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An Ultrastructural Study on the Desert Locust (*Schistocerca gregaria*) as Affected by Tebufenozide (RH-5992)

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ABSTRACT

Tebufenozide exhibited some histopathological effects on the integument of last instar nymphs of desert locust (*Schistocerca gregaria* Forsk) such as: detachment of the cuticle from epidermis, undistinguishable epicuticle. Tebufenozide treatments affected the ultrastructural configuration of thoracic muscles such as disrupted organization of A, I and H bands and the degeneration of Z disc varied from one half of the disc to small central or peripheral degenerated areas. Tebufenozide treatments resulted in the loss of normal architecture of the majority of mid-gut epithelial cells, which had dwarf and deformed microvilli. The most important ultrastructural changes in the intracellular organelles by the action of Tebufenozide, as shown in the electron micrographs, were elongation of mitochondria, which appeared with prominent cristae, the lysosomes were supposedly autophagic, Golgi bodies had a bullet shape and the cytoplasm contained scattered granules.

Kew Words: *Schistocerca gregaria*; Tebufenozide; Nymphal instar; Ultrastructure; Histopathology; Integument; Muscles

INTRODUCTION

Development and reproduction in insects are affected by a number of hormones, including juvenile hormone and ecdysone. Insect growth regulators (IGRs) are synthetic analogues, which mimic the naturally occurring hormones for affecting the physiological processes of insects and are generally classified as juvenile hormone analogues and ecdysone agonists (Sehnal, 1983; Mohandass *et al*., 2006). The ecdysone agonists, or ecdysteroids, are synthetic products interfering with the natural insect moulting hormone (20-hydroxyecdysone), which controls several physiological and biochemical processes of growth and development. They have attracted the attention of many researchers all over the world (Hagedron, 1985; Silhacek *et al*., 1990; Ghoneim *et al.*, 1991 & 98; Smagghe *et al*., 1996; Cadogan *et al*., 1997). Tebufenozide (RH-5992) represents a novel class of IGRs, which directly stimulate the ecdysteroid receptors as the molecular level initiating the moulting process by gene regulation especially in larval Lepidoptera (Wing *et al*., 1988).

Although considerable interest has been shown in the use of hormone analogues as agents for insect pest control, studies have tended to concentrate on morphometric changes and on assessing pesticidal activity. Hence, information concerning the effects of such compounds at the cellular or subcellular level is somewhat limited. In the present work, the histopathological effects of Tebufenozide, as well as the ultrastructural changes in the desert locust (*Schistocerca gregaria* Forsk) were investigated aiming to shed some light on its roles at the cellular and subcellular levels.

MATERIALS AND METHODS

Experimental insect. A gregarious stock culture of desert locust [*Schistocerca gregaria* Forsk. (Orthoptera: Acrididae)] was raised by a sample from the established culture of Locust and Grasshopper Res. Division, Agric. Res. Center, Giza, Egypt. The insects were reared under crowded breeding conditions outlined by Hunter-Jones (1961) and Hassanein (1965). Newly hatched hoppers were kept in wooden cages with wire-gauze sides (40 x 40 x 60 cm) and small door in the upper-side to allow the daily feeding and cleaning routine. The bottom was covered with 20 cm layer of sterilized sand. Cages were equipped internally with 60 W electric bulb for lightening (17:7 LD) and warming (32±2°C.). Successive generations were raised before obtaining the nymphs for the present experimental work. Fresh food plant was clover *Medicago sativa* along the period of study except few weeks every year, because of the absence of this plant species. During these weeks, insects were fed on *Sesbania egyptiaca*. All experiments were conducted with *Medicago sativa* only.

Administration of the hormone analogues. A technical grade of the non-steroidal ecdysone agonist Tebufenozide

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(RH-5992) was used. Its chemical name is 1-N-t-butyl-1 (3, 5-dimethyl benzoyl)-2-(4-ethylbenzoyl) hydrazine (Rohm & Haas Company, Philadelphia, PA). Fresh clean clover leaves (*M. sativa*) were dipped for 3 min in 1000 mg kg^{-1} Tebufenozide and introduced to one-day penultimate instar nymphs. Three replicates (10 nymphs per replicate) were carried out for each treatment and controls. Each individual nymph was kept in a suitable glass vial whose bottom covered with a thin layer of sterilized sand. The nymphs, treated or control, were kept individually.

Histopathological and ultrastuctural techniques. The histopathological and ultrastuctural effects were examined in the cuticle, muscles, mid-gut of one-day old last instar nymphs. A representative nymph of each group was dissected and transected, fixed as soon as possible in 3% phosphate buffered glutaraldehyde (pH 7.3) for 2 h. After two rinses in the buffer (for a period of 4 h.) the specimens were fixed in 1% buffered osmium tetraoxide for 1 h at 4°C (Brissan *et al*., 1996).

The tissue pieces were washed twice in a buffer for 30 min. The specimens were then dehydrated in grades of ethanol; 50, 70, 80, 90 and 100%. The specimens were cleared in toluene for 10 min and then embedded in the resin of choic Epon. Semithen sections are cut from these blocks (stained with toluidine blue) and examined by the light microscope (Spnrr, 1969).

Ultrathin sections obtained from selected blocks were mounted on copper grids stained with uranyl acetate and lead citrate and then examined with Jol 1010 transmission electron microscope (Reynolds, 1963). This technique was carried out at Regional Center for Mycology and Biotechnology, Al-Azhar University, Madenit Nasr, Cairo.

RESULTS

The histopathological and ultrastructural investigations were carried out for one-day old last instar nymphs of desert locust after feeding the one-day old penultimate instar nymphs on a fresh food plant treated with 1000 mg kg^{-1} of Tebufenozide, as given above.

Histopathology and ultrastucture of integument. The basic structure of control integument of the last instar nymphs is shown in (Fig. 1a & b). The available electron micrograph clearly shows several changes in the integument of Tebufenozide treated last instar nymphs (Fig. 2). Tebufenozide treatments resulted in detachment of the cuticle from the hypodermis, undistinguishable appearance of epicuticle and exocuticle.

Histopathology and ultrastructure of thoracic muscles. At least, two kinds of filaments exist in the fibrils: prominent thicker filaments running along the A band and represent the protein, myosin; and less obvious filamentous thinner filaments extending from Z line to the edge of the H band, which represent the actin (Fig. 3).

Feeding of one-day old penultimate instar nymphs on 1000 mg L^{-1} Tebufenozide treated fresh food caused many

Fig. 1. Electron micrographs of transverse sections through two sites (a & b) of the integument of control 1- day old last instar nymphs of desert locust. Ep: epicuticle, Ex: exocuticle, En: endocuticle, Mt: mitochondria, Cr: mitochondrial cristae

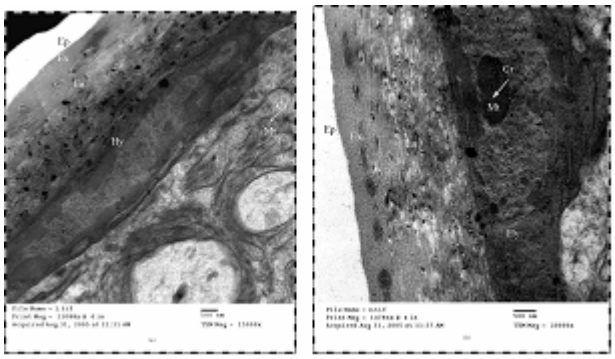
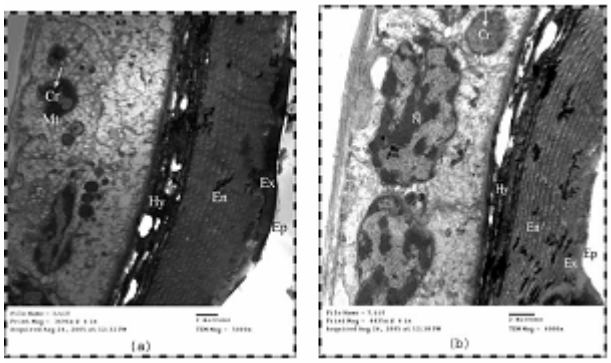


Fig. 2. Electron micrographs of transverse sections through two sites (a, b) of the integument of Tebufenozide treated last instar nymphs of desert locust. Tebufenozide treatments resulted in: detachment of the cuticle from the hypodermis, undistinguishable appearance of epicuticle and exocuticle. Abbreviations: Ep: epicuticle, Ex: exocuticle, En: endocuticle, Hy: epidermis (or hypodermis)



histopathological and ultrastuctural disorders in the thoracic muscles of the last instar nymphs as demonstrated in the electron micrograph (Fig. 4). The degeneration of Z-disc varied from one half of the disc to small central or peripheral degenerated areas. The micrograph also shows a disrupted organization of A, I and H bands.

Histopathology and ultrastructure of mid-gut. As seen in (Fig. 5), the control (normal) mid-gut of desert locust appears with luminal surface of the epithelium, which is provided with a striated border constituting long microvilli. Such microvilli protrude inwards into the lumen to increase the absorption surface of the cells, as well as, the spaces between them act as a kind of sieve.

The ecdysone agonist Tebufenozide exerted several histopathological effects on the mid-gut of last instar hoppers. The majority of epithelial cells lost their normal

Fig. 3. Electron micrograph of a longitudinal section of thoracic muscles of control one day old last instar nymphs of desert locust. Abbreviations: I: isotropic band, A: anisotropic band, M: middle band, H: narrow light band, Z: marked limits of sarcomere

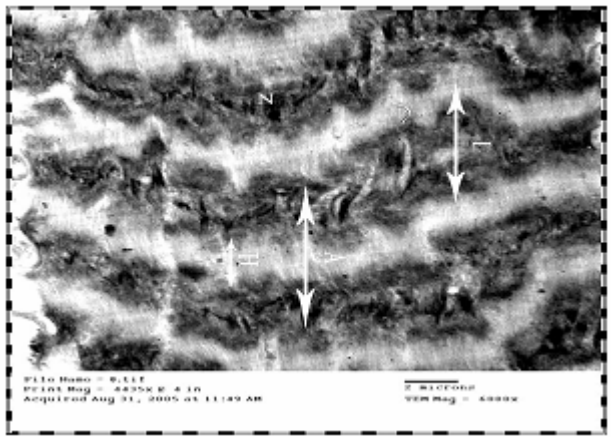
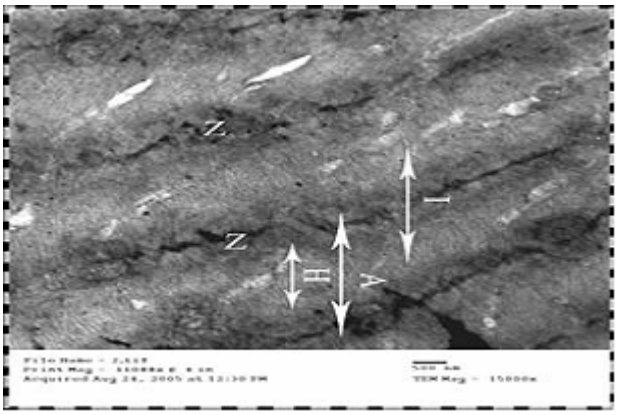


Fig. 4. Electron micrograph of a longitudinal section of thoracic muscles of Tebufenozide treated last instar nymphs of desert locust. The degeneration of Z-disc varied from one half of the disc to small central or peripheral degenerated areas. Also, disrupted organization of A, I and H bands can be observed. Abbreviations: I: Isotropic band, A: anisotropic band, H: narrow light band, Z: marked limits of sarcomere



architecture, because the microvilli appeared dwarf and had been severely deformed. Also, autopathogenic vacuoles and residual bodies, as well as vacuolization and ruptured cell wall were obtained.

Changes in the organelles. The orientation of the mitochondria is said to depend on the nature of the cytoplasmic matrix, vacuolar system and the direction of the diffusion currents of the cell. Also, the number of mitochondria in a cell depends on the type and functional state of the cell. They vary from cell to cell and from other to species. The mitochondria may be filamentous or granular in the shape and may change from one form to another depending upon the physiological condition of the cells. Thus they may be of club, racket, vesicular, ring or round-shape. The size of the mitochondria ranges from 0.2 to 2.0 um and the length may range from 0.3 to 40 µm.

Fig. 5. Electron micrographs of transverse sections through two sites (a & b) of the mid gut of control one day old last instar nymphs of desert locust. Abbreviations: L: lumen cavity, Gb: Golgi bodies, N: nucleus, Ly: lysosome, Mv: microvilli

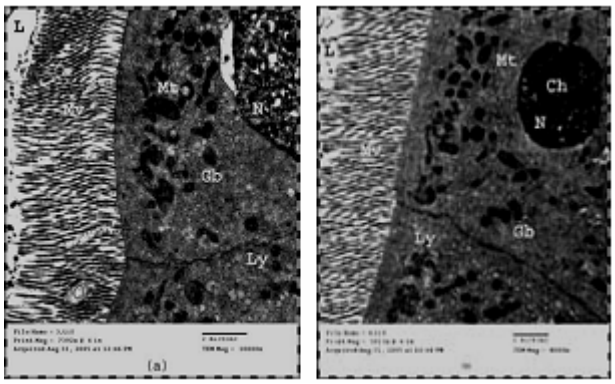
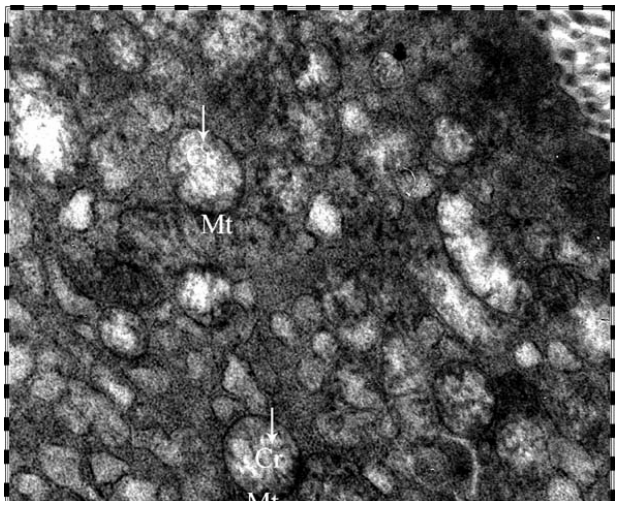


Fig. 6. Electron micrograph of mitochondria in control one day old last instar nymphs of desert locust. Abbreviation: Mt: mitochondria, Cr: Cristae



In addition, the mitochondria are bounded by a double membrane envelope, which provides good tensile strength, stability and flexibility to them. The outer and inner mitochondrial membranes are 0.6-0.7 µm thick. Each of them consisted of outer and inner dense osmiophilic layers composed of protein molecules of 200 to 250 nm thicknesses. Also, there was a middle (250 nm thick) biomolecular layer composed of lipids (Fig. 6). Existence of rounded bodies, lysosomes, was noted in the form of sac-like structures, each surrounded by a single thin lipoprotein membrane, which contained the broken cellular materials such as protein, nucleic acid and polysaccharides. The

Fig. 7. Electron micrograph of mitochondria in Tebufenozide treated last instar nymphs of desert locust. Some mitochondria were elongated with prominent cristae. Abbreviations: Mt: mitochondria, Cr: cristae

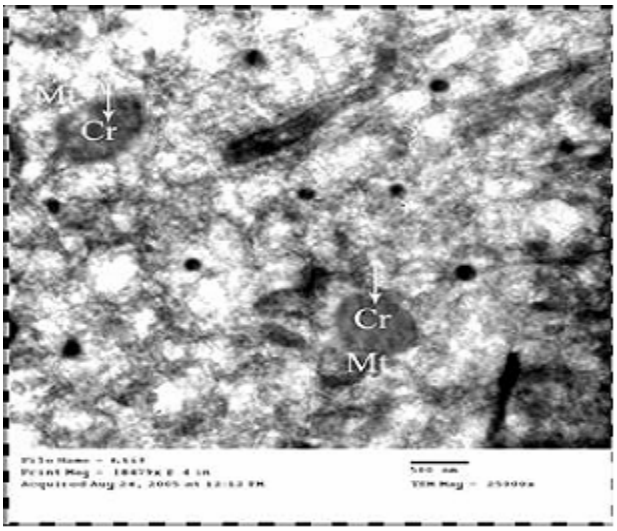
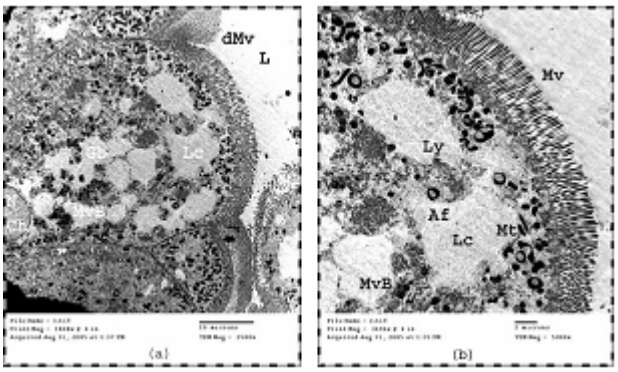


Fig. 8. Electron micrographs of transverse sections through two sites (a & b) of the mid gut of Tebufenozide treated last instar nymphs of desert locust. The microvilli appeared as dwarf and severely deformed. Vacuolization and ruptured cell wall were obtained. Also, there are proposingly autophagic lysosomes. Golgi bodies appear in a bullet shape. The cytoplasm contains scattered granules. Abbreviations: dMv: destruction of microvilli, Gb: Golgi bodies, L: lumen cavity, N: nucleus, Ch: nuclear chromatin, Lc: lysed cytoplasm, MvB: multivesicular bodies



dictyosomes appear as flattened oval sac together with cluster of small vesicular bodies at their edges (Fig. 7). The ultrastructural examination, in the present study, revealed several effects of the ecdysteroid Tebufenozide on the last instar nymphs of desert locust. Some mitochondria were elongated with prominent cristae (Fig. 7). There were autophagic lysosomes. The dictyosomes appeared in a bullet shape and the cytoplasm contained scattered granules (Fig. 8).

DISCUSSION

Histopathological Changes in the Body Tissues

The integument. In the present study, one-day old penultimate instar nymphs of desert locust after feeding on Tebufenozide-treated food resulted in several histopathological changes in the integument of last instar nymphs. The most important changes were detachment of the cuticle from the epidermal layer, the epicuticle could not be distinguished from the exocuticle. Wing *et al.* (1988) obtained precocious and lethal moults in various insect larvae by the ecdysteroid Tebufenozide. Disturbance in some steps of chitin deposition and histopathological changes in the epidermal cells were also reported by several authors using different hormone analogues (Gnatzy & Romer, 1984; Retnakaran & Oberlander, 1993).

Electrophoretic investigation revealed the effect of Tebufenozide on the production of cuticular proteins in larvae of *Spodoptera littoralis* and *Spodoptera exigua* (Smagghe & Degheele, 1996). In addition, the inhibition of normal endocuticle synthesis in tomato looper *Chrysodeixis chalcites* may have been caused by residual levels of Tebufenozide and this in turn may have prevented formation of a functional cuticle and may explain the fragility of the incompletely formed new cuticle (Smagghe *et al*., 1997).

The thoracic muscles. All the insect muscles are built on a similar plan with elongate cells housing the contractile elements and in many cases, inserted into the integument at either end. Each muscle is made up of a number of long fibers. Multinucleate cells usually run along the whole length of the muscle. The sarcolemma, myofibrils, sarcoplasm, molecular filaments, striation pattern (including discs & bands) and other structures are detailed in Elder (1975). In desert locust, in the present study, a complete set of adult muscles was present in the nymphal form. Most muscles were in use during nymphal stage. Flight muscles, however, remained small and functionless until the last larval instar and developed rapidly just before and after the imaginal moult (Novicki, 1989; Shiga *et al*., 2002).

Excision of the corpora allata (CA) producing juvenile hormones (JH) from reproductive beetles causes muscle degeneration. Reimplantation of the active CA causes them to regenerate (Stegwee *et al*., 1963). In contrast, implantation of the CA from reproductive females into diapausing ones induces degeneration of flight muscles in the plant bug *Dysdercus intermedius* (Edwards, 1970). These studies have shown a close association between the endocrine system and flight muscle degeneration. In *Modicogryllus confirmatus* and *Gryllus firmus*, exogenous applications of JH or a JH analog confirmed its role (Tanaka, 1994; Zera & Cisper, 2001). Topically treated 5th instar nymphs of *Locusta migratoria* with the juvenoid methoprene caused profound changes in the muscle fiber fine structure (Cotton & Anstee, 1990).

The mid gut. Tebufenozide treatments of the tomato looper

C. chalcites larvae resulted in cell degeneration of the fore gut (Smagghe *et al*., 1997). In the present study, the Tebufenozide treatments to one-day old penultimate instar nymphs of desert locust resulted in several serious histopathological changes in the mid gut of one-day old last instar nymphs. Majority of epithelial cells lost normal architecture and had dwarf and deformed microvilli. Also, obvious autophagic vacuoles scattered in the cytoplasm containing many vesicles. These results are in accordance with those of various other insect species (Williams, 1968; Birkenbeil, 1983; Gnatzy & Romer, 1984; Locke, 1984 & 85; Hanton *et al*., 1993).

Influence of hormonal analogues on intracellular ultrastructure. Mitochondria are considered as the 'power house' of the living cell, which supply energy for many chemical reactions and transport mechanisms in the cell. Orientation of mitochondria depends on the nature of the cytoplasmic matrix, vascular system and the direction of diffusion of currents in the cell. Their number and shape depend on the cell type and its physiological state varying from insect species to another. Each mitochondrium is bounded by a double membrane envelope, which provides its tensile strength, stability and flexibility (Bakr, 1986).

Smagghe *et al*. (1997) observed a dramatic increase in endoplasmic reticulum, scattered throughout the whole epidermal cell cytoplasm, hypertrophy of Golgi complexes, increase in the volume of the nuclei, and the presence of numerous oval and elongated mitochondria of *C. chalcites* larvae. Also, the epidermal cell cytoplasm of treated larvae contained numerous vacuoles and vesicles, myelin figures and glycogen clusters. On the other hand, Bakr *et al*. (1997) examined various ultrastructural effects of some IGRs on the larvae and pupae of *Culex pipiens* such as the loss of cristae in some mitochondria, swelling of others with prominent cristae and loss of demarcation of the mitochondria membranes with obvious increase in the mitochondria granules.

Tebufenozide, in the present study, remarkably affected the ultrastructure of the last instar nymphs of desert locust such as: elongation of mitochondria with prominent cristae, appearance of lysosomes supposedly autophagic, bullet-shaped Golgi bodies and scattered granules in the cytoplasm.

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