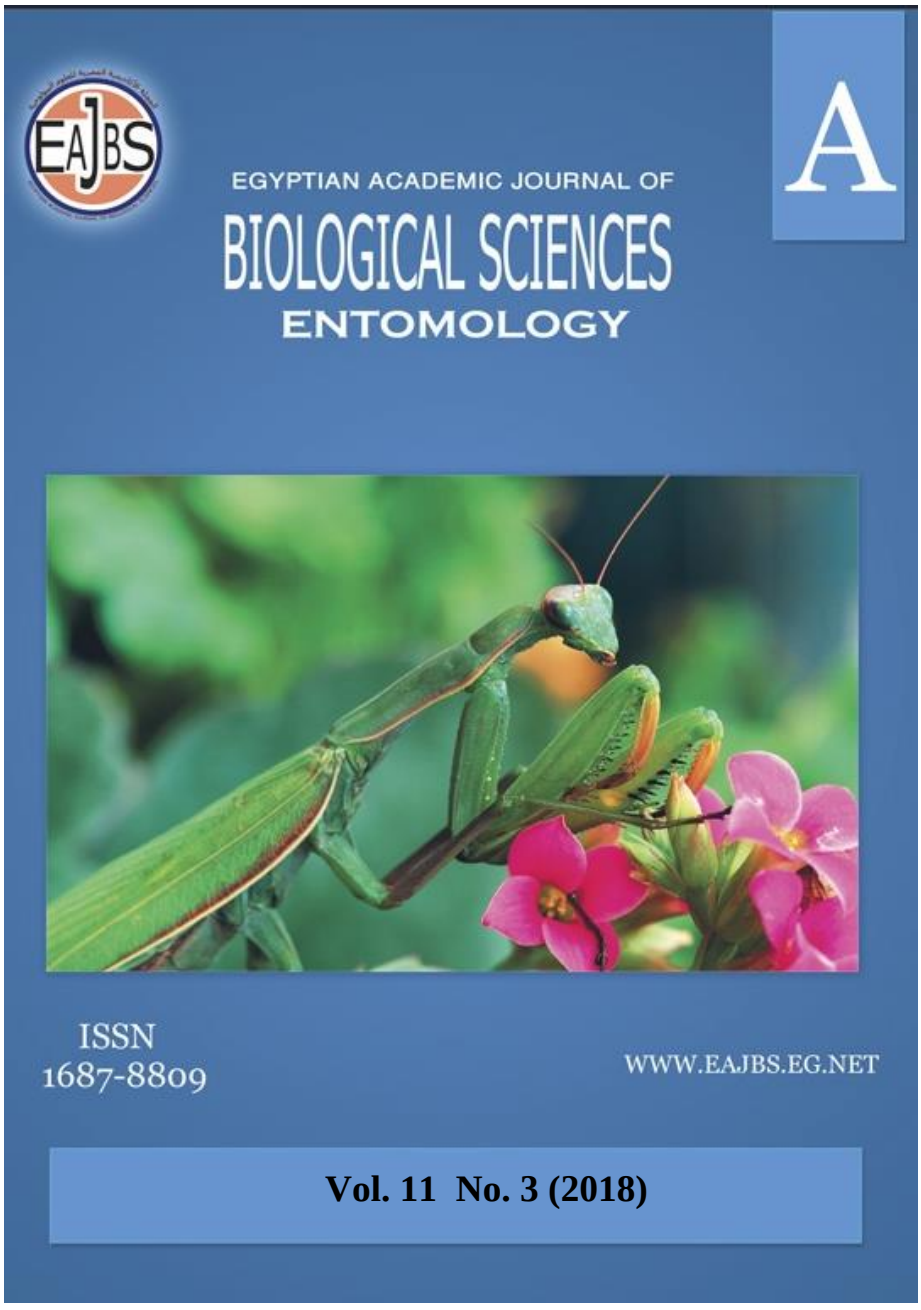

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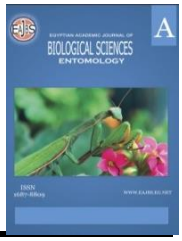

Ultrastructural Changes of the Cotton Leaf Worm, *Spodoptera littoralis* (Boisd.) Ovaries Induced by the two IGRs; Diflubenzuron and Chromafenozide.

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ABSTRACT

The present study was designed to investigate the possible effects of the two insect growth regulators, Diflubenzuron (Dimilin®) and Chromafenozide (Virtu®), on the ultrastructure of the cotton leaf worm, *Spodoptera littoralis*, ovaries. Therefore, the sublethal concentrations "LC_{50}" (3 ppm of diflubenzuron and 0.1 ppm of chromafenozide) were applied to the 2nd and 4th larval instars. After the Dimilin®-treatment of the 2nd instar larvae (D-II), the ovary of the emerged adult moth showed the separation of outer sheath and shrunk of tunica propria as well as pyknotic follicular nuclei. By the Dimilin®-treatment of the 4th instar larvae, the emerged adults' ovary revealed irregular shaped and pyknotic follicular nuclei, degenerated microvillar region, degenerated yolk granules and signs of autolysis. Regarding the 2nd instar larvae treated with Virtu® (V-II), pyknotic follicular nuclei with irregular shape, ruptured nuclear envelope and degenerated yolk granules were observed in moth's ovary. However, Virtu®-treated 4th instar larvae (V-IV) showed follicular nuclei with irregular shape and pyknosis and the cytoplasm appeared with fragmentation of rough endoplasmic reticulum with loss of ribosomes in moth's ovary. Results showed that the effects of tested IGRs resembled the effects of insecticides on *S. littoralis* ovaries through distorting the cell organelles which may lead to the disruption of the ova production. These events would lead to metamorphosis failure resulting in unhealthy adults. In conclusion, results suggested that Diflubenzuron and Chromafenozide were promising, effective and safe insecticides and can be used with integrated pest management programs for controlling the Egyptian cotton leafworm *S. littoralis*.

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), is considered the major pest of cotton and a lot of important crops in Egypt. The extensive use of chemical insecticides to control the cotton leafworm produce many drawbacks as resistance to various insecticides, residual toxicity, environmental

pollution and negative effects on non-target organisms (Rashad *et al.*, 2015). A new approach in insect control programs is the use of insect growth regulators (IGRs). IGRs are diverse groups of chemical compounds that are species-specific and highly active against immature stage of insects. They have a good margin of safety to most non-target organisms. Thus, they will play an important role in control programs in the future (Mulla, 1995; Darvas and Polgar, 1998). The main groups of IGRs used commercially are juvenile hormone analogs and chitin synthesis inhibitors (Parrella and Murphy, 1998). Diflubenzuron interferes with chitin synthesis in insects and kills larval insects by disrupting their growth (Anwar and Abd El-Mageed, 2005; Abdel Rahman *et al.*, 2007). Besides, it leads to high destructions in fibrous ovariole membranes (Abdel-Ghany *et al.*, 1985), decreases thickness of the follicular epithelium and reduces protein content and number of oocytes per ovary (Soltani and Soltani-Mazouni, 1992). However, Chromafenozide, non-steroidal moulting hormone agonists, has an insecticidal activity by disrupting insect moulting process. It is very potent against Lepidoptera, but weak or inactive against other insect orders such as Diptera and Coleoptera (Nakagawa *et al.*, 2005). Further, the use of chromafenozide at recommended dose did not pose any hazards to consumers when applied in strawberry under open field conditions (Malhat *et al.*, 2014).

The ovary in insects consists of functional units, the ovarioles. Ovaries of the cotton leafworm *S. littoralis* are polytrophic meroistic, meaning that the oocyte is interconnected with several nutritive cells, the trophocytes. The oocyte–trophocyte complex, surrounded by somatic epithelial cells, follicle cells, constitutes an ovarian follicle. Each ovariole is formed of a single linear array of ovarian follicles, each larger and more mature than the developmentally preceding one. Posterior to the germarium layer, a growth phase area followed by the vitellarium in a series of follicles, in which the oocytes accumulate yolk materials. Each egg follicle matures gradually while moving through the vitellarium, until it attains the final stage of its development.

Insect ovary, due to its essential role in insect reproduction, has been a perennial topic of investigation. Shalaby et al. (1987) postulated that the application of juvenoid ZR-520 reduced the number of oocytes and caused variations in their sizes and shapes which leads to difficulty to distinguish between oocytes and nurse cells in *S. littoralis* ovarioles. Sorge et al. (2000) suggested that a crucial role for ecdysone agonist, 20-hydroxyecdysone, in reduction of vitellogenesis in the noctuid *S. frugiperda* and may trigger biosynthesis in the ovary. Therefore, the ultrastructural examination of the ovarioles may provide morphological information describes the mode of action of the selected IGRs.

Accordingly, the aim of the present study is to examine the ultrastructural changes occurred in the ovaries of *S. littoralis* adults developed from treated of the 2nd and 4th larval instars with sublethal concentrations (LC_{50}) of the two IGRs; diflubenzuron and chromafenozide.

MATERIALS AND METHODS

Maintenance of Insect Colony:

The stock colony of *S. littoralis* was obtained from Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza. This strain was reared under the technique described by (El-Defrawy *et al.*, 1964).

Insect Growth Regulators (IGRs):

Two analogues of IGRs were used:

1- Diflubenzuron (Product name: Dimilin® 48% SC) is a benzamide insecticide, mainly against lepidopteran larvae, was discovered and developed under cooperative works by Chemtura Co., Ltd. USA.
2- Chromafenozide (Product name: Virtu® 5%) is a novel non-steroidal agonist of the insect moulting hormone 20hydroxyecdysone, specific for lepidopteran larvae, was discovered and developed under cooperative works by Nippon Kayaku Co., Ltd. and Sankyo Co., Ltd. Japan.

IGRs Application:

Freshly moulted 2nd and 4th larval instars of S. *littoralis* were used. The target instars were starved for 8 hours till experiment. Then, freshly castor oil beans leaves were dipped for 30 sec. in each of the previously estimated LC_{50} of Diflubenzuron and Chromafenozide (Ahmed *et al.,* 2015). The LC_{50} of Diflubenzuron was 1.3 and 3 ppm against 2nd and 4th larval instars, respectively, but it was 0.1 for both instars treated with Chromafenozide. The treated leaves were left for 30 min. at room temperature before being introduced to *S. littoralis* larvae. The treated castor leaves were applied to the target instars for 24 hours and then replaced by untreated leaves. Control experiments were done as above without any treatment. The living larvae reared till adult emergence, first generation (F1), which were studied as treated groups. The ovaries of the control and treated F1 females were dissected in Ringer's saline solution. These ovaries were used for ultrastructural studies. The following groups were subjected to ovary-ultrastructural studies:

Control 2nd and 4th instar larvae (C), Dimilin®-treatments of 2nd instar larvae (D-II), Dimilin®-treatments of 4th instar larvae (D-IV), Virtu®-treatments of 2nd instar larvae (V-II), Virtu®-treatments of 4th instar larvae (V-IV).

Transmission Electron Microscope Techniques:

Ultrastructural studies by transmission electron microscopy were performed as described by Dykstra *et al.* (2002). Freshly dissected ovaries, of control and different treatments, were cut into small blocks (1x1 mm3) and fixed directly in cold 4F1G (i.e. 4% formalin + 1% glutaraldehyde adjusted at pH 2.2) for 24 hours. After that, samples were post-fixed with 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.3), dehydrated in an ethanolic series culminating in 100% acetone, and infiltrated with epoxide resin. After polymerization, overnight a 60°C, semithin sections (0.5 µm) were stained with 1% toluidine blue in 1% sodium borate then examined with light microscope. Areas of interest for target tissues were selected and the blocks trimmed accordingly. Ultrathin sections (80-90 nm) were cut, mounted on 200 mesh copper grids, and stained with uranyl acetate and lead citrate. The stained grids were examined and photographed by JEOL.JEM-1400-EX-ELECTRON MICROSCOPE at the Central Laboratory of Faculty of Science, Ain Sham University.

RESULTS

The Control Moth Ovary:

Ovaries of the cotton leafworm *S. littoralis* are polytrophic meroistic, meaning that the oocyte is interconnected with several nutritive cells, the trophocytes. The oocyte–trophocyte complex, surrounded by somatic epithelial cells, follicle cells, constitutes an ovarian follicle. Each ovariole is formed of a single linear array of ovarian follicles, each larger and more mature than the developmentally preceding one. Posterior to the germarium layer, a growth phase area followed by the vitellarium in a series of follicles, in which the oocytes accumulate yolk materials. Each egg follicle matures gradually while moving through the vitellarium, until it attains the final stage

of its development. The ovariole is enclosed separately by a continuous ovariole sheath which is composed of the cellular epithelium on the outside, a layer of isolated lumen cells and the amorphous tunica propria on the inside. In the growth phase, the epithelial sheath is surrounded by continuous basal membrane. The cytoplasm shows tracheoles, mitochondria, rough endoplasmic reticulum, small vesicles with flocculent material and free ribosomes (Figs. 1 a-f).

At the onset of vitellogenesis, the follicle cells surrounding the oocyte undergo striking changes in shape and structure. These changes are associated with transfer of yolk precursors from the haemolymph to the surface of the oocyte. A system of intercellular spaces generated between the follicle cells has the dominant role in this transfer (Figs. 1 a-c). The follicle cells are cuboidal, columnar in shape with spherical, slightly elongated or polygonal nuclei contain well-developed nucleoli and numerous chromatin aggregations (Fig. 1 a). Cytoplasm contains elements of smooth endoplasmic reticulum (SER) and rough endoplasmic reticulum (RER) which is shown in a curved shape with electron-dense granules in some dilated vesicles (Fig. 1 b). Also, rich mitochondria and free ribosomes are seen in the ground cytoplasm. At this stage, the oocyte surface is furnished with numerous, short microvilli, whereas pinocytosis takes place in the oocyte cortex. The oocyte microvilli are immersed in a substance fills the entire peri-oocyte space. Concurrently, the apical parts of these cells become pointed and enter corresponding depressions in the oocyte surface (Fig. 1 c).

The ooplasm contains abundance smooth endoplasmic reticulum (SER) which consists of tubules sometimes branch forming a network of dilated areas (Fig. 1 d). Also, well-developed network of sac-like membranes forms the rough endoplasmic reticulum (RER) which appears as whorl bodies (Fig. 1 e). Numerous mitochondria and abundance of free ribosomes are seen. In addition, the ooplasm contains Golgi complex which is made up of a series of compartments consisting of a collection of fused, flattened membrane-enclosed disks known as cisternae. The cisternae at the convex end called cis while at concave called trans face. Between cisternae are usually present in a stack or sacculus. Small vesicles may also be discharged from the margins of cisternae between the cis and trans faces (Figs. 1 d,e).

The ooplasm appears well developed and more deposition of yolk vesicle containing proteinous yolk, cell membrane and other cell organelles. Through haemolymph circulation vitellogenin (Vg), the precursor of vitelline (Vn), is transported to the follicle cells (Figs. 1 b,c) and is deposited in the form of Vn in the ooplasm. The egg obtains its yolk from extraovarian source in a way called hetero-synthetic strategy. A hormone usually stimulates the fat body to secrete a specific protein, Vg, into the blood. This protein is taken from the circulation and incorporated into yolk granules in the oocyte. Post-translational modifications may accompany this process, altering the Vg to become Vn, the mature yolk protein.

The oocyte continues to grow, sequesters yolk from the hemolymph and ribosomes from the nurse cells. The nurse cells become smaller and finally disappear as they collapse and dump their contents into the oocyte. As the follicle matures, the follicle cells synthesize and secrete first a product that is endocytosed by the oocyte. The oocyte nucleus is located near the trophocytes and is surrounded by a delimited nuclear envelope which enclosing the nucleoplasm. The later has two different types of chromatin; the lightly stained partially condensed euchromatin and the darkly stained in the condensed state heterochromatin (Fig. 1 f).

The ovariole of Dimilin®-Treated Insects :

The TEM micrographs of the Dimilin®-treated 2nd and 4th larval instars with LC_{50} (1.3 & 3 ppm), respectively, induced advanced signs of damage in the adult

moths' oogenesis. Figures 2 (a-d) reveal the D-II treatment while figures 3 (a-d) show the D-IV samples.

The Dimilin®-treated ovarioles appeared suffering from changes in the vitellarium region. Separation of the outer sheath was recorded (Figs. 2 a & 3 a,b). The outer membrane including the epithelial sheath, lumen cells and the tunica propria. The later became thinner and closed to the lumen cells and composed of a homogeneous layer of finely filamentous material (Fig. 1 a). Sometimes, epithelial sheath moves away from the tunica propria (Figs.3 a,b) and many tracheae appeared in the outer sheath.

Most of the oocytes appeared with altered structure after Dimilin® treatments. Their pyknotic follicular nuclei appeared elongated with dispersed heterochromatin in a highly condensed electron dense beside presence of more than one nucleolus (Fig. 2 a,b). The ground cytoplasm suffered from signs of damage. The more extensive rough endoplasmic reticulum (RER) appeared fragmented (Figs. 2 a & 3 a,b) and lost their ribosomes.

As a sign of destruction, many lysosomes were found in the cytoplasm. Several different lysosomal forms were observed in the cytoplasm. Large lysosomes that formed by the fusion of small ones with vacuoles contained extracellular substances They bind with degraded mitochondria, endoplasmic reticulum, microbodies, glycogen particles or other cytoplasmic structures (auto-phagy or auto-lysosomes). Lysosomes may also combine with residual bodies which resulted from undigested secondary lysosomes and transferred to the cytoplasm within vacuoles as residues (Figs. 2 a-d & 3 c,d).

Dimilin® treatment decreased the amount degenerated lipids during vitellogenesis (Figs. 2 a & 3 c,d). In this stage, the metabolism in the Dimilin® treated oocytes seem very active than in the control ones. Besides, the changes in rough endoplasmic reticulum, mitochondria and other cellular organelles in ovarioles of treated insects might have reflected action the decrease in vitellogenin. However, the number of lipid droplets and yolk granules appeared less than those in the control mature oocyte. Despite of this decreased amount and volume, the vitelline membrane developed in case of D-II samples (Fig. 2 c). The microvilli between follicle cells and oocytes revealed signs of damage among ovarian tissue. Few small pinocytotic vesicles existed between follicle cells and oocyte (Figs. 2 a,c & 3 c,d). Different profiles of vitelline degeneration because of degenerated yolk granules, besides, abundant lysosomes and rickety lipid droplets led dramatically to failure of chorion formation in the treated oocyte (Figs. 3 b,c).

The Ovariole of Virtu®-Treated Insects:

The ovarian system in the treated cotton leafworm *S. littoralis* (2^{nd} and 4^{th} larval instars) with LC_{50} (0.1 ppm) Virtu® appeared suffering from advanced signs of damage in the adult moths' oogenesis. Figures (4 a-f) revealed the V-II treatment, while, figures (5 a-f) showed the V-IV ones.

In the vitellarium region, the separation of the outer sheath is recorded. Sometimes, epithelial sheath left a wide space away from the tunica propria and many tracheae appeared in the outer sheath (Fig. 5 a). Altered structures appear in most of the oocytes after Virtu® treatments. The pyknotic follicular nuclei appeared in an irregular shape with heterochromatin dispersant in a highly condensed electron dense (Figs. 4 a,b & 5 a-c), sometimes elongated and ruptured nuclear envelope was also observed (Fig. 4 b).

In addition, the cytoplasm of the follicular cells suffered from sings of different alterations. The rough endoplasmic reticulum revealed fragmentation (Figs. 4 b-d & 5

b-d) and lost their attached ribosomes. Numerous of lysosomes and degenerated mitochondria were found in the cytoplasm. Mitochondrial particles with low electron density in proximity of vacuole membrane indentations (Figs. 4 a-d); mitochondrial particles are engulfed by vacuoles (Figs. 4 b,c) and its shape was diversely among spherical, elongated, rod and triangular-shaped and lost many cristae and fluctuant matrix (Fig. 4 b). Lipid droplets and yolk granules showed reduction in sizes, sometimes appeared degenerated and damaged (Figs. 4 d,e). However, the number of lipid droplets and yolk granules appeared less than those in the control in the mature oocyte.

The pre-ovulatory oocytes were induced to form a distorted chorion that comprised two layers: the very thin exo-chorion and endo-chorion. The endo-chorion is a thin, non-lamellate layer. It lies adjacent to the vitelline membrane. The exo-chorion consists of closely arranged plate-like structures forming a network interposed with a fine granular material. The irregular thickness of the exo-chorion varies considerably in the different areas of the pre-ovulatory oocyte surface. In newly laid eggs of many insects a mucous sheath, referred to as epi-chorion is also present externally to the chorion. (Figs. 4 b,c & 5 e,f).

The microvilli between follicle cells and oocytes revealed signs of damage among ovarian tissue. Different profiles of vitelline degeneration appeared because of degenerated yolk granules. Besides, abundant lysosomes and rickety lipid droplets led dramatically to distorted chorion formation in pre-ovulatory oocytes (Figs. 4 f & 5 e,f).

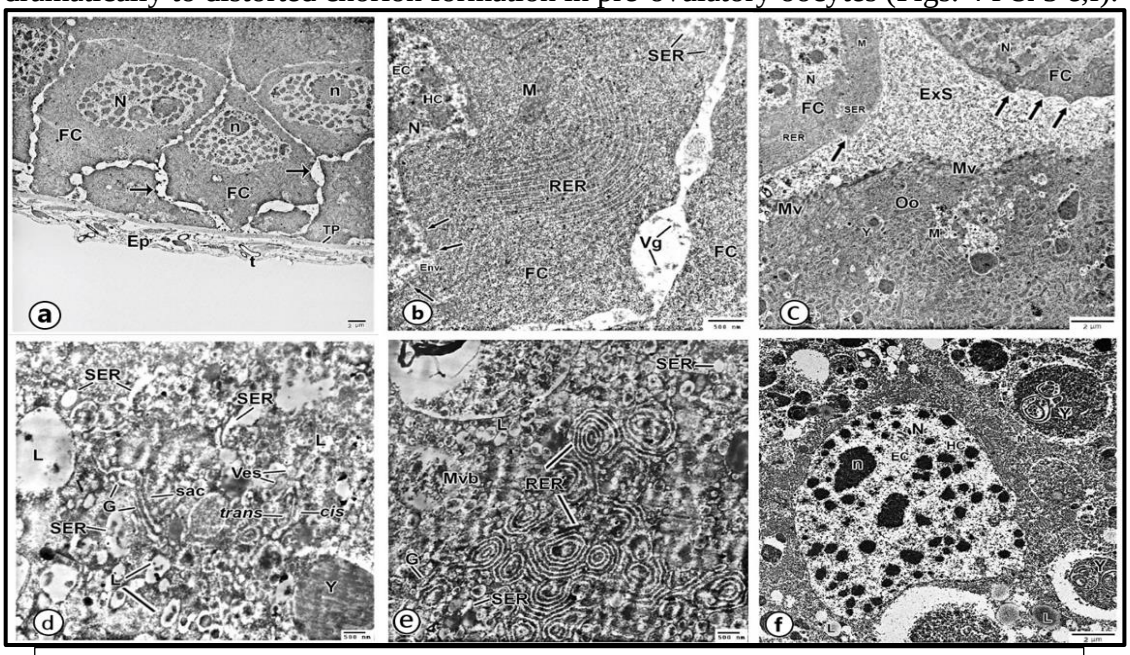

Fig. 1 (a-f): Electron micrographs of the control moth ovarioles. **(a)** A part of growth phase oocyte showing the outer epithelial sheath (Ep), the follicle cells (FC) with semispherical nucleus (N), a prominent nucleolus (n) thick tunica propria (TP), extracellular spaces (arrows) separating neighboring follicle cells and tracheoles (t) within the covering sheath. **(b)** An enlarged portion of the growth phase showing vitellogenin particles (Vg) and follicular cell cytoplasm contains mitochondria (M), free ribosomes scattering in the ground cytoplasm, rough endoplasmic reticulum (RER), smooth (SER), a polygonal nucleus (N), bilayer nuclear envelope (Env) with nuclear pores (arrows) and two types of chromatin; the peripheral heterochromatin (HC) and the scattered euchromatin (EC). **(c)** Showing an extrafollicular space (ExS) restricting two neighboring follicle cells (FC) and developing oocyte (Oo), and short microvilli (Mv). **(d,e)** Ooplasm contains abundance lipid droplets (L), yolk granules (Y), Golgi complex (G) with two faces; convex immature face (cis) and concave mature face (trans), stalks of Golgi saccules (sac) and vesicles (Ves) at the forming face, and multivesicular bodies (Mvb). **(f)** Part of the vitellogenic oocyte with large polygonal nucleus.

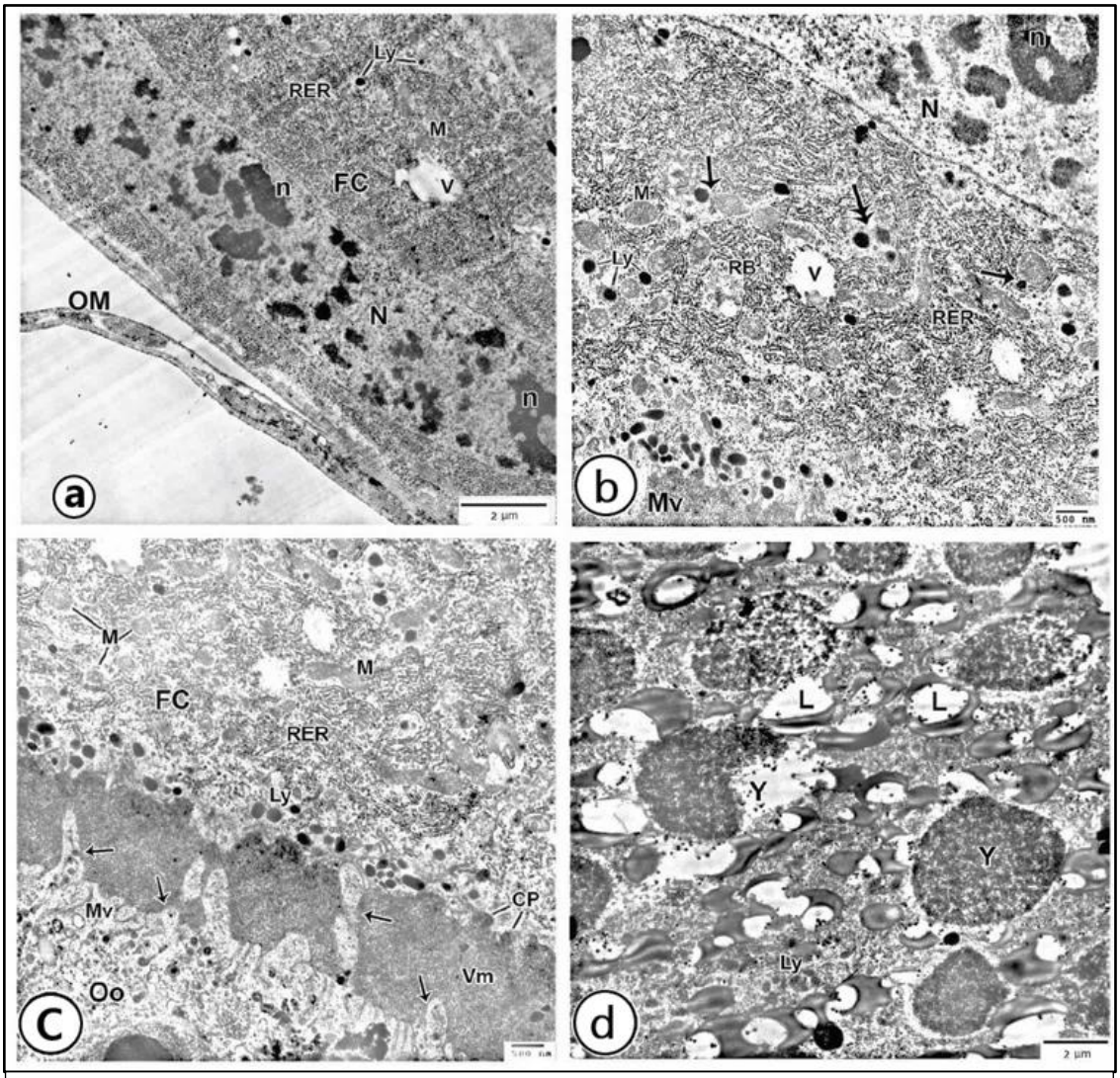

Fig. 2 (a-d): Electron micrographs of the moth ovarioles treated with Dimilin© as 2nd instar (**D-II**). **(a)** A follicle cell (FC) with separated outer membrane (OM), pyknotic elongated-shaped nucleus (N) has more than one nucleolus (n). and the ground cytoplasm has many fragmented rough endoplasmic reticulum (RER), degenerated mitochondrion (M) and vacuoles (V). **(b)** Nucleus (N) of degenerated follicle cell (FC) shows pyknosis, ring shaped nucleolus (n) and dispersed heterochromatin. The cytoplasm contains abundant Lysosomes (Ly) with different types of primary (arrow), secondary (double headed arrow) (autolysosomes) and residual bodies (RB), autolysis finally leads to empty vacuoles (V), and few microvilli (Mv) appears between follicular epithelium and oocyte. **(c)** Showing abundant small sized mitochondria (M) a well-developed vitelline membrane (Vm) contains coated particles (CP) which expel toward the growing oocyte as coated vesicles through pinocytosis (arrows). **(d)** Showing degenerated vitellogenic oocyte (Oo) with dilapidated yolk granules (Y), many lipid droplets (L) and dispersed digested granules scattered among the ground ooplasm.

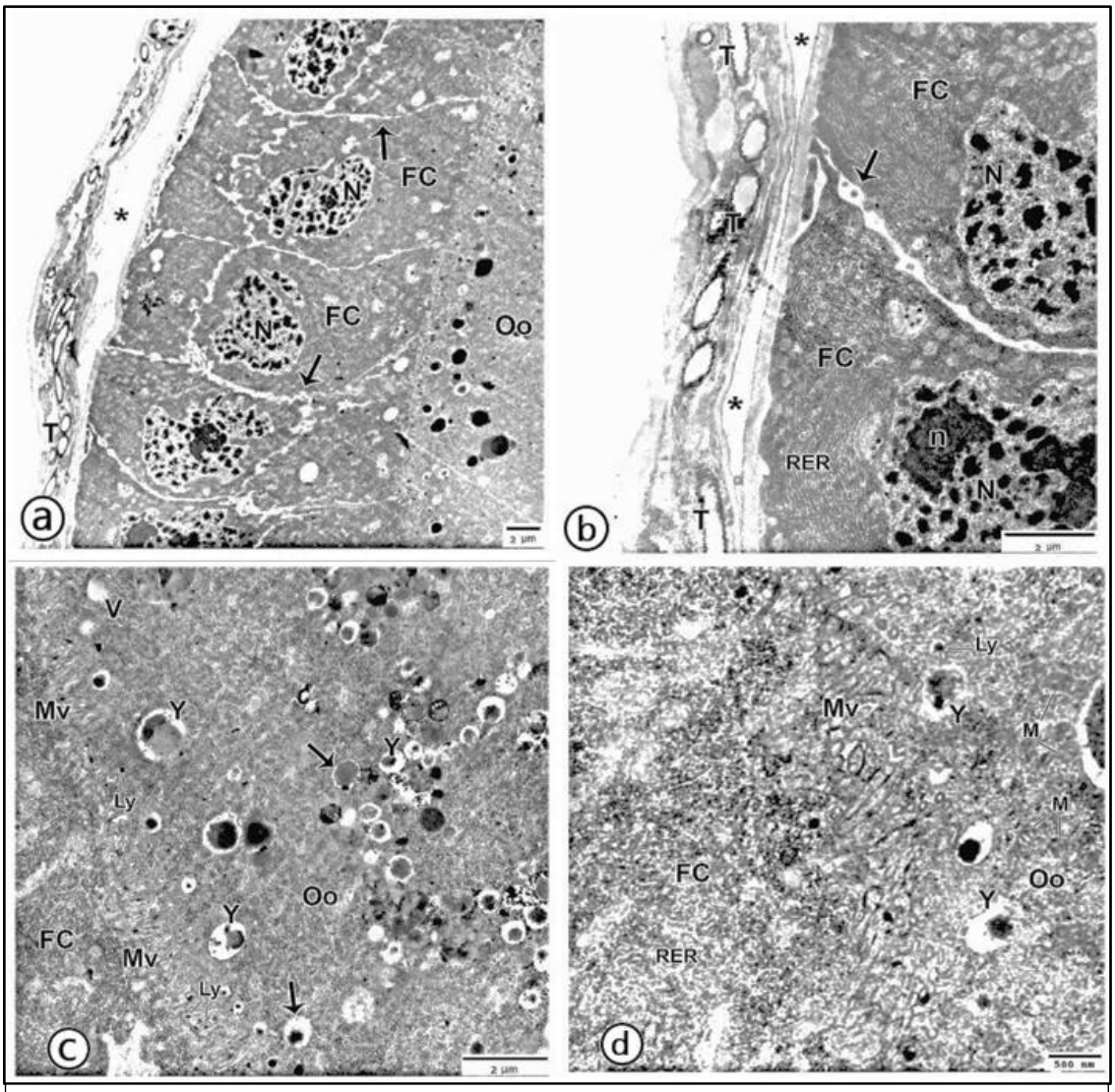

Fig. 3 (a-d): Electron micrographs of the moth ovarioles treated with Dimilin© as 4th instar (**D-IV**). **(a)** A vitellarium region showing signs of degenerated follicle cells and oocyte; the outer sheath contains many tracheae (T) and appearing separated from follicular epithelium leaving wide space (asterisk) and the follicle cells (FC) separating from each other by extracellular spaces (arrow). Their nuclei (N) showing signs of pyknosis and irregular shape. Oocyte (Oo) is suffering from sings of degeneration with few yolk spheres. **(b)** Showing nuclei containing numerous dispersed heterochromatins, the ground cytoplasm containing rough endoplasmic reticulum (RER) in a relatively healthy state, and a few passing particles through the extracellular space (arrow) from haemolymph. **(c)** Showing a narrow microvillar region (Mv) appears between follicle cells (FC) and degenerated oocyte (Oo) The cytoplasm containing abundant lysosomes (Ly) and degenerated yolk granules (Y), sometimes completely decayed (arrow). **(d)** Showing advanced stage of degeneration of microvillar region (Mv); the outer follicular cell containing fragmented rough endoplasmic reticulum (RER) lost their ribosomes, many dense granules appear scattered in the ground cytoplasm. In the oocyte cytoplasm, the dispersed lysosomes, cristae disorganization in mitochondria (M) and degenerated yolk granules (Y).

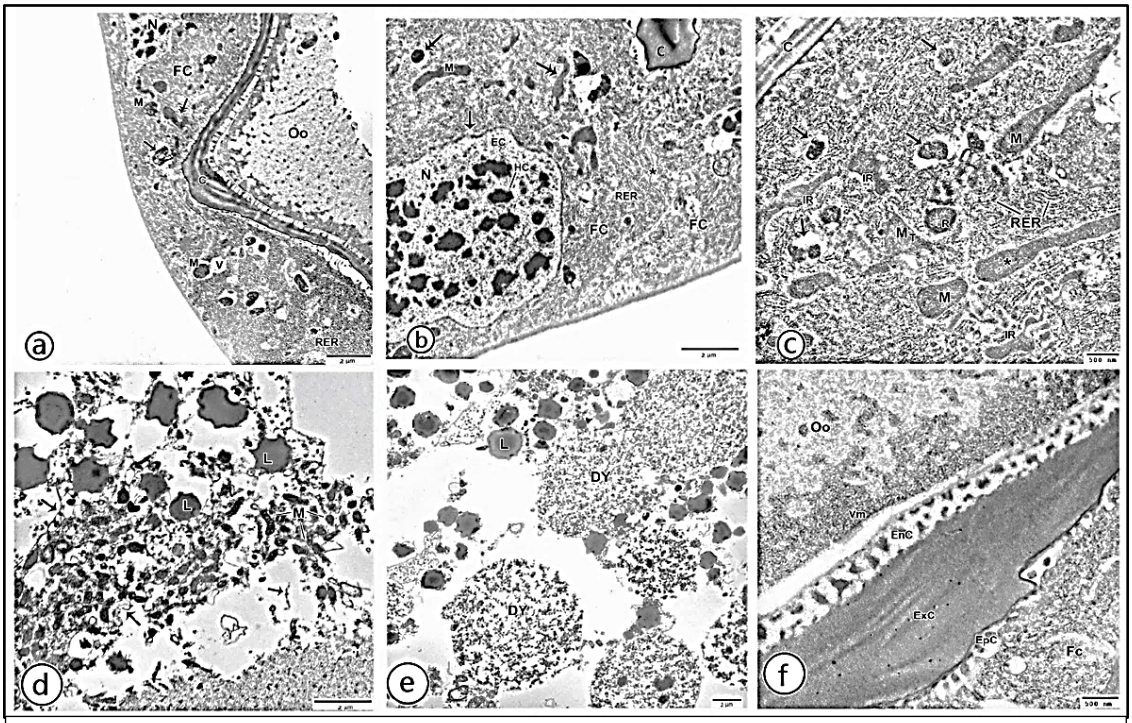

Fig. 4 (a-f): Electron micrographs of the moth ovarioles treated with Virtu© as 2nd instar (**V-II**). **(a)** A degenerated follicle cell (FC) and oocyte (Oo) showing pyknotic follicular cell nucleus (N), the ground cytoplasm has numerous vacuoles (V), fragmented rough endoplasmic reticulum (RER), mitochondrial cisternae (M) are lost (arrow) and matrix become dense (double head arrow) and finally, they become vacuoles. The degenerated oocyte (Oo) is completely separated from outer follicular epithelium by irregular malformed chorion (C). **(b)** Showing two neighboring degenerated follicular cells (FC) with a tightly contact (*) and no extracellular spaces. The follicular cell pyknotic nucleus (N) has discontinuous nuclear envelope (arrow). Heterochromatin (HC) is dispersed among euchromatin (EC). **(c)** Great aggregation of distorted mitochondria (M) in numerous shapes; triangular (MT), rounded (R), elongated (E), irregular (IR) and rod-shaped ones (*) and final step of mitochondrial autolysis (arrows). In the upper left corner, the irregular chorion (C) is noticed. **(d)** Degenerated ooplasm with numerous of degenerated and irregular lipid droplets (L), aggregation of different shapes of electron dense mitochondria (M) and fragmentation of endoplasmic reticulum (arrow) and great dilapidation. **(e)** Signs of dilapidated ooplasm organelles. Degenerated yolk granules (DY), lipid droplets (L) are suffering from damage. **(f)** Showing irregular chorion, irregular end-ochorion (EnC), vitelline membrane (Vm), exo-chorion (ExC) and epichorion (EpC).

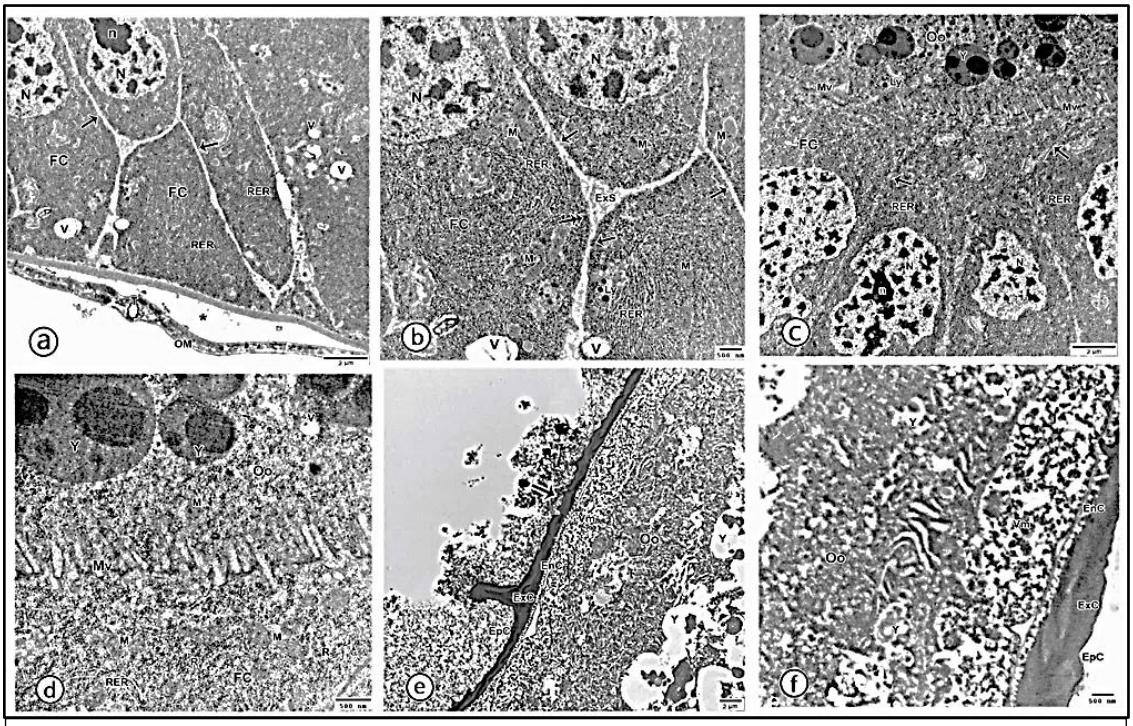

Fig. 5 (a-f): Electron micrographs of the moth ovarioles treated with Virtu© as 2nd instar (**V-IV**). **(a)** Showing the follicular cells outer membrane (OM) separating from tonica propria with a wide space (*), follicular cells (FC) separate from each other by narrow extracellular spaces (arrow). The irregular pyknotic nuclei (N) have large nucleoli (n) and dispersed heterochromatin. The ground cytoplasm contains many degenerated rough endoplasmic reticulum (RER), dark granules and numerous vacuoles (V). **(b)** Enlarged follicular region showing extracellular spaces (ExS) separating follicle cells where the vitellogenin pass to the growing oocyte via extracellular space (arrows). The cell cytoplasm contains fragmented rough endoplasmic reticulum (RER) with fragmentation and lost ribosomes. Degenerated mitochondria (M), multi-vesicular bodies (Mvb), numerous of lysosomes (Ly) and few vacuoles (V). **(c)** The microvillar region (Mv) between follicular epithelium and oocyte having irregular shapes and high electron dense, scattering lysosomes, and extracellular spaces (arrows) became narrow or absent. The ooplasm (Oo) contains few nutrients and electron dense yolk granules (Y). **(d)** Enlarged microvillar (Mv) region showing scattering ribosomes (R) in the follicular cell cytoplasm. The ooplasm containing ribosomes, mitochondria (M), vacuole (V) and the yolk granules (Y). **(e)** Degenerated oocyte (Oo) surrounded by irregular chorion, induced to form the trilaminar chorion including vitelline membrane (Vm), endo-chorion (EnC), exo-chorion (ExC) and epi-chorion (EpC). The outer exo-chorion has an irregular shape, sometimes serrate (arrow). The dilapidated ooplasm appears devoid of minimal nutrients need to develop healthy chorion. **(f)** Dilapidated oocyte (Oo) shows irregular chorion. The innermost thin vitelline membrane (Vm) revealing a degree of degeneration. The thin endochorion (EnC) is situated beneath the exo-chorion (ExC) and has a single row of air space. The edges are thickened and folded to form a strengthened rim of the outermost heterogeneous layer, exo-chorion. The latter is covered with wavy epi-chorion (EpC) layer and masking the sculptures of the chorion surface. These chorion envelopes enclose the ooplasm which contains degenerated yolk components (Y).

DISCUSSION

The insect' ovary is the most important organ in the female reproductive system. It is characterized by two main functions; synthesis of steroid hormones and production of ova (Carreau *et al.,* 2002). Various factors could affect oogenesis; among these factors are chemical agents, such as insecticides and toxic elements in environmental pollution.

The female reproductive system of cotton leafworm, *S. littoralis*, composed of a pair of ovaries of the meroistic polytrophic type. Each of which consists of four ovarioles where the ova are produced. Each ovariole possesses four components: a terminal filament, germarium, vitellarium and an ovariole pedicel connecting the ovariole to the lateral oviduct. Near the distal end (the terminal filament) of ovariole, there are groups of germ cells, oogonia, which divide by mitosis and increase in size to form oocytes. Each oocyte undergoes meiosis, which yields four cells, one egg and three polar bodies that may disintegrate or accompany to egg as trophocytes.

The normal ovariole contains a chain of developing ova which had defined spherical shape. The oocyte is enclosed with follicular cells and has well-differentiated and branched nuclei contained dispersed chromatin granules. The trophocytes supply the growing oocytes with needed nutrients. The follicle cells surround the oocytes forming the follicular epithelium. For vitellogenin (Vg) to enter the oocyte, intercellular follicular spaces are formed, in a process called patency, which is induced by juvenile hormone (JH). As the Vg enters the oocyte it converted to vitelline (Vn) which is one of the main components of yolk used by the embryo, surrounding the nucleus and replacing the cytoplasm. The trophocytes provide the developing eggs with nursing materials, while the cells surrounding oocyte, follicle cells, assist in producing the egg membranes.

Light microscopic examination of the ovarioles of control moths showed that each ovariole is composed of the terminal filaments, followed by the germarium with mast cells and pro-follicles. The later contains trophocytes, nurse cells, and follicular epithelial layer. Trophocytes characterized by their peculiar nuclei.

The treatment of Diflubenzuron and Chromafenozide against 2^{nd} and 4^{th} larval instars induced several structural changes at the ultrastructural level of moths' ovary as compared to controls. In most lepidopterans, last larval instar had found to have an initial peak of JH at the beginning of the instar that inhibits larval-pupal transformation (Zimowska *et al.,* 1989). Among several possible mechanisms, diflubenzuron affected endogenous JH titer indirectly by blocking endogenous JH degradation or directly by acting as a JH analog (*Kim et al.,* 2002). However, Chromafenozide is nonsteroidal-ecdysteroid agonist industrialized to interrupt the development of lepidopteran larvae. Its substantial effects had been observed on the reproduction of lepidopteran insects (Gelbic et al., 2011). In the present study, the ultrastructural alterations of the oogenesis might be interpreted as evidence of the diminished focal action of JH of damaged germ cells.

The tunica propria has a supporting function and plays an important role in ovulation (Chapman, 1985). In addition, it has the potential of serving as a selective membrane regulating chemical exchanges between ovariole cells and the haemolymph (Ahmed, 1987). In the present investigation, the ovariole surrounding basal lamina was ruptured and may be thickened. Such alternations of the boundary tissue interfered with and inhibited the function of its contractile activity and the transfer of vitellogenin toward the developing oocyte. Altered basal lamina structure was associated with severe functional impairment of the ovariole.

The follicular epithelium is essential for the development and maintenance of oogenesis. Oogenesis is a complex and dynamic process that results in the continual production of oocytes. Follicle cells are largely responsible for scoring the germ cells through sequential phases of mitosis, meiosis, and differentiation. Therefore, it seems that any agent impairs the viability and function of follicle cells may have profound effects on oogenesis.

Results revealed that follicle cells were particularly vulnerable to IGRs. They had multiple functions which protect and nourish the growing oocyte, control chorion formation and involved in the spatial patterning of the egg supporting and hormonal activities (McKearin et al., 2005). Thus, alterations in follicle cells would be reflected in the development of the growing oocyte.

Follicle cells also played important roles in vitellogenesis. They produced yolk proteins precursors, (Vgs), which accumulated in yolk spheres of maturing oocytes and provided energy reserved for embryonic development (Zhang *et al.,* 2018). Additionally, follicle cells control the oocyte uptake of yolk proteins (vitellogenins) which produced by the fat body (Bebas *et al.,* 2008).

Moreover, the most important feature in the taxonomic and phylogenetic studies, as the oo-taxonomy, was the egg chorion (Margaritis and Mazzini, 1998), because its structural details would provide valuable information that might be employed for pest management (Srivastava and Kumar, 2016).

Secretory activities of the follicle cells led to characteristic surface sculpturing of developing oocyte (Fehrenbach, 1995). In the same run, the differential secretory activity of follicle cells in various regions control the egg shell stratification as radial complexity (Srivastava and Kumar, 2016).

The present study showed ultrastructural changes in follicle cells of treated insects with IGRs. These changes include a large number of vacuoles, lysosomes and dilated endoplasmic reticulum. Moreover, follicle cells were damaged first followed by degeneration of the oocytes.

Mitochondria are responsible for ATP synthesis (Junqueira *et al.,* 1995) and Ca++ storage which is vital for ATP formation (Jouaville et al., 1999). The mitochondria' matrix and cristae are the sites where the enzymes catalyze the final oxidation of sugars and lipids and synthesis of ATP molecules (Lodish *et al.,* 1995). Consequentially, the decrease in mitochondria cristae or deformed mitochondria presumably led to the reduction in ATP synthesis (Reger and Fitzgerald, 1983).

Nutrients were transported via the inter-follicular spaces from the haemolymph under JH control. Protein yolk precursors were formed in the fat body and released into the haemolymph where they passed through the follicular epithelium and then, taken into oocyte by pinocytosis (Telfer, 1965; Hagedorn and Kunkel, 1979; Ahmed, 1987 and Peel and Akam, 2007).

The present study indicated that because of IGRs application, there are narrow inter-follicular spaces suggesting that the majority of vitellogenin cannot be allowed to enter to the oocyte, and then oocyte development becomes affected. Consequentially, endoplasmic reticulum, Golgi apparatus and other organelles suffered from hypotrophy after treatment with IGRs.

Also, results showed that most of the developing oocytes were possessing many vacuolation, shrinkage and finally, collapsed. Moreover, degenerated oocytes were enclosed within irregular chorion despite the loss of their nutrients and normal structure. These findings agree with those of Banerjee and Saadany (1986) when *P. pictus* treated with tepa and metepa and Shalaby and Diab (2004) after application of cyfluthrin and lambda-cyhalothrin to *C. pipiens*. On the other hand, the present results

disagreed with those of Badr *et al.,* (2000) after pyriproxyfen treatment against *S. littoralis*.

As shown in the present investigation, the mitochondria and smooth endoplasmic reticulum became numerous throughout the cytoplasm of follicle cells and developing oocyte in the two different treatments. The close association between enzymes and the SER, as an important site involved in steroid biosynthesis, besides, the role of mitochondria in the yolk formation, consider these organelles as an indicator for the synthetic activity of follicle cells and developing oocytes.

Therefore, the two IGRs, Diflubenzuron and Chromafenozide, proved to reduce the vitellogenic activity in the cotton leafworm and sometimes disrupt it. This may explain the irregular scattering yolk deposits in the ooplasm resulting after IGRs treatment. Moreover, this argument elucidates the interfere action of the juvenile hormone which regulates the vitellogenin.

It can be concluded that the tested IGRs appeared affecting *S. littoralis* as insecticides through distorting the cell organelles; endoplasmic reticulum, mitochondria, lysosomes, Golgi apparatus and even nuclei which may lead genetically disrupt the produced protein; to detoxify the IGRs or consequence translated protein from mutant genes. However, these events would finally lead to the observed failure in metamorphosis which is characterized the evaluation of unhealthy adults.

All above results suggest that Diflubenzuron and Chromafenozide as promising effective and human-safe insecticides which can be used in integrated pest management programs for controlling the Egyptian cotton leafworm, *S. littoralis*.

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RABIC SUMMERY

تغيرات التراكيب الدقيقة لمبيض فراشة دودة ورق القطن، سبودوبترا ليتوراليس (بويسد)، بواسطة منظمى النمو الحشرى دايفلوبنزورون وكرومافينوزيد.

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أجريت الدراسة الحالية للتحقق من التأثيرات المحتملة لمنظمى النمو الحشرى: دايفلوبنزورون (ديميلين) وكرومافينوزايد (فيرتو) على التركيب الدقيق لمبيض فراشة دودة ورق القطن (سبودوبترا ليتوراليس). لهذا، تم تطبيق التركيز النصف مميت (٣ جزء في المليون من الديميلين، و٠,١ جزء في المليون من الفيرتو) على الأعمار اليرقية الثانية والرابعة. بعد معالجة العمر اليرقى الثانى بالديميلين حدث انفصال للغمد الخارجي بالإضافة لظهور علامات النكرزة على أنوية خلايا الجريب، وعلى مستوى عضيات الخلية بدت الأجسام المحللة (الليسوسومات) بكثافة، مع تمدد الشبكة الإندوبلازمية الخشنة، وتلف الميتوكوندريا وكثافة مادتها الخلالية وفقد أعرافها. ومع معالجة العمر اليرقى الرابع بالديميلين ظهر انفصال الغمد الخارجي مخلفا وراءه مساحة واسعة، وظهور أنوية الجريب بعلامات النكرزة، وظهور نطاق الخميلات الدقيقة مدمرا، مع تجزؤ الشبكة الإندوبلازمية الخشنة وفقدانها الريبوسومات، وتحلل المح كعلامة على تحلل الخلية ذاتيا. وظهر انفصال الغمد الخارجي، وظهور أنوية الجريب بشكل غير منتظم وبغلاف ممزق وعليها علامات النكرزة، وكذلك بدأ تحلل كريات المح جليا بعد معالجة الطور اليرقى الثانى بالفيرتو. وبدا انفصال الغمد الخارجي مع ظهور النواة الجريبية بشكل غير منتظم وبعلامات النكرزة، وتواجد كثيف للكروماتين المغاير، كما لوحظ تجزئة الشبكة الإندوبلازمية الخشنة مع فقدانها الريبوسومات. النتائج أوضحت أن تأثير منظمات النمو المستخدمة مشابه لتأثير المبيدات الحشرية على مبايض فراشة دودة ورق القطن وذلك من خلال تشويه عضيات الخلية والذي قد يؤدى إلى اختلال انتاج البيض. وهذه الشواهد يمكن أن تؤدى إلى فشل التحول وإنتاج أفراد بالغة غير سليمة. وبناء على ذلك فإن النتائج تقترح الديفلوبنزورون والكرومافينوزايد مبيدات واعدة ومؤثرة وآمنة ويمكن أن تستخدم في برامج المكافحة المتكاملة لمقاومة فراشة دودة ورق القطن المصرية.