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Histological and ultrastructural aspects of larval corpus allatum of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) treated with diflubenzuron and chromafenozide

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Abstract
The present investigation was undertaken to follow the two insect growth regulators (IGRs), diflubenzuron and chromafenozide, possible effects on the histology and ultrastructure aspects of corpus allatum (CA) of 6th instar larvae of *Spodoptera littoralis*. Therefore, the LC_{50} (3 ppm of diflubenzuron and 0.1 ppm of chromafenozide) were applied to the 4th larval instar. The CA of 6th larval instar treated with LC_{50} of diflubenzuron appeared with rounded shape and decreased size (265.625 µm width & 331.25 µm length) and the capsular fibrous sheath (3.380 µm) also reduced. Contradictory, cellular cortex was increased in size (71.875 µm), as well as glandular cell numbers were increased, and their nuclei were lost their spheroid shape. Disturbance in cytoplasmic organelles pushed glandular cell to switch off or to be inactive. Also, damage was pronounced in the CA of the LC_{50}- chromafenozide treated larvae. The present work investigates the high potency and efficacy of the two IGRs towards *S. littoralis* corpora allata.

Keywords: *Spodoptera littoralis*, corpus allatum, chromafenozide, diflubenzuron, histology, ultrastructure

1. Introduction
In insect larvae, three endocrine glands are responsible for the release of neurohormones essential for growth, development and differentiation: the prothoracic gland, the corpus allatum (CA) and corpus cardiacum (CC) [1]. The cerebral neuroendocrine system of insects comprises the brain and retrocerebral complex [2]. The retrocerebral complex involves a pair of corpora cardiaca and corpora allata [3]. The corpus allatum is a glandular organ which engages in the release of hormonally active materials [4]. It is the major organ responsible for Juvenile hormone (JH) synthesis and release, which, maintains the larval characteristics at each moult until the adult metamorphosis takes place. JH stimulates both the synthesis of vitellogenin by the fat body and its uptake by the developing oocytes. In females, cyclic patterns of reproductive activity and vitellogenic cycles are associated with synthesis and release of JH [5-7].

The CA is ectodermal in origin and is in the posterior regions of the head on both sides of the oesophagus. The main regulators of JH synthesis, in most insect species, are neuropeptides from insect brain, allatotropins, and allatostatins. These are considered the stimulators or inhibitors of the JH synthesis in CA. The CA contains both intrinsic glandular cells as well as neurosecretory cells. They are generally innervated from the brain by two pairs of nerves, the nervicorporisallati I (NCA I) that originate in the brain and pass through the corpus cardiacum (CC) on their way to the CA; and the (NCA II) that originate in the sub-esophageal ganglion [8]. Three other nerves, the nervicorporiscardiaci I, II, and III (NCC I, II, and III) originate in the brain and enter the CC till reaching the CA. Because JH is not stored in the CA, its release is dependent upon synthesis, and this synthesis is rigidly controlled along several avenues.

IGRs are diverse groups of chemical compounds that are highly active against immature stage of insects. They have a good margin of safety to most non-target biota including invertebrates, fishes, birds, domestic animals and other wild life. Thus, they will play an important role in control programs in the future [9-10]. Diflubenzuron interferes with chitin synthesis in insects and kills larval insects by disrupting their growth [11-15].

Also, Chromafenozide has an insecticidal activity by disrupting insect moulting. It is very potent against Lepidoptera, but weak or inactive against other insect orders such as Diptera and Coleoptera [16].

Research of endocrinology in insects is important because it may offer new methods for disrupting the insect life cycle without harm to environment. Accordingly, the aim of the present study was to examine the histological and ultrastructural changes occurred in the CA of the *S. littoralis* 6th larval instar developed from treated of the 4th larval instars with sublethal concentrations (LC_{50}) of the two IGRs; diflubenzuron and chromafenozide.

2. Material and Methods

2.1 Maintenance of insect colony

The stock colony of *S. littoralis* was obtained from Plant Protection Research Instiute, Agricultural Research Center, Dokki, Giza. This strain was reared under the technique described by El-Defrawy *et al.* [17].

2.2 Insect growth regulators (IGRs)

Two analogues of IGRs were used: Diflubenzuron (Product name: Dimilin® 48% SC) and Chromafenozide (Product name: Virtu® 5%).

2.3 IGRs Application:

The 4th larval instars of S. *littoralis* were treated with the previously estimated LC_{50} of diflubenzuron and chromafenozide [18]. The CA of the control and treated 6th instar larvae were dissected. Histological and ultrastructural studies of CA were performed by using the light and transmission electron microscopes.

2.4 Light and Transmission electron microscope techniques:

The 6th instar larvae were dissected out in 4% formaldehyde and 1% glutaraldehyde (FG) in phosphate buffer solution as described by Dykstra *et al*. [19]. The CA were isolated from the freshly dissected larvae and fixed directly in cold FG (adjusted at pH 2.2) for 24 hours, then were post fixed in 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 at 60°C, semithin sections (0.5 µm) were stained with 1% toluidine blue in 1% sodium borate and examined with light microscope [20-21]. Areas of interest for CA 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. Statistical analysis section is missing. Provide it.

3. Results

3.1 Light microscope observations

Examination of the histological sections, obtained from the CA of control specimens revealed normal histological architecture. The CA appeared as a compact oval-shaped organ (259.375 µm width & 390.625 µm length) contains glandular cells, axons and neurons. A rich tracheal network supplies CA cells afforded their needs of ventilation. These tracheae diffused underneath the outer shell into tracheoles to make it wavy, sometimes roughly surfaced gland. The CA can be easily differentiated into three main regions; the outermost capsule, the cellular cortex and the innermost medulla. Surrounding the gland, an acellular fibrous area (5.222 µm) delimits the cortex and medulla and consists of an obvious fibrous sheath with a rich supply of tracheal network. The gland comprises the cortex (37.5 µm) containing neurons with axons running parallel with the surface of the gland and the glandular cells which contain distinct spheroid nuclei. The lucent appearance innermost medulla (167.188 µm width & 353.125 µm length) has an internal parenchymal matrix which contains axons of neurons filling the rest of the gland (Fig. A 1, 2).

Examination of the CA sections of diflubenzuron-treated larvae showed that the size get reduced (265.625 µm width, 331.25 µm length) than control and the tracheal supply became less. The boundary capsule sheath, became narrower (3.38 µm) compared to control and lost its roughly shape. In addition, the gland cortex became wider (71.875 µm) and had multiple layers of smaller glandular cells and many nerve cells. Moreover, neurons appeared reduced in size and migrated little inward to the medulla. Also, axons number were reduced and sometimes disappeared in medulla (112.745 µm width, 225.49 µm length) (Fig. A 3, 4).

Corpora allatum sections from larvae treated with chromafenozide revealed numerous histological changes. The CA gland appeared severely shrinked (180.625 µm width, 290 µm length), compared to control with lower tracheal supply. Moreover, the fibrous sheath thickness was reduced (3.87 µm). Also, the gland cortex was increased in size (74.878 µm) and the glandular cells became smaller than control. Neurons migrated inward far from the sheath. Besides, parenchymal matrix of medulla was severely reduced (69.375 µm width, 178.125 µm length) because of treatment. In addition, the glandular cells are appeared in one corner of the gland (Fig. A 5, 6).

3.2 Ultrastructural observations

Transmission electron microscopy of CA of normal 6th instar larvae displays that the external surface is covered with a flocculent, intact acellular capsule or sheath. Frequently, narrow electron lucent spaces were seen among the stroma (Fig. B7). Following the sheath, the outer part of the gland, the cortex, composed of electron lucent cells, the neurons. They are irregular in shape with several branching prolongations which interdigitate and associated with other glandular cells (Figs. B7, 8). Each neuron contains flattened nucleus with several scattered hetero-chromatin and euchromatin and enclosed by nuclear envelope. Their cytoplasm has numerous mitochondria, they are appeared in different shapes; spherical, elongated, dumbbell, oblong and rocket-shaped, some of these in state of division (Fig. B8). In addition, few intercellular spaces are observed. Multi-vesicular bodies and smooth endoplasmic reticulum are also observed (Fig. B9). Axons of neurons are distributed in the parenchymal matrix singular or in groups (Fig. B7). The glandular CA cells are lying beneath the outer neurons and have a well distinct plasma membrane. The latter encloses the ground cytoplasm which containing secretory granules and organelles, close to the nucleus. The cell boundaries are easily distinguished in most of the preparations and the cytoplasm contains free ribosomes, others in cluster form. Scarce rough endoplasmic reticulum (RER) is found besides smooth endoplasmic ones (SER). The latter is abundantly distributed. It is aggregated, sometimes appears winding. Moreover, it is found almost near Golgi apparatus and RER to present an aid in production of secretory granules (Fig. B10).

Mitochondria occur in great numbers, indicating a high metabolic activity of the cells. The shape and size of mitochondria are varying. They are found near the Golgi apparatus, SER, RER and secretory material. Electron dense mitochondria is a characteristic feature of the ultrastructure of the corpora allata cell cytoplasm. The well-developed cristae commonly traverse the mitochondrion as oblique, longitudinal, interconnecting and concentric arrangements were also seen (Fig. B7-10).

Many Golgi apparatus are scattered in cytoplasm. They usually appeared as stacks of flattened cisternae, frequently small vesicles and saccules. The SER, located close to the Golgi apparatus, produce small components toward the Golgi cisternae, then, it is translocated into Golgi saccules which finally joined to produce the secretory granules (Fig. B10). The secretory vesicles, found in the ground cytoplasm, aggregate and then migrate to the plasma membrane till discharging. Frequently, a large granule is surrounded and connected with smaller granules. The CA cell nuclei are granular bodies of regular spheroid shapes and occupy the middle area of cells. Moreover, they have nucleoli and irregular chromatic bodies (chromosomes) are also seen, often near to their envelopes. Chromatin is scattered into clusters of heterochromatin and great distribution of euchromatin. Distinct double layer nuclear envelope is distinguished with its nuclear pores where it is delimited (Fig. B10).

Treatment with LC_{50} of diflubenzuron for 4th larval instars induced advanced signs of damage in the CA ultrastructure (Figs. C11-14). The diflubenzuron treatment caused changes in the capsule, cellular cortex and medulla regions of the CA. The fibrous capsule sheath lost its rough shape. Little numbers of tracheal supplies were recorded. Neurons of CA appeared with no secretory granules. The number of electron dense mitochondria was reduced. The neuron nucleus appears suffering from pyknosis. Also, extracellular spaces are separating neighboring glandular cells with pyknotic nuclei (Fig. C11, 12). Few axons were appeared in one group inside the cortex and became free of neurosecretory materials. Finally, they became aggregated and bundled. The neuron cytoplasm showing distribution of different lysosomes; primary and secondary lysosomes, which attacking the cellular organelles. The glandular cell cytoplasm gives a real description of its damage. Glandular cells appeared inactive as they are lacking neurosecretory granules. There were few fragmented rough endoplasmic reticula vacant from their ribosomes. Ribosomes disperse randomly in the ground cytoplasm and multi-vesicular body are seen (Fig.C13). Golgi apparatus was reduced in number and their cisternae became swollen. SER, in turn, were also reduced in number as well as mitochondria. Degenerated nuclei of glandular cells displayed irregular shapes with ruptured nuclear envelopes which reflects the great degree of cellular damage (Fig. C 13, 14).

CA gland of treated-larvae with Chromafenozide, 20E agonist, appeared suffering from damage signs (Fig. D15-17). The outer fibrous coating sheath was reduced in size and partially separated from basal lamina leaving large space in between (Fig. D16). The fibrous sheath became smooth as tracheae and tracheoles reduced and migrated inside the cortex. Intracellular spaces were scattered in large areas of the gland. A characteristic highly electron dense of small-sized mitochondria were present. Death of CA was represented in scattering of electron dense of different lysosomes (L_1, L_2 and L_3) scattered within neuron cytoplasm in great numbers. Nuclei of glandular cells appeared pyknotic with electron lucent chromatin (Fig. D15). Additionally, cytoplasmic prolongations extend parallelly on the neuron cytoplasm and large intracellular space is also recorded (Fig. D16). Axons were devoid from neurosecretory materials. Multi-vesicular bodies were also distributed among the gland cells. Also, tracheae and tracheoles are seen in irregular sizes (Figs. D 16, 17).

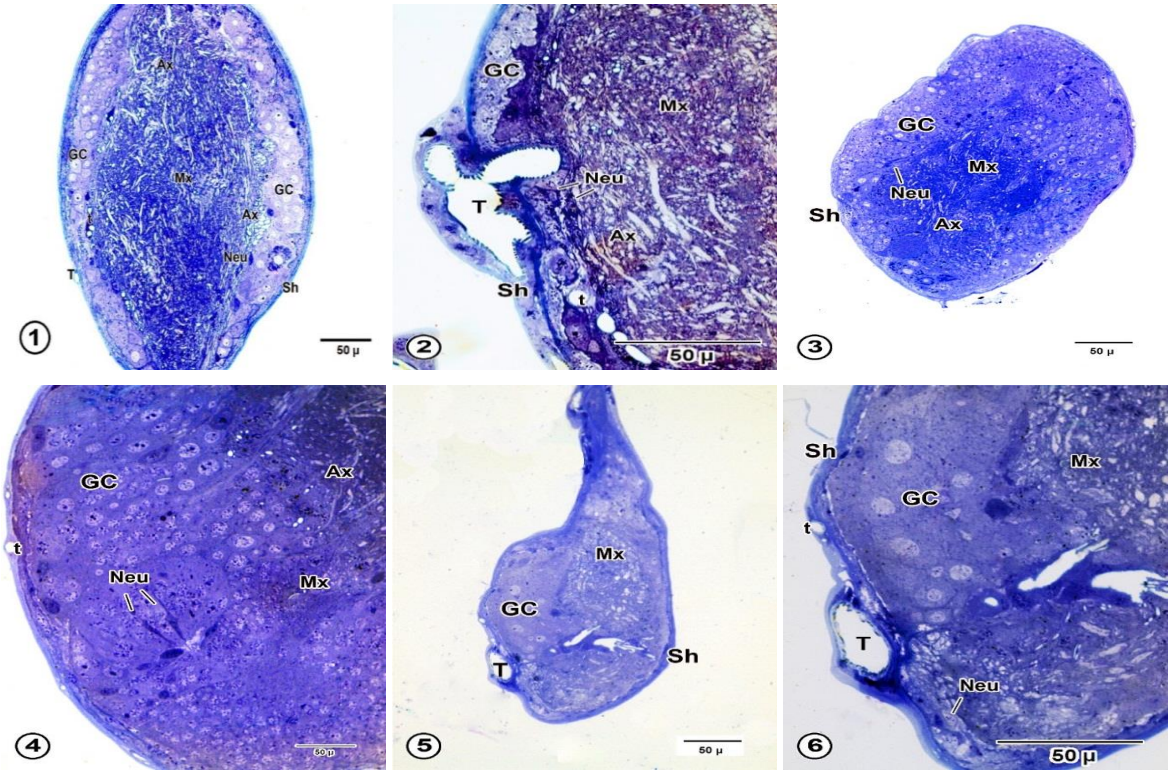


Fig A (A1-A6): Photomicrographs of Corpus allatum of 6th instar larva, control CA of 6th instar larva (Fig. 1,2), diflubenzuron - treated CA of 6th instar larvae (Fig. 3,4) and chromafenozide -treated CA of 6th instar larvae. Showing Fibrous sheath (Sh), glandular cells (GC), parenchymal matrix (Mx), neurons (Neu), Axons (Ax), tracheae (T) and tracheoles (t).

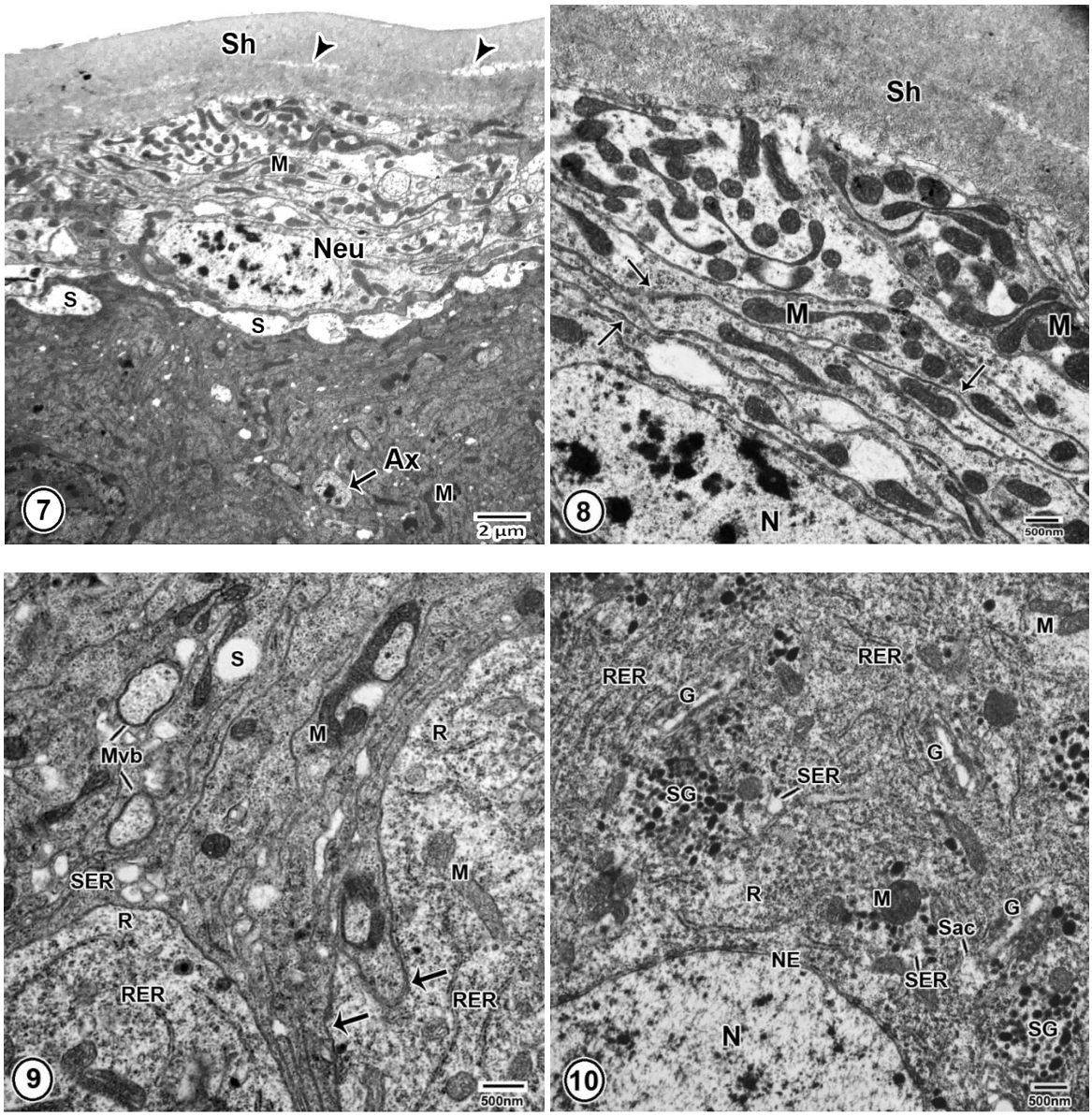
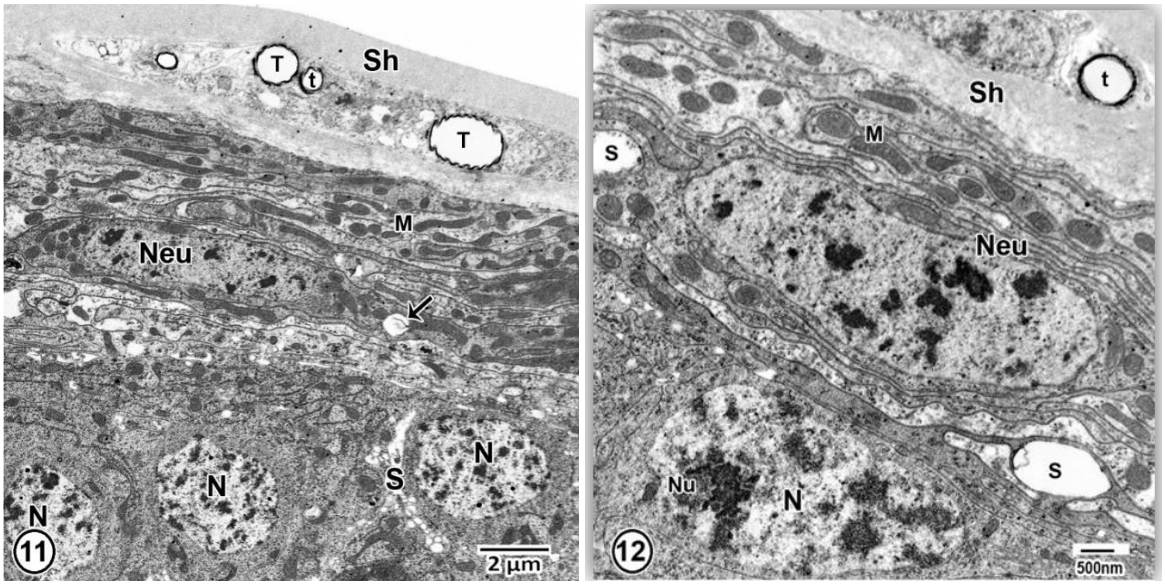


Fig B (B7-B10): Electron micrographs of control corpus allatum of 6th instar larvae. showing fibrous sheath (Sh), neurons (Neu), axons (Ax), mitochondria (M), spaces (S), smooth endoplasmic reticulum (SER), rough endoplasmic reticulum (RER), ribosomes (R), multi-vesicular bodies (Mvb), nucleu (N) and nuclear envelope (NE).



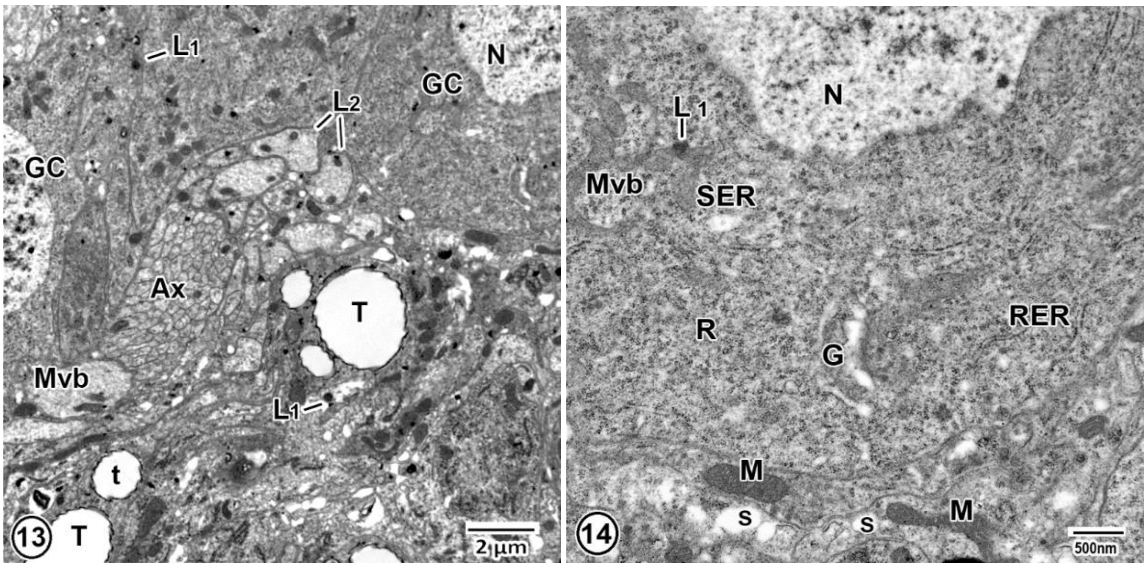
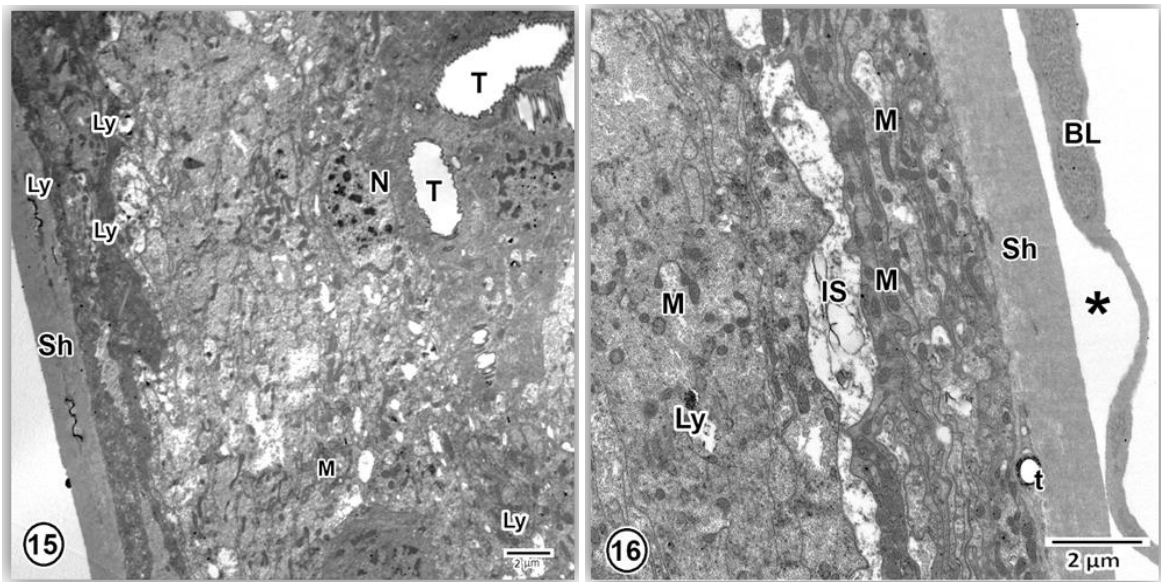


Fig C (C11-C14): Electron micrographs of diflubenzuron - treated corpus allatum of 6th instar larvae.
Abbreviations: fibrous sheath (Sh), tracheae (T), tracheoles (t), neurons (Neu), mitochondria (M), spaces (S), nucleus (N), tracheae (T), tracheoles (t), primary lysosomes (L1), secondary lysosomes (L2), multi-vesicular body (Mvb), axons (Ax), rough endoplasmic reticulum (RER) and smooth endoplasmic reticulum (SER)



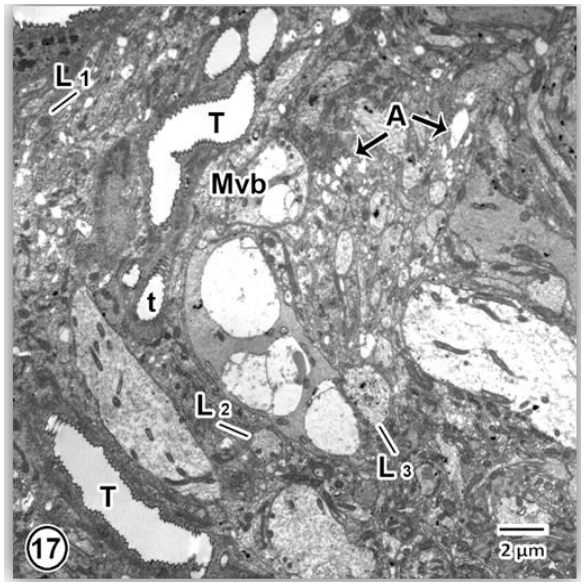


Fig D15-D17: Electron micrographs of chromafenozide -treated corpus allatum of 6th instar larvae.
showing fibrous sheath (Sh), nucleus (N), mitochondria (M), tracheae (T), lysosomes (Ly), basal lamina (BL), tracheoles (t), different lysosomal forms (L1, L2 and L3), multi-vesicular bodies (Mvb), axons (Ax)

3. Discussion

The corpus allatum is known as the main source for juvenile hormone. Both juvenile hormone and ecdysteroid are necessary for insect normal development and for vitellogenin (Vg) production in the female reproductive system [6]. Juvenile hormone increases the sensitivity of the vitellogenin-producing tissues to ecdysteroid. Indeed, JH and 20-hydroxyecdysone (20E) play a gonadotropic role in adult insects [22, 23]. The JH regulated 20E titer via the activation of the enzyme of its synthesis, ecdysone 20-monooxygenase, 20E agonist [23].

In Lepidoptera, female reproduction is regulated either by JH or ecdysteroids and it is well-known that JH stimulates vitellogenesis [24]. For example, in *Psuedaletia unipuncta*, there is a correlation between release rate of JH from CA and Vg synthesis [25] while, vitellogenesis is inhibited in decapitated females of *Heliothis virescens*, which then restored after JH treatment [5, 25]. Besides, it was found that decapitation of female *Choristoneura fumiferana* and *C. rosaceana* reduces egg production, while treatment with methoprene restores egg production [26]. Similarly, in Diptera, it was found that the presence of juvenile hormone is required for the ovarian maturation in adult females of *Musca domestica* [27]. Also, it was found that injection of 20E elevated vitellogenin levels in ovary-ectomized flies [28] and in decapitated flies [29]. In addition, JH is required for oocyte development in mosquito, which was blocked by CA removal and restored by implantation of CA or JH application [30].

The present results revealed that many similarities exist between the structure of CA gland in *S. littoralis* larvae and that of diverse insect species. Similar features were reported with CA of *Mamestra configurat* [31]. King *et al.* = [32] and Aggarwal and King = [33] reported similar structures in *Drosophilla melanogaster* larvae and prepupae. Also, in stable fly (*Stomoxys calcitrans*) and tsetse fly (*Glossina morsitans*) [34].

Concerning normal CA cells, the presence of neural components in the capsulated corpora allata of *S. littoralis* larvae, indicates that the activity of this gland may partially regulated by the nervous system, as they were found in the corpora allata capsule of immature and adult stages of various insect species [6, 31, 35].

Also, normal CA features with characteristic narrow intercellular spaces. Aggarwal and King (1969) [32] suggested that spaces may represented repositories of juvenile hormone. Therefore, during periods with active JH secretion, hormone accumulate temporarily in the cytoplasm. Moreover, Thomsen and Thomsen (1970) [36] found intracellular spaces in the cytoplasm of CA in females of *Calliphora erythrocephala*, and they suggested that these spaces may represent deposits for hormones.

The presence of mitochondria, with great numbers and different sizes and forms, indicates a high metabolic rate of gland activity which helps in getting energy to facilitate their duty. Whereas, Golgi apparatus is involved in many different cellular processes; packaging of secretory materials, processing of proteins, synthesis of certain polysaccharides and glycolipids, sorting of proteins in the cell, and proliferation of membranous elements for the plasma membrane. Amino acids are used to produce proteins from the RER, then conveyed to the Golgi apparatus for incorporation into secretory vesicles as reported by Baehr *et al.* [37] and Sayah [38] in the earwig *Lebidura riparia*. The presence of neurosecretory granules in the ground cytoplasm indicates the hormone-release function of CA. As Agui *et al.* [39] stated that the CA in some Lepidoptera species was the release site for prothoraciotrophic hormone (PTTH). Ultrastructural study of the corpus allatum has revealed that it contains many glandular elements. These results agree with Kou *et al.* [40] who observed very large numbers of mitochondria, abundant whorled smooth endoplasmic reticulum, irregularly shaped nuclei, Golgi bodies and free ribosomes in the CA cells of adult females of *Leucania ioreyi*.

IGRs can disturb CA neuro-secretions, among these regulators are; Diflubenzuron, a chitin synthesis inhibitor, and Chromafenozide, a non-steroidal agonist to the insect molting hormone 20E. Rentakarn *et al.* [41] stated that endocrine system was found to be affected by IGRs such as Tebufenozide which causes damage for the cell organelles because of imbalance in hormone secretions.

In this work, Diflubenzuron and 20E agonist, Chromafenozide, affect the JH production via changes in corpus allatum structure. The present work showed that tested IGRs caused histological and cytopathological changes in the CA. Morphological changes in the corpora allata strongly suggest variations in the synthetic activity of the gland. These results were in accordance with Ronderos [42] who studied the corpora allata changes along the Chagas disease vector *Triatoma infestans* 4th instar.

After administration of the two IGRs, CA cells exhibit ultrastructural features of degenerative glands, overall abnormal intercellular spaces, pyknotic nuclei, multi-vesicular bodies, and malformed of SER and RER. These cytotoxic features may lead to decrease of JH levels in the insect haemolymph. These results agree with that of the CA cells of *P. turionellae* which had poor organelles such as mitochondria, Golgi complex, RER [3].

Almost the same observations were reported by Dutta *et al.* [43] who studied the effect of two hormones (JH-III and 20E) on ultrastructure of muga silkworms, *Antheraea assamensis*, and found that the oocytes have more empty spaces and there are increase in number and size of the mitochondria and trachea in the neurosecretory cells. Also, they observed that Neuroplasm contained large number of free ribosomes with increased deposition of endoplasmic reticulum.

Bonetti *et al.* [44] analyzed CA ultrastructure in bees and found abundant mitochondria in all stages, larvae, pupae and adults. But after application of JH, mitochondria became less which possibly indicated the negative effects of hormone application.

IGRs treatments showed reduction in follicular epithelium thickness which agreed with the results of Raina *et al.* [45], when they studied the CA ultrastructure of *Coptotermes formosanus*, and found reduction in follicular epithelium thickness with extensive vacuolation under it.

Sedlak [46] stated that lysosomes are responsible for the elimination of enzymes and hormone precursors needed for hormone production. The present results emphasize that action as lysosomes were abundant with different types in the treated samples.

A lower chromatin condensation indicates higher activity of nuclear activity of CA, and vice versa [47, 48]. That finding was in accordance with the present result where the treated cells appeared suffering with pyknotic nucleus.

Consequently, these cytotoxic effects in larval CA may link to the appearance of multi-vesicular bodies, and finally, complete inhibition of vitellogenesis in adult stages. These findings were like those of Joly [49] on *Locusta migratoria*, and [38] on the earwig *Lebidura riparia*. The appearance of multi-vesicular bodies may be resulting from the

transformation of SER, RER, mitochondria and Golgi complex, so, low levels of JH may occur.

Previous researches showed that treatment with the anti-allatal drug, precocene, blocks ovarian maturation, which indicates JH secreted by CA controls maturation of insect ovary [50, 51]. Unnithan *et al*. [52] reported that treatment with precocene, an anti-JH, inhibited the egg maturation and CA was degenerated in *Oncopeltus fasciatus*. The same hormone showed degenerative effects in CA of *L. migratoria* nymphs [53]. Parallel to our results, precocene-treated *L. migratoria* showed electron dense cells and little cytoplasm with increased extracellular spaces. Also, Unnithan *et al.* [54] found segregation of various cytoplasmic organelles, vacuoles, residual bodies, pleomorphic mitochondria, irregularly Golgi apparatus, clumping of SER in CA of treated precocece II bugs.

The present observation showed that cytoplasm of CA cells was filled by secretion granules. Similar result was obtained by Rankin *et al*. [55] who found that CA cells in earwig (*Euborellia annulipes)* are full of secretion granules.

On the other hand, the present findings are contrary to Muszynska-Pytel *et al.* [56] who found that the ecdysone mimic RH 5849 caused allatotrophic activity leading in supernumerary larval molts in *Galleria mellonella*. Besides, Ergen [57] did not find any cell destruction in CA of *A. aegyptium* after treatment with precocene II. Similarly, treatment by halofenozide against the ground beetle, *Harpalus pennsylyanicus*, had no adverse effect on CA [58].

In the present study, Analysis of the corpora allata ultrastructure revealed that the application of IGRs altered the ultrastructure by decreasing the activity, as seen with fewer and small-sized mitochondria and greater chromatin condensation, as compared with the control. Application of IGRs may interfere with the synthesis of JH by CA.

The present findings support the idea that the modified corpora allata could induce disturbances in oogenesis which led to degrees of fecundity reduction. These results were in accordance with our previous findings [18]. In which Ovariole growth was stunted and vitellogenesis and chorion formation were inhibited after the application of IGRs.

4. Conclusion

From the present study, it can be concluded that treatment with diflubenzuron and chromafenozide altered the ultrastructure of the corpora allata and can efficiently be used in integrated pest management strategies of Egyptian cotton leafworm.

5. References

1. Meurant K, Sernia C. The Ultrastructure of the Prothoracic Gland/Corpus Allatum/Corpus Cardiacum Ring Complex of the Australian Sheep Blowfly Larva *Lucilia cuprina* (Wied.) (Insecta: Diptera). Insect Biochemistry and Molecular Biology. 1993; 23(1):47-55.
2. Raina A, Murphy C. A new gland associated with the retrocerebral complex of the adult corn earworm, *Helicoverpa zea*. Arthropod Structure and Development. 2008; 37(6):535-538.
3. Özlük A, Gül N, Muhammed A. Ultrastructural description of the corpora allata of *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae). African Journal of Biotechnology. 2011; 10(19):3784-3789.
4. Tombes AS, Smith DS. Ultrastructural studies on the corpora cardiaca-allata complex of the adult alfaalfa weevil, Hypera postica. Journal of Morphology. 2005; 132(2):137-147.
5. Ramaswamy SB, Shu SQ, Park YI, Zeng FR. Dynamics of juvenile hormone-mediated gonadotropism in the Lepidoptera. Archives of Insect Biochemistry and Physiology. 1997; 35:539-558.
6. Sharaby AM, El-Hawary FM, Moawad S. Zinc sulfate as a growth Disruptor for *Spodoptera littoralis* with reference to histological changes in larval endocrine glands. IOSR Journal of Agriculture and Veterinary Science. 2013; 5(3):67-74.
7. Belles X. Vitellogenesis directed by juvenile hormone. In: Raikhel, A.S., Sappington, T.W. (Eds.), Reproductive Biology of Invertebrates. Part B: Progress in Vitellogenesis. Science Publishers, Enfield, USA/Plymouth, UK. 2004; 12:157-198.
8. Odhiambo TR. The fine structure of the corpus allatum of the sexually mature male of the desert locust. Journal of Insect Physiology. 1966; 12:819-828.
9. Mulla MS. The future of insect growth regulators in vector control. Journal of the American Mosquito Control Association. 1995; 11(2):269-273.
10. Darvas B, Polgar LA. Novel type insecticides: specificity and effects on non-target organisms. In: Ishaaya, I., Degheele, D. (Eds.), Insecticides with Novel Modes of Action. Springer, Berlin, 1998, 188-259.
11. Gijswijt MJ, Deul DH, Dejong BJ. Inhibition of chitin synthesis by benzoylphenylurea insecticides, III. Similarity in action in *Pieres brassicae* (L.) with polyxin D. Pesticide Biochemistry and Physiology. 1979; 12:84-94.
12. Hammock BD, Quistad GB. Metabolism and mode of action of juvenile hormone, Juvenoids and other insect growth regulators, in the Juvenile hormone. (Ed. L. I. Gilbert). Plenum press New York, 1981, 374-394
13. Tripathi RK. Information sheet on Dimilin. Richmond, Virginia. 1996; 23218(804):786-1763.
14. Anwar EM, Abd El-mageed AEM. Toxicity impacts of certain insect growth regulators on some biochemical activities of the cotton leafworm. Egyptian journal of agricultural research. 2005; 83(3):915-935.
15. Abdel- Rahman SM, Hegazy EM, Elwey AE. Direct and la-tent effects of two chitin synthesis inhibitors to *Spodoptera littoralis* larvae (Boisd). American-Eurasian Journal of Agricultural & Environmental Sciences. 2007; 2(4):457-467.
16. Nakagawa Y, Takahashi K, Kishikawa H, Ogura T, Minakuchi C, Miyagawa H. Classical and three-dimensional QSAR for the inhibition of [3H] ponasterone A binding by diacylhydrazine-type ecdysone agonists to insect Sf-9 cells. Bioorganic & Medicinal Chemistry. 2005; 13:1333-1340.
17. El-Defrawy M, Toppozada A, Mansour N, Zeid M. Toxicological studies on the Egyptian cotton leaf worm, *Prodenia litura* L. Susceptibility of different larval instars to insecticides. Journal of Economic Entomology. 1964; 57:591-593.
18. Ahmed KS, El-Bermawy SM, Ismail EH, Abdel-Gawad RM, Saleh TA. Histopathological effects of Diflubenzuron and Chromafenozide (IGRs) on egg development in the cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera, Noctuidae). Journal of Bioscience and Applied Research. 2015; 1(4):147-153.
19. Dykstra MJ, Mann PC, Eiwell MR, Ching SV. Suggested standard operating procedures (SOPs) for the preparation of electron microscopy samples for toxicology/pathology

studies in a GLP environment. Toxicologic Pathology. 2002; 30:735-43.
20. Mercer EH. A scheme for section staining in electron microscopy. Journal of the Royal Microscopical Society. 1963; 81(3-4):179:186.
21. Burns WA. Thick Sections: Technique and Applications, Diagnostic Electron Microscopy, Ch. 4, B.F. Trump and R.J. Jones, eds., John Wiley & Sons, New York, USA, 1978.
22. Bownes M. The roles of juvenile hormone, ecdysone and the ovary in the control of Drosophila vitellogenesis. Journal of Insect Physiology. 1989; 35:409-413.
23. Gruntenko NE, Bogomolova EV, Adonyeva NV, Karpova EK, Menshanov PN, Alekseev A *et al.* Decrease in juvenile hormone level as a result of genetic ablation of the *Corpus allatum* cells affects the synthesis and metabolism of stress related hormones in Drosophila. Journal of Insect Physiology. 2012; 58:49-55.
24. Raikhel AS, Brown MR, Belles X. Hormonal control of reproductive processes. In: Gilbert LI, Iatrou K, Gill SS, editors. Comprehensive molecular insect science. 2005; 3:433-491.
25. Cusson M, Yu CG, Carruthers K, Wyatt GR, Tobe SS. Regulation of vitellogenin production in armyworm moths, *Pseudaletia unipuncta*. Journal of Insect Physiology. 1994; 40:129-136.
26. Delisle J, Cusson M. Juvenile hormone biosynthesis, oocyte growth and vitellogenin accumulation in *Choritoneura fumiferana* and *C. rosaceana*: A comparative study. Journal of Insect Physiology, 1999; 45:515-523.
27. Adams TS, Filipi PA. Interaction between juvenile hormone, 20-hydroxyecdysone, the corpus cardiacum-allatum complex, and the ovaries in regulating vitellogenin levels in the housefly, *Musca domestica*. Journal of Insect Physiology. 1988; 34(1):11-19.
28. Adams TS, Hagedorn HH, Wheelock GD. Haemolymph ecdysteroid in the housefly, *Musca domestica*, during oogenesis and its relationship with vitellogenin levels. Journal of Insect Physiology. 1985; 31:91-97.
29. Agui N, Bollenbacher WE, Granger NA, Gilbert LI. Corpus allatum isrelease site for insect prothoracicotropic hormone, Nature (London). 1980; 285:669-670.
30. Raikhel AS, Lea AO. Control of follicular epithelium development and vitelline envelope formation in the mosquito – role of juvenile-hormone and 20-hydroxyecdysone. Tissue Cell. 1991; 23:577-591.
31. Luo M. Morphology and ultrastructure of an isolated cell type corpora allata in adults of the bertha armyworm, *Mamestra configurata* Wlk. (Lepidoptera: Noctuidae). International Journal of Insect Morphology and Embryology. 1987; 16(2):143-l5l.
32. King RC, Aggarwal SK, Bodenstein D. The comparative submicroscopic cytology of the corpus allatum-corpus cardiacum complex of *Drosophila melanogaster*. Journal of Experimental Zoology. 1966; 161:151-176.
33. Aggarwal SK, King RC. A comparative study on ring glands from wild type and 1(2)gl mutant *Drosophila melanogaster*, Journal of Morphology. 1969; 129:171-200.
34. Meola SM, Pendleton MW, Langley PA, Lovering SL. Ultrastructural Localization of unique neurosecretory granules in the corpora cardiaca of the stable fly, *Stomoxys calcitrans* and tsetse fly, *Glossina morsitans*. Journal of Morphology. 1999; 240:155-168.
35. Tobe SS, Stay B. Structure and regulation of the corpus allatum. Advances in Insect Physiology. 1985; 18:305-432.
36. Thomsen E, Thomsen M. Fine structure of the corpus allatum of the female blowfly, *Calliphora erythrocephala*, Z. Zellforsch. Mikrosk. Anat. LIO, 1970, 40-60.
37. Baehr JC, Cassier P, Caussanel C, Porcheron P. Activity of corpora allata, endocrine balance and reproduction in female *Labidura riparia* (Dermaptera). Cell Tissue Resesrch. 1982; 225:267-282.
38. Sayah F. Ultrastructural changes in the corpus allatum after azadirachtin and 20-hydroxyecdysone treatment in adult females of *Labidura riparia* (Dermaptera). Tissue & Cell. 2002; 34(2):53-62.
39. Agui N, Izumi S, Tomino S. The role of ecdysteroids and juvenoids in vitellogenin levels and follicle development in the housefly, *Musca domestica*. Applied Entomology and Zoology. 1985; 20:179-188.
40. Kuo CT, Jan LY, Jan YN. Dendrite-specific remodeling of Drosophila sensory neurons requires matrix metalloproteases, ubiquitin-proteasome, and ecdysone signaling. Proceedings of the National Academy of Sciences of U.S.A. 2005; 102(42):15230-15235.
41. Rentakarn A, Macdonald A, Tomkins W, David C, Brownwright A, Palli SR. Ultrastructure effects of non-steroidal ecdyson agonist, RH-5992, on the 6th instars larvae of the spruce budworm, *Cheristoneure fumiferana* (Bibliographic citation). Journal of Insect Physiology. 1997; 43(1):55-68.
42. Ronderos JR. Changes in the corpora allata and epidermal proliferation along the fourth instar of the Chagas disease vector *Triatoma infestans*. Biocell. 2009; 33(3):149-154.
43. Dutta, Effect P, Dutta P, Rai AK, Neog K, Yadavm RN S *et al*. Effect of hormones (JH-III AND 20E) on biochemical and ultrastructural aspects of muga silkworm *Antheraea assamensis* (Lapidoptera: Saturniidae). International journal of recent biotechnology. 2014; 2(1):11-23.
44. Bonetti AM, Silva Siquieroli AC, Santana FA, Vieira CU, Bezerra M, Pereira GB *et al.* Effect of juvenile hormone III on the ultrastructure of the corpora allata in *Melipona quadrifasciata* (Hymenoptera, Apidae, Meliponını). Brazilian Journal of Science and Technology. 2006; 23(3-4):393-400.
45. Raina AK, Park Y, Hruska Z. Ecdysone agonist halofenozide affects corpora allata and reproductive physiology of the Formosan subterranean termite, *Coptotermes formosanus*. Journal of Insect Physiology. 2003; 49(7):677-683.
46. Sedlak BJ. The ultrastructure of interacting endocrine and target cells. R. C. King *et al*. (eds.), Insect ultrastructure. Plenum Press, New York, 1984.
47. Silva de Moraes RLM, Brochetto-Braga MR, Azevedo A. Electrophoretical studies of proteins of the hypopharyngeal glands and of the larval food of *Melipona quadrifasciata anthidioides* Lep. (Hymenoptera, Meliponinae). Insects Sociaux. 1996; 43(2):183-188.
48. Akahira Y, Beig D. Comparative study of corpora allata in Brazilian stingless bee. Papéis Avulsos de Zoologia. 1967; 20:165-190.
49. Joly L. Fonctions des corpora allata chez *Locusta migratoria* L. Thesis, University of Strasbourg, 1960,

103p.
50. Bitsch C, Bitsch J. Antigonadotropic and antiallatal effects of precocene II in the firebrat, *Thermobia domestica* (Thysanura: Lepismatidae). J Insect Physiol. 1984; 30:463-470.
51. Bitsch C, Delbecque JP, Mathelin J, Bitsch J. Effects of precocenes on intermoult length and ecdysteroid titers in relation to ovarian maturation in *Thermobia domestica* (Thysanura: Lepismatidae). J. Insect Physiol. 1986; 32:535-541.
52. Unnithan GC, Bern HA, Nair KK. Ultrastructural analysis of the neuroendocrine apparatus of *Oncopeltus fasciatus* (Heteroptera), Acta Zool. 1977; 52:117-143.
53. Schooneveld H. Precocene-induced collapse and resorption of corpora allata in nymphs of *Locusta migratoria*. Experientia. 1979; 35:363-364.
54. Unnithan GC, Nair KK, Syed A. Precocene-induced metamorphosis in the desert locust, *Schistocerca gregaria*. Experientia. 1980; 36:135-156.
55. Rankin SM, Stay B, Chan K, Jackson ES. Cockroach allatostatin-immunoreactive neurons and effect of cockroach allatostatin in earwigs. Insect Biochemistry and Physiology. 1998; 38:155-165.
56. Muszynska-Pytel M, Mikolajczyk P, Pszczolkowski MA, Cymborowski B. Juvenilizing effect of ecdysone mimic RH 5849 in Galleria mellonella larvae. Experientia. 1992; 48:1013-1017.
57. Ergen G. The effect of precocene II on the fine structure of corpus allatum in adult female *Anacridium aegyptium* L. (Orthoptera: Acrididae). Turkish Journal of Zoology. 2001; 25:95-103.
58. Kunkel BA, Held DW, Potter DA. Lethal and sublethal effects of bendiocarb, halofenozide and imidacloprid on *Harpals pennsylvanicus* (Coleoptera: Carabidae) following different modes of exposure in turfgrass. Journal of Economic Entomology. 2001; 94:60-67.