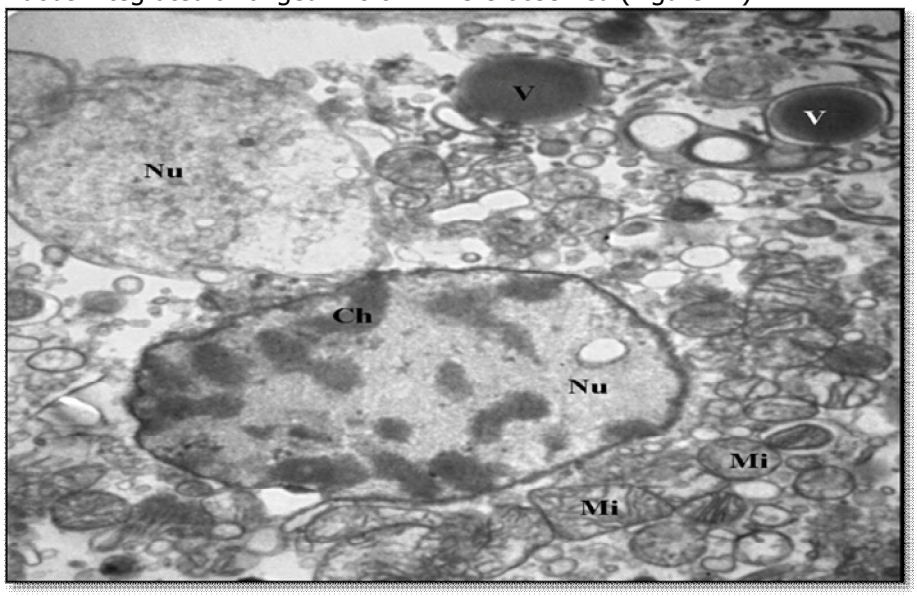

Figure 9. Transmission electron micrograph of the midgut of the late 6th instar larva treated with LC_{50} of Chlorfluazuron. The nucleus (Nu) lost its oval shape and the nuclear membrane appeared to be sinuous. The chromatin materials (Ch) appeared scattered and condensed. Mitochondria (Mi) are available in large number, malformed and clumped (x8000)

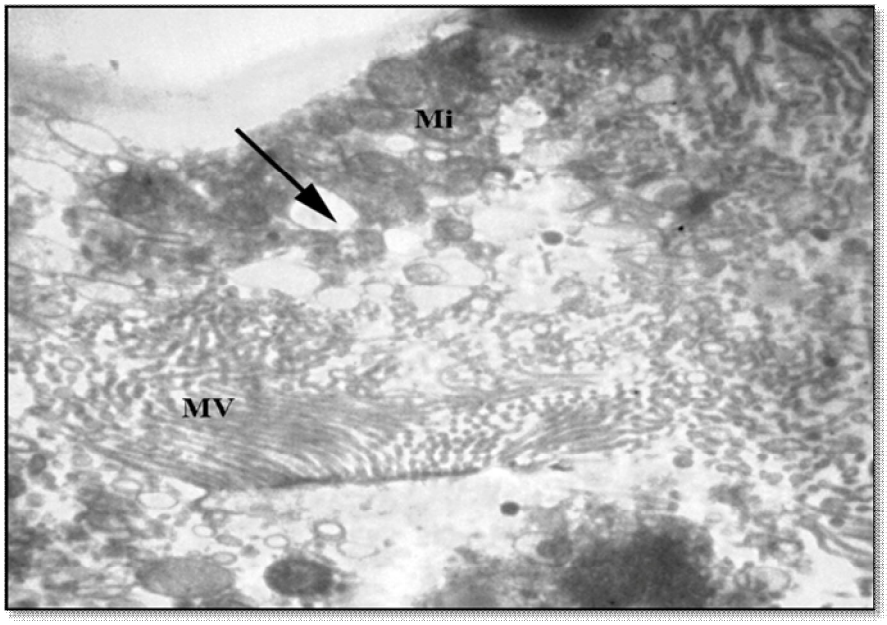

Figure 10. Transmission electron micrograph of the midgut of the late 6th instar larva treated with LC_{50} of Chlorfluazuron. The microvilli (MV) were arranged integrated. Laceration in midgut tissue is observed (black arrow) (x6000)

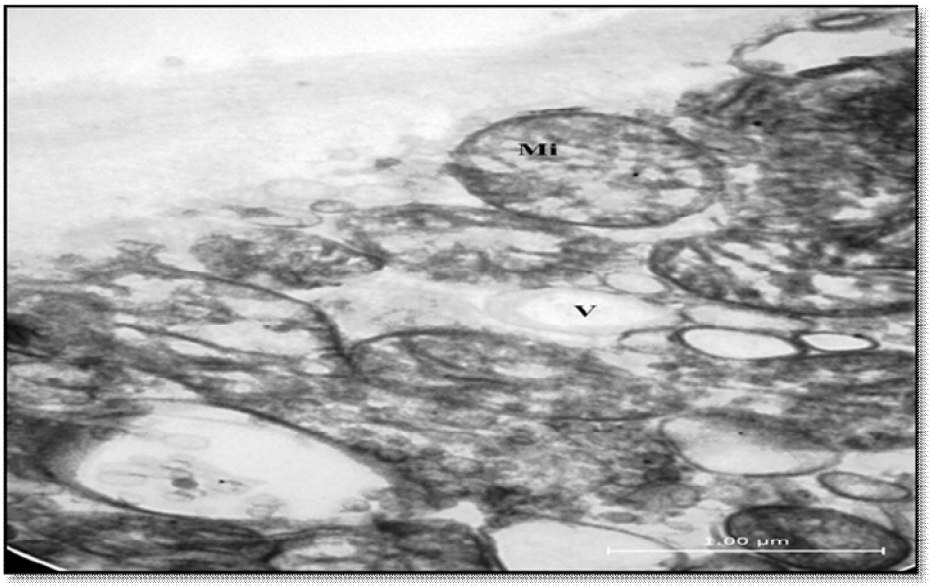

Figure 11. Transmission electron micrograph of the midgut of the late 6th instar larva treated with LC_{50} of Chlorfluazuron. Mitochondria (Mi) are available in large number, malformed and clumped. Vacuoles (V) are recognized (x30000)

The histopathology and ultrastructure of the alimentary canal, and the mid gut in particular have revealed various effects of several CSIs. The vacuolation, elongation and disintegration of the epithelial cells, as well as the disappearance of muscularis and regenerative cells, detachment of the basement membranes, ect … had been observed in the mid gut of *S. exigua* by the action of diflubenzuron (Younes *et al.,* 2002). In addition, similar or other histopathological changes had been reported for some CSIs on such as *Spodoptera exigua* Hübner (Younes *et al*., 2002); triflumuron against *Tribolium castaneum* Herbst (Parween, 1997); Flufenoxuron and Lufenuron against *Schistocerca gregaria* Forsskål (Bakr *et al*., 2008); Triflumuron on *Agrotis ipsilon* Hufnagel (Abdel-Hakim *et al*., 2016); Lufenuron against *Anthonomus grandis* Boheman (Costa *et al*., 2017); and chlorpyrifos on the midgut of 3rd larval instar of *Chrysomya megacephala* (Fabricius) (Shagufta and Mohammad, 2018).

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التأثيرات الهيستوباثولوجية لمركبين من مانعات الانسلاخ على المعي المتوسط ليرقات دودة ورق القطن الكبرى

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تم فحص التأثيرات الهيستوباثولوجية لمثبطين تخليق الكيتين (CISs) على يرقات دودة ورق القطن معملياً. وقد أظهرت النتائج أن معاملة يرقات العمر الرابع بالمركبين محل الدراسة قد أدت إلى العديد من التغيرات النسيجية الخلوية فى نسيج المعي المتوسط ليرقات العمر السادس. وقد ظهرت هذه التغيرات فى في السيتوبلازم ، النواة ، وبعض عضيات الخلية. وقد أظهرت النتائج التي تم الحصول عليها أن مثبطات تكوين الكيتين يمكن أن تكون بدائل جيدة للمبيدات الحشرية الكيميائية لمكافحة دودة ورق القطن حيث أن التأثيرات الهيستوباثولوجية من الممكن أن تكون قد أحدثت سوء التغذية مما إدى إلى الموت فى النهاية.