



**Histopathological Changes in the Muscle of the Desert Locust,
Schistocerca gregaria (orthoptera: acrididae) Treated with
Insect Growth Regulator (IGR), Lufenuron**

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ABSTRACT

The present study aimed to minimize the pollution effects of traditional insecticides on the environment by development and synthesis of bio-insecticide and to evaluate insect growth regulators, Lufenuron as insecticidal agents. Stock colony of *Schistocerca gregaria* was used in this study which were kept in cages and fed on leaves of Castor plant. Treatment of the fifth nymphal instar of locusts by using insect growth regulator, Lufenuron were carried out.

Results showed that, the treatment with different concentrations of Lufenuron (50, 75 and 100 ppm) resulted in nymphal mortality of 0.0, 12.33 and 21.01% respectively and percentages of adult malformed with 89.99, 73.34 and 72.33 respectively comparing with control. The current study showed that Lufenuron caused malfunction and decomposition of the components in the muscles of the desert locust.

INTRODUCTION

The desert locust (*Schistocerca gregaria*) is a wide spread pest that cause untold and terrible damages to our crop plants in the field. *S. gregaria* is a species of locust discovered by Forskal in 1775 and considered to be *S. Americana gregaria* (Dirsh, 1974). *S. gregaria* was generally recognized as polyphytophagous acridid that causes damage to pastures and crops during the desert locust upsurge in 2004 (Tarai and Doumandji, 2009). The preference of food by grasshoppers is determined by many factors such as toughness of the leaf, hairs and water content frequently are believed to influence feeding behavior, chemical differences are also vital, sugars phospholipids, organic nitrogen compound, tannins, and others influence host preference; generally feeding is limited to temperature between 15°C and 30°C and little time is spent in feeding approximately 15% (Uvarov, 1977).

Desert locust feed on all sorts of plants; consuming approximately equivalent of their body mass each day. Nearly all crops and non-crops plants are at risk including millet, rice, sorghum, maize, sugar cane, badly, cotton, fruit trees, vegetable, grasses, alacia pines and banana (OECD, 2004).

Desert Locust has been reported to feed on more than 400 species of plants (Uvarov, 1977).

Chitin synthesis inhibitors (CSIs) interfere with chitin biosynthesis in insects (Gijswijt *et al.*, 1979) and thus prevent moulting or produce an imperfect cuticle (Hammock and Quistad, 1981). These compounds are effective suppressors of development for the entire life cycle on insects (Verloop and Ferrell, 1977). However, these compounds, also, affect the hormonal balance in insects, thereby resulting in physiological disturbances, such as inhibition of DNA synthesis (DeLoach *et al.*, 1981); alteration of carbohydrates (Ishaaya and Ascher, 1977); increase in phenyoxidase activity (Deul *et al.*, 1978); cuticular lipids (Salama *et al.*, 1976) and microsomal oxidase (Yu and Terriere, 1977).

However, the histopathological and/or ultrastructural changes in some insect species had been investigated by some CSIs such as Dimilin against *Locusta migratoria* (Clarke *et al.*, 1977), *Pectinophora gossypiella* (Saad *et al.*, 1985), *Chironomus decorus* (Pelsu, 1985), *Musca domestica* (Bakr, 1986), *Culex pipiens* (Bakr *et al.*, 1997) and *Spodoptera exigua* (Younes *et al.*, 2000); triflumuron against *C. decorus* and *Tanypus grodhaus* (Pelsu, 1985) and *Tribolium castaneum* (Parween, 1997); buprofezin against *Trialeurodes vaporariorum* (De Cock and Degheele, 1991).

IGRs represent the newest of all approaches to operational and commercial insect control. Their species or stage-specificities that were higher than those of conventional insecticides offer a good alternative for a selective insect pest control that is in harmony with existing IPM programs. IGRs generally have a good margin of safety for most non-target biota

including invertebrates, fish, birds, and other wildlife. They are relatively safe for human beings and domestic animals (Siddall, 1976).

Locust flight muscles are the most active known muscles which are suited for sustaining the prolonged muscular activity of locust

The present work is an attempt to evaluate the toxicity of IGR (Lufenuron) against fifth nymphal instar of *S. gregaria* as well as to study the fine structure of flight muscles after treatment with LC₅₀ of the tested compound. In addition, the study aimed to minimize the pollution effects of the traditional insecticides on the environment by the development and synthesis of bio-insecticide.

MATERIALS AND METHODS

Toxicology Evaluation:

The stock colony of *Schistocerca gregaria* was provided from the Locust Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza. The insects were reared and handled under the following technique described by Abbassi *et al.* (2003).

Leaves of Castor plant (*Ricinus communis*) were daily placed as feeding material. The cages were incubated in a constant room temperature (32±2°C) and (30-50% RH).

The experimental nymphs were segregated from the gregarious stock colony at the beginning of the first nymphal instar and held up in cages (30x30x30 cm) in diameter. The cages were a wooden framed equipped with zinc bottom covered by thin layer of sand, glass covered sides and a wire-gauze top provided with a little door. Unconsumed food, dead locusts and feces were removed daily.

The whole cage was thoroughly washed and effectively sterilized with

an antiseptic agent (every 4-6 weeks) or whenever it becomes empty or at the end of any experiment.

One of the insect growth regulators (IGRs) Lufenuron (EC) 10% was used.

Treatment of Experimental Insects:

Both sexes of nymphs of one-day old of the 5th nymphal instars of *S. gregaria* during synthesis and deposition of the newly adult cuticle (Taha and El-Gammal 1990) were treated by feeding technique with one of insect growth regulators, the Lufenuron as follows: leaves of *Ricinus communis* were dipped in 50, 75 and 100 ppm of the Lufenuron for two minutes, then leaves were air dried before being offered to the nymphs for feeding on it. Three replicates of 20 nymphs were subjected to each of the treated leaves. After feeding for 24 hours on the treated leaves, alive nymphs were transferred onto untreated leaves and left to feed for additional 24 hours, after that mortality counts or malformed individuals were recorded.

Histopathological Studies:

Adult females of *Schistocerca gregaria* were prepared for electron microscopy. The insects were killed by twisting of the head to break the neck membrane. The posterior tip of the abdomen was cut off and the head, with the gut attached, was removed. The carcass was cut open ventrally and the fat body overlying the flight muscles was removed with tissue paper.

Flight muscles were dissected in ice-cold (0-5 °C) Karnovsky fixative, pH 7.3 (Karnovsky, 1965). The tissues were transferred to fresh ice-cold fixative for 1h. After washing for 30 min in 0.1M sodium cacodylate buffer, pH 7.3, the tissues were post-fixed for further 1h in 1% osmium tetroxide in 0.1M sodium cacodylate buffer, pH 7.3 at 4 °C (Brissanet *et al.*, 1996).

The muscles were dehydrated at room temperature via a graded series of ethanol solutions to propylene oxide prior to embedding in Araldite epoxy resin. Semithin sections were 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 Joel 1010 transmission electron microscope (Reynolds, 1963) at the central laboratory, Faculty of Science, Ain Shams University.

RESULTS

Toxicity Evaluation:

Results in Table (1) and graphically illustrated in Figure (1) show the effects of Lufenuron on the one day old 5th nymphal instar of *S. gregaria* during feeding technique.

Data cleared that the percentages of nymphal mortality of the 5th nymphal instars of *S. gregaria* were 0.0, 12.33 and 21.01% after one day treatment with 50, 75 and 100 ppm of Lufenuron, respectively compared to control (0.0%), whereas, the percentages of adult malformed were 89.99, 73.34 and 72.33%, respectively compared to control (0.0%). While the percentage of adult emergency was 10.0, 0.0 and 0.0% for the three concentrations, respectively. On the other hand, the percentage of total inhibition adult emergence was 90, 100 and 100% for the Lufenuron concentrations compared to control (0.0%).

Statistical analysis in Table (1) shows highly significant differences among Lufenuron concentrations compared to control in % nymphal mortality, and percent malformed adults after one day old of the 5th nymphs of *S. gregaria* treatments ($F =$

370.74, and 127.01 & LSD = 0.96, and 0.43), respectively.

Table (1): Biological activities of Lufenuron against 1 day old of the 5th nymphal instar of *schistocerca gregaria* using feeding technique.

Conc. (ppm)	% Nymphal mortality Mean \pm SE	% Malformed adult Mean \pm SE	% Adult emergence Mean \pm SE	%Total inhibition of adult emergence Mean \pm SE
Control	0.0 \pm 0.0	0.0 \pm 0.0	100 \pm 0.0	0 \pm 0.0
50	0.0 \pm 0.0	89.99 \pm 0.0	10.0 \pm 0.0	90 \pm 0.0
75	12.33 \pm 0.33	73.34 \pm 0.30	0.0 \pm 0.0	100 \pm 0.0
100	21.01 \pm 1.0	72.33 \pm 0.33	0.0 \pm 0.0	100 \pm 0.0
F 0.05	370.74***	127.01***	-	-
LSD	0.96	0.43	-	-

F: Measurement of distance between individual distributions

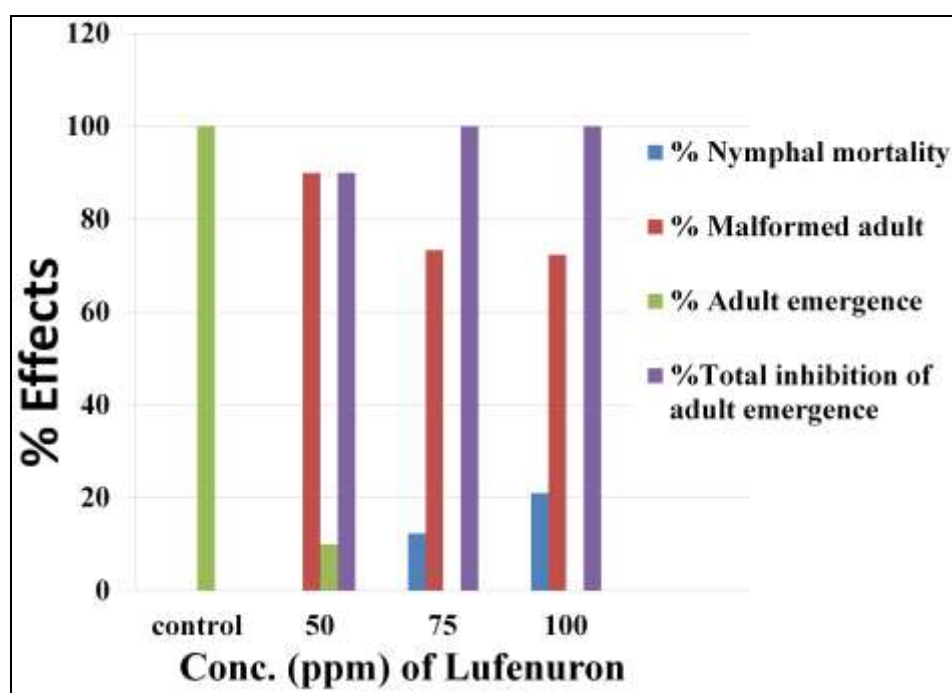


Fig. (1): Effect of Lufenuron on some biological aspects of the desert locust (*S. gregaria*) nymphs treated as 1-day old of the 5th nymphal instar.

Ultrastructure Study of Lufenuron on *S. gregaria*:

Histopathological effects induced by different concentrations of Lufenuron were studied in adult muscles treated as 5th nymphal instar

of *S. gregaria*. These effects were illustrated in figures (2-6) and explained as follows:

Muscles of Untreated Adult Stage:

Ultrastructure of muscles of untreated adult *S. gregaria* was shown

in Figures (2 & 3). The contractile fibrils that filled the cytoplasm of each large fiber demonstrate their patterned organization.

The muscle fibers having a radial arrangement of strap-like myofibrils separated from each other by mitochondria and with peripheral nuclei. Myofibrils were surrounded by an extensive sarcoplasmic reticulum which formed dyad connections.

The fine structure of myofibrils revealed the presence of at least two kinds of filaments in the fibrils. The fibrils are clearly constructed of filaments and the distribution of those filaments is related to the alternating light and dark bands.

From the several bands in the striation pattern, the Z-line is commonly selected as marking the limits of the sarcomere. This line is comparatively denser, especially in contracted fibrils, and may be correctly regarded as a kind of septum that is continuous transversely across the fibril. Other bands are: isotropic band

I, is bisected by the Z-line and anisotropic, A, is the more dense and is bisected by the narrow light band (H band). The ultrastructure of muscle fibers from mature locust was clearly showed myofibrils which had regular appearance and were, at the level of the A-band, clearly defined by surrounding sarcoplasmic reticulum. Mitochondria appear as spherical or oval shaped organelle. Each mitochondrion was delimited by an outer membrane and inner membrane. The inner membrane was folded to form cisternae in various directions.

Muscles of Adult Treated with Lufenuron:

Treatment of adults muscles in desert locust with Lufenuron showed distribution and disintegration of the fibers of these muscles in electron micrograph of longitudinal section, were the bands and zones less defined. I bands became like grid interspersed other components of fibers. Whereas mitochondria that are distinct (Fig 4, 5 & 6).

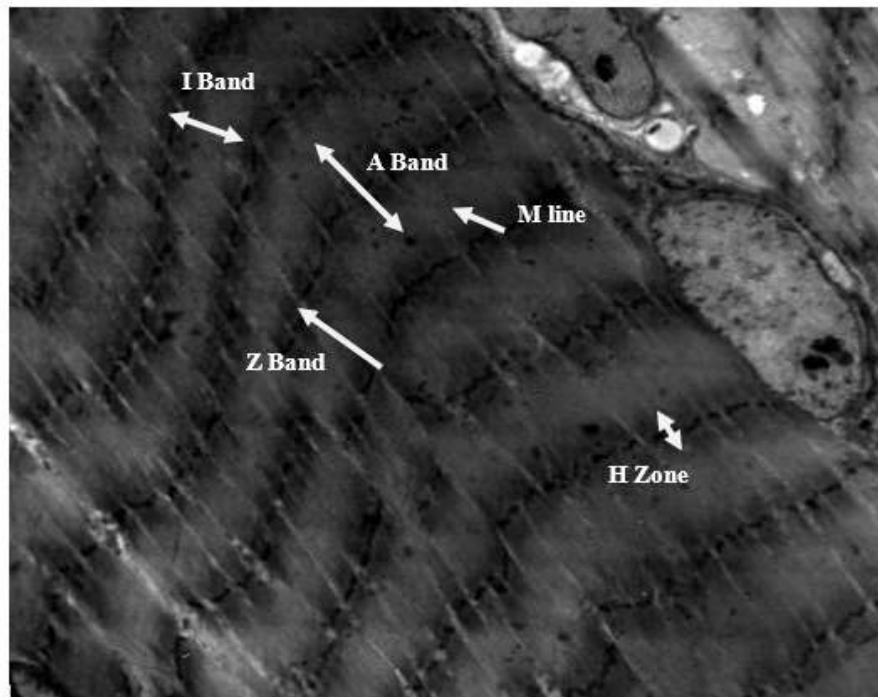


Fig. 2: Electron micrograph of longitudinal section through myofibrils of untreated adult *Schistocerca gregaria* showing fine structure of muscle

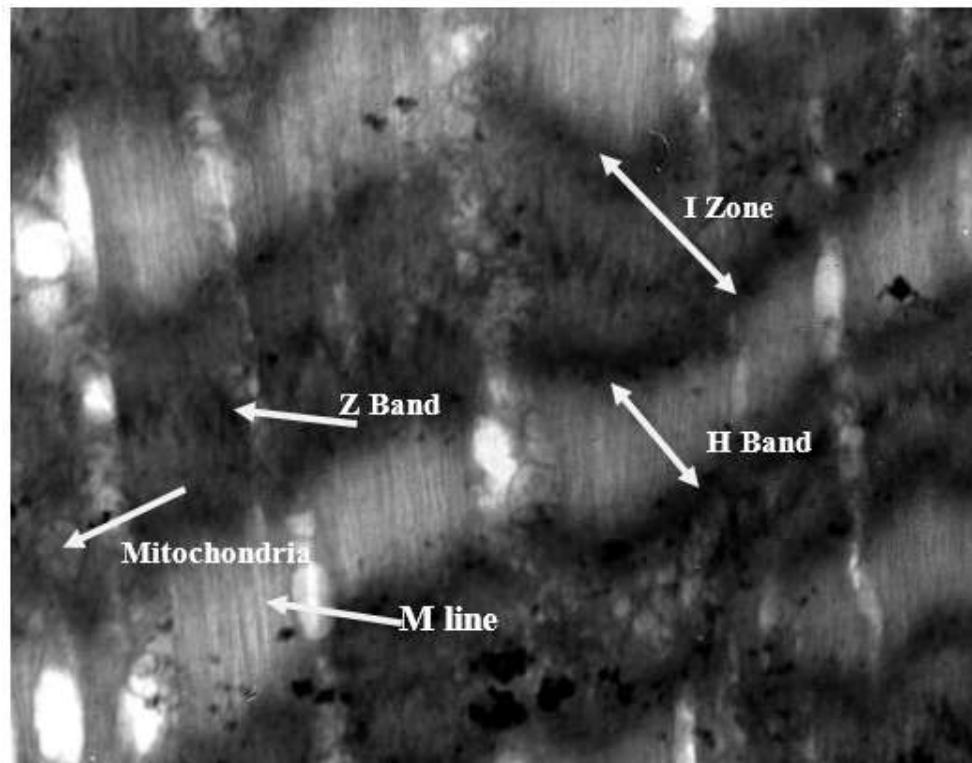


Fig. 3: Electron micrograph of longitudinal section through myofibrils of untreated adult *Schistocerca gregaria* showing M-line (Z), I, and H -bands of sarcomere and mitochondria

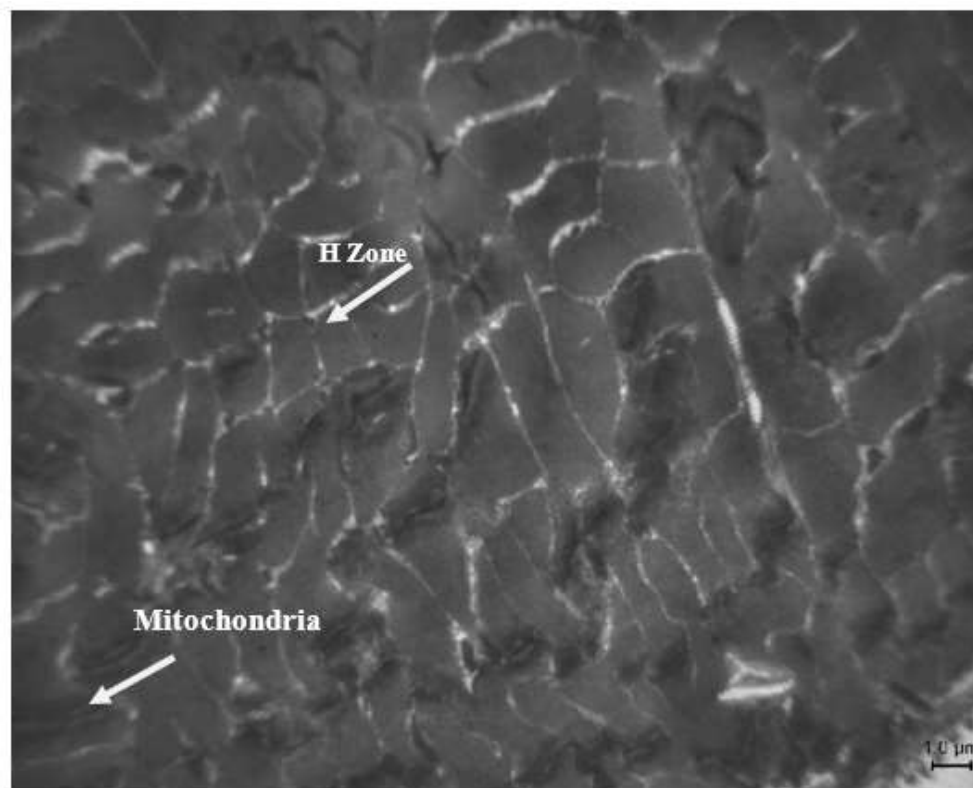


Fig. 4: Electron micrograph of longitudinal section through myofibrils of adult *Schistocerca gregaria* treated with Lufenuron showing less clear (Z), I, and H -bands of sarcomere and mitochondria. X= 4000.bmp

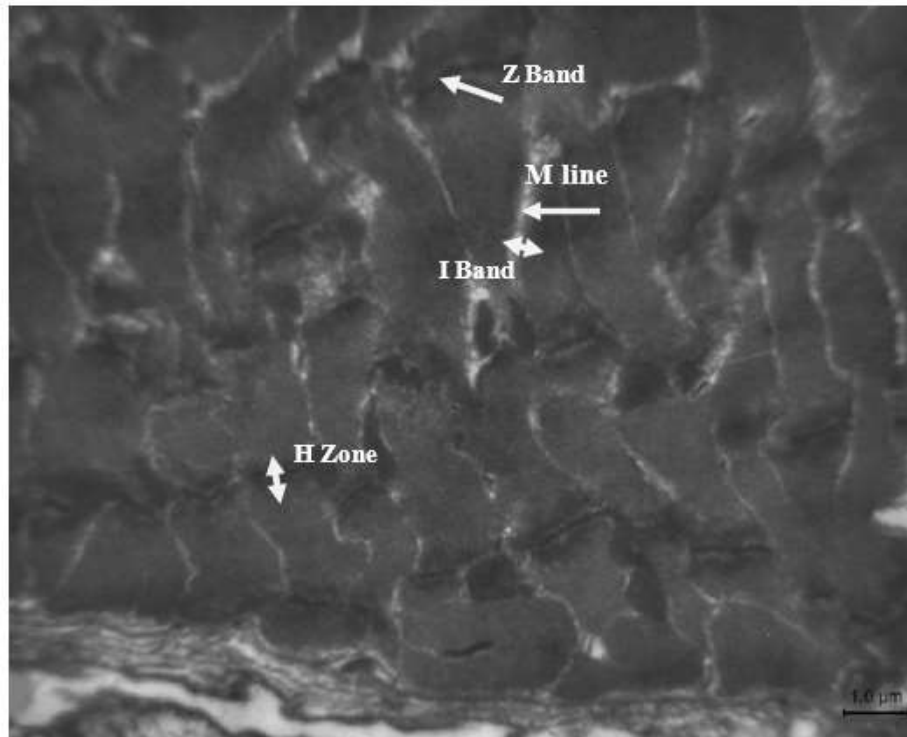


Fig. 5: Electron micrograph of longitudinal section through myofibrils of adult *Schistocerca gregaria* treated with Lufenuron showing M line (Z), I, and H - bands of sarcomere. X= 8000.bmp

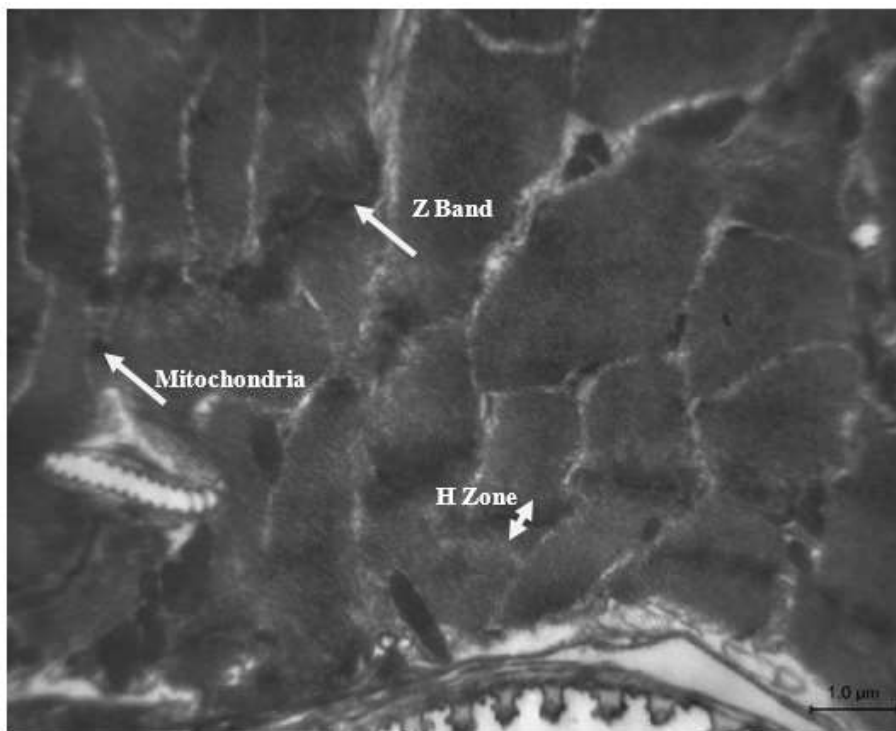


Fig. 6: Electron micrograph of longitudinal section through myofibrils of adult *Schistocerca gregaria* treated with Lufenuron showing (Z), I, and H -bands of sarcomere and mitochondria. X= 10000.bmp

DISCUSSION

Toxicological Studies:

The present investigation revealed that, the treatment with insect growth regulators has toxic effects on the desert locust, *Schistocerca gregaria*. These effects depended on the concentrations of the compound and the age of the treated insects. In the present study, Lufenuron acted as chitin synthesis inhibitor (CSI), was used against the 5th nymphal instar of *S. gregaria* during one day by feeding technique. The present study showed that, the treatment with different concentrations against one day old of the 5th nymphal instar of *S. gregaria* caused nymphal mortality and failure to ecdysis to adult increased with the increase of Match concentrations. Also, the percentage of total inhibition of adult emergence reached to 100%. More or less, the present results are agreed with those finding by several chitin synthesis inhibitors against the same acridide species, *S. gregaria* such as Diflubenzuron, which interfered with the chitin synthesis during the nymphal ecdysis to the last instar causing some mortalities (Taha and El-Gammal, 1985), also Diflubenzuron when injected to the 5th nymphs of *S. gregaria* was observed that, some treated instars were unable to moult and died without completing the moulting process, some were able to split the old cuticle but unable to wriggle out of the exuvia, some were able to complete moulting process but the resulting adults were deformed to varying degrees and some were able to moult without deformity in the resulting adults (Roa and Mehrotra, 1986), Pyriproxyfen when injection to last-nymphal instar of *Locusta migratoria* induced malformations of the wings and green pigmentations (Kort *et al.* 1991), the greatest mortality was recorded during ecdysis

of early the 4th nymphal instar to the 5th nymphal instar of *S. gregaria* when treated with Chlorfluazuron (Abo El-Ela *et al.*,1993), also Chlorfluazuron induced appreciable failure in ecdysis to adult stage when applied on the last nymphal instar (El-Gammal *et al.*, 1993), Coppen and Jepson 1996a) when treated the 2nd nymphal instars of *S.gregaria* with Diflubenzuron, hexaflumuron and Teflubenzuron, they recorded mortality after all other treatments. Triflumuron caused different mortalities after 5 to 15 days of the barrier application in Mauritania (Wilps and Diop, 1997). Diflubenzuron and Teflubenzuron caused abortive moult, and most survivors developed twisted wings (Wakgari 1997), also, Lufenuron exhibited an inhibitory effect on the adult emergence after treatment of last instar nymphs , regardless of the timing of treatment (Bakr, R.F. *et al.* 2008). When treated the newly moulted last 5th instar nymphs of the desert locust *Schistocerca gregaria* with pyriproxyfen (juvenoid), tebofenozone (ecdysone agonist) and lufenuron (chitin synthesis inhibitor) an inhibitory action on haemolymph proteins was generally exhibited by all these IGRs along the nymphal stage with an exception of the day after treatment (1-day old nymphs) (Ghoneim, et al 2012).

Histopathological Studies:

The current study showed that the toxic effects of growth regulators when treated of *Schistocerca gregaria* by Lufenuron on tissues. The Lufenuron caused malfunction and decomposition of the components in the muscles of the desert locust. More or less, the present study are agreed with those finding by several insect growth regulators against same species, *Schistocerca gregaria* such as delta-philanthotoxin (delta-PTX) of the

venom from the wasp *Philanthus triangulum* blocks transmission postsynaptically at excitatory synapses on locust muscle. delta-PTX depresses both the iontophoretic glutamate potential and the excitatory junctional current (e.j.c.) in a glutamate receptor activation-dependent manner (Clark, *et al* 1982), also exposed newly moulted 4th nymphal instars of *Schistocerca gregaria* in the laboratory to precocene II at 20 µg/cm² or treating them topically with Cycloheximide at 10 µg/locust and these reduced the respiratory rate of the flight muscles (Salem, *et al* 1982a), the effect of juvenile hormone (JH) on the process of tracheolization of the dorsolongitudinal flight muscles of the migratory locust is studied during larval flight muscle development and used two treatments of precocene (20 .mg/cm²) by contact method and cycloheximide (10 .mg/insect) by topical application on the newly moulted fourth instar nymphs that depressed the respiratory rate of flight muscles (Abdel Hamid M, 1982), GABA and the trans isomer of 4-aminocrotonic acid were equally potent at inducing increases in Cl⁻ conductance when applied to distal extensor tibia muscle fibres of the locust (*Schistocerca gregaria*) (Scott, and Duce; 1985). No Fabp, however, was found in last instar nymphs, methoprene induced supernumerary nymphs and azadirachtin induced over-aged nymphs (Chen, *et al* 1993), (Mutwally, 1993) reported that tyramine alone was un effective on the foregut muscle, suggesting a lack of either pre- or postsynaptic receptors responding exclusively to this monoamine, The mitochondrial portion of the tissue enlarged from 17 to 30%. Similar growth of all components was observed in precocious adults, obtained after treatment of 3rd-instar nymphs

with ethoxy-precocene (Wang, Z; *et al*1993), (Blau, and Wegener; 1994) injected the biogenic amine octopamine into the haemolymph of 20-days old male locusts, *Locusta migratoria*, and the content of fructose 2,6-bisphosphate, a potent activator of glycolysis, was measured in the flight muscle after various time. the effect of the endogenous FMRFamide-like neuropeptide SchistoFLRFamide on the heart and skeletal muscle of *Schistocerca gregaria* (Robb, Sandra; *et al*1994), the effects of philanthotoxin-343 (PhTX-343; tyrosyl-butanoyl-spermine) and photolabile analogues of this synthetic toxin on locust (*Schistocerca gregaria*) skeletal muscle (Sudan, *et al* 1995), the effects of ryanodine, 9, 21-didehydroryanodine and 9, 21-didehydroryanodol on channels of skeletal muscles that are K⁺ channel; a maxi, Ca²⁺-activated 170 pS channel (BK channel) and an inward rectifier of 35 pS conductance (IK channel) (Vais, *et al* 1996). The juvenile hormone (JH) was assessed by comparing muscle properties in immature and mature females and showed that JH production was inhibited by allatectomy early in adult life (Rose, (2004), the inhibitory innervation of the intersegmental (body wall) muscles between the first and the second thoracic segment of the migratory locust, *Locusta migratoria*, which investigated using neuroanatomical, immunocytochemical and electrophysiological techniques (Braunig, P; *et al* 2006), Ttebufenozide treatments affected the ultrastructural configuration of thoracic muscles (Ghoneim; *et al* 2018), Flufenoxuron caused several dangerous effects on the thoracic muscles such as distortion shape of the Z line and disorganization of A, I and H bands (Bakr, R.F; *et al* (2008), showed that the effects of a methanolic extract of the

plant *Haplophyllum tuberculatum* (ME-Ht) and of Teflubenzuron (TFB) which compared on several reproductive variables and ecdysteroid titers in the females of *Locusta migratoria*.

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