Physiological and Cytological Effects of Abamectin in the Desert Locust, *Schistocerca gregaria* Forsk. (Orthoptera, Acridiidae)

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ABSTRACT

Application of abamectin as a bioinsecticide to newly molted 5th instar nymphs of *Schistocera gregaria* Forsk, almost caused different levels of reductions in Na^+ and K^+ ion concentrations, resulted in imbalance in Na/K ratio. It caused diversified effects on PO^-_4 ion, treatments at lower (LC_{10} and LC_{30}) and higher (LC_{70}) levels, significantly increased and decreased its concentration, respectively. A significant increase in the acetylcholinesterase (AChE) activity was recorded at the LC_{30} level. Ultrastructural studies revealed a marked atrophy or destruction of the corpus allatum (CA). Disintegrating nuclei, degenerating mitochondria, increase of lysosomes, reduction in cytoplasm, and absences of cell membrane were observed on treatment with lower concentrations (LC_{10} and LC_{30}). The degenerative changes were much more pronounced at a higher concentration of abamectin (LC_{70}).

Key words: Corpus allatum, Abamectin, Ultrastructure, haemolymph, *Schistocerca gregaria*.

INTRODUCTION

Abamectin, belongs to a new class of bio-insecticides, classified by the U.S. Environment Protection Agency (EPA) as class IV toxicity, as insecticidal/acaricidal compounds derived as metabolite from a natural fermentation of the soil actinomycete *Streptomyces avermitilis* (Burg *et al.*, 1979). Physiologically, abamectin blocks post-synaptic potentials of neuromuscular junctions (Kass *et al.*, 1980) leading to paralysis (Turner and Schaeffer, 1989). In addition to its toxic activity, abametin has been shown to inhibit feeding (Pienkowski and Mehring, 1983) and may exhibit growth-regulating activity (Wright, 1984). Abamectin has been shown to be effective against a broad spectrum of arthropod pests (Lasota and Dybas, 1991). Abamectin is rapidly degraded in the environment through photo-degradation and absorption to semi-dents. Also, it is rapidly degraded in water and plants don't absorb it from the soil, and could be used safely in the pest management programs.

Desert locust, *Schistocerca gregaria* Forsk., is a major pest in most regions of the world. A dedicated search is being made for new and non-disruptive control agents effective against this pest.

To obtain more information on the mode of action of abamectin, the oral toxicity of this compound on *S. gregaria* 5th nymphal instar was evaluated. Also, the activity of the target enzyme of neurotoxic insecticides, acetylcholinesterase (AChE), and changes in the concentration of haemolymph ions were studied. It is particularly important in this time to establish the underlying cellular changes in the corpora allatum (CA) of *S. gregaria*.

MATERIALS AND METHODS

Insect:

Schistocera gregaria 5th nymphal instar, used for the present investigation was selected from a standard stock culture maintained in the Entomology Department, Faculty of Science, Cairo University, Egypt.

Toxicity tests:

Abamectin was applied at different concentrations; 20, 50, 75, 100, 150, 200 ppm; and given to the tested insects after soaking the plant leaves of clover in each abamectin concentration and drying at room temperature. Control locusts were provided with untreated food. Three replicates each containing 10 newly molted nymphs were used per each treatment and control as well. Mortality was assessed at 72h post-treatment and corrected (Abbott, 1925). Bioassay of data were subjected to probit analysis (Finney, 1971) and estimated lethal concentrations were determined (SAS, 1990). LC_{10}, LC_{30}, and LC_{70} were calculated for further studies.

Haemolymph preparation:

Haemolymph of normal *S. gregaria* 5th instar nymphs and survivors from abamectin treatments at the LC_{10}, LC_{30}, and LC_{70} levels were collected after 72 h of treatment. Insects were pre-cooled on ice for 2 min to slow down coagulation of heamolymph. Haemolymph was collected in a small test tube coated with ice to prevent melanization. The blood cells were removed by centrifugation of the haemolymph at 3000 rpm for 10 min. The resulting supernatant was aliquoted and used for the haemolymph AChE preparation and for determination of haemolymph ions concentrations.

Enzyme assay:

Three concentrations corresponding to LC_{10}, LC_{30}, and LC_{70} were evaluated on enzyme activity in the haemolymph of 5th nympal instar of *S. gregaria*. The AChE activity was carried out following the method of Ellman *et al.* (1961) using acetylcholine as a substrate. Measurements were conducted at a wavelength of 412 nm with a run time of 20 min. Total protein content was determined according to Bradford (1976) using bovine serum albumin (sigma) as a standard. Enzyme activity was expressed as µM/min/mg protein.

Haemolymph ions determination:

Sodium (Na^+) and potassium (K^+) were made by flame photometer as described by Amin and EL-Halafawy (2002). Inorganic phosphate (PO^-_4) was determined as described by Rockstein and Herron (1951). According to Düchateau *et al.* (1953) Na^+/K^+ ratio was determined.

Ultrastructure studies:

Corpora allata (CA) of 5th nymphal instar previously treated with LC_{10}, LC_{30}, and LC_{70} abamectin were fixed immediately in cacodylate-buffer glutraldehyde (pH 7.4) for 3 h at 25 °C. The fixative was removed and CA washed by cacodylate-buffer solution (pH 7.4) for 30 min. CA were then post-fixed in 1% osmium tetraoxide at pH 7.4 for 1 h at 25°C. CA were washed, and dehydrated by passing through increasing concentrations of ethanol (30, 50 and 70 % for 5 min each; twice in 90 % for 10 min. and thrice in 100 % for one min.). Propylene oxide was then substituted for alcohol with two changes (2 min each). Embedding in epon resin (1:1) for 24 h. Resin blocks were adequately dried in an oven at 60°C for 24 h. The blocks were sectioned using an ultra-microtome. Semi-thin sections, 900-1000 nm thick, were obtained for preliminary investigations by light microscopy and for proper orientation. Ultra-thin sections (70 nm) were then prepared, mounted on copper grids and double stained with lead citrate for 15 min and with 10% methanol uranyl acetate for 10 min (Reynolds, 1963). These sections were examined by Philips CM 100 electron microscopy at 70 KV. Corpora allata from untreated 5th nymphal instar were similarly processed and used as a check.

Statistics:

Results are expressed as means ± standard deviation (SD). The significance between control and treated series was estimated using Duncan's multiple range test (Duncan, 1955) at 1% level. Data from enzyme activity and haemolymph ions concentrations were subjected to one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Bioassay:

Abamectin exhibited insecticidal activity against 5th nymphal instar of *S. gregaria*. The earlier symptoms of abamectin poisoning were erratic and disoriented movements of locusts followed by tremors and paralysis. Percentage of corrected mortality of *S. gregaria* was determined as a function of abamectin concentration. LC_{10}, LC_{30}, and LC_{70} were 50, 89, and 149 ppm, respectively. The toxic effects of abamectin increased with the increase in the concentration of the bio-insecticide.

Heamolymph ions determination:

The plasma of the normal *S. gregaria* 5th nymphal instar composed of 85± 3.6, 10.1± 0.65, and 168± 11.5 mg/ml of Na^+, K^+ and PO_4^{--}, respectively. These values were significantly different at p= 0.01 (Table 1). Obtained data showed that sodium ion concentration was significantly decreased in a dose-dependant manner by increasing abamectin concentration. While potassium ion concentration increased significantly with LC_{10} abamectin dose as compared to the control, then decreased significantly with further increase in abamectin concentration (Table 1). Phosphate ions showed significant increase, only, at LC_{10} and LC_{30} levels reaching a maximum concentration on using abamectin at the LC_{10}. While, higher abamectin (LC_{70}) caused considerable reduction in its concentration (Table 1). There was no significant difference in the phosphate ion concentration, when *S. gregaria* 5th nymphal instar was treated with abamectin at the LC_{10} and LC_{30} levels.

Table (1): Effect of ingested abamectin administrated to newly molted 5th nymphal instar of *S. gregaria* on the heamolymph ion concentrations and acetylcholinesterase activity in surviving insects as a function of the dose.

Abamectin Conc.	Na^+ (µg/ml)	K^+ (µg/ml)	Na/K ratio	PO_4^{--} (µg/ml)	AChE (µg/min/mg protein)
Control (0 ppm)	85 [a] ±3.6	10.1 [a] ± 0.65	8.4	168 [a] + 11.5	3.18 [a] ± 0.16
LC_{10} (50 ppm)	49.5 [b] ± 2.3	12.9 [b] ± 0.31	3.8	245 [b] ± 25.23	3.28 [a] ± 0.14
LC_{30} (89 ppm)	29 [c] ± 1.15	7.63 ± 0.62 [c]	3.8	220 [b] ± 19.5	4.68 [b] ± 0.21
LC_{70} (149 ppm)	16.8 [d] ± 1.3	2.43 [d] ± 0.32	6.98	39.8 [c] ± 4.03	3.91 [c] ± 0.19

Means in column followed by different letter are significantly different at P<0.01, Duncan's multiple range test.

Enzyme activity:

Results of the AChE activity determination are shown in table (1). In control, the mean AChE activity was 3.18 ± 0.163 µg/min/mg protein. Abamectin showed insignificant increase at the LC_{10} (3.28 ± 0.141 µg/min/mg protein, $P < 0.01$). While significant increases in the mean values of activity of AChE, as compared to the control, were recorded at LC_{30} and LC_{70} concentration levels (4.68 ± 0.21 and 3.91 ± 0.193 µg/min /mg protein, respectively). It is worth mentioning that AChE activity reached its optimum activity at LC_{30}. Further increase in abamectin concentration (LC_{70}) decreased the AChE activity, but it was significantly higher than the control.

Effect of Abamectin on CA ultrastructure:

Electron microscopic studies have contributed to characterize the structure of normal and abamectin-treated corpora allata (CA) in the 5th nymphal instar of *S. gregaria.*

Normal glandular cells have extremely complex forms (Fig. 1). The allata cells contain well developed mitochondrial system and endoplasmic reticulum which are involved in the biosynthesis of juvenile hormone (JH). Also, well developed nuclei with large and compact nucleoli, Golgi complex, primary lysosomes, vacuoles, and residual dense bodies were observed in the normal CA cells of *S. gregaria* 5th nymphal instar. The CA was surrounded by a basal lamina (Fig 1a) that prevents the intrusion of haemocytes and holds the gland together.

Treatments with different concentrations of abamectin, LC_{10}, LC_{30} and LC_{70}, resulted in varying degrees of deterioration of CA ultra-structure.

Treatment with the sub-lethal dose (LC_{10}) led to extensive damage to CA cyto-architecture. Figure (2 a, b and c) show that nuclei appeared abnormal, but only remnants of other organelles remained. Membranous organelles appeared degenerated with aggregated membranes, forming irregular masses (Fig 2b). Eventually nuclei disintegrate, lose their shape and much of their interchromatin substances clump together. Plasma membranes deteriorated leaving no boundaries between cells and cytoplasm became more narrow than that reported in the normal CA cells (Fig. 2 a and c). The extent of degradation was varied in different cells.

Conspicuous degradative changes were evident in the CA treated with LC_{30} abamectin (Fig. 3 a, b, c and d). These include disintegrated nuclei; secondary lysosome, large vacuoles, and the intercellular spaces became very large and sometimes contain a highly dense homogenous material. In addition, a number of nuclei showed disrupted or disintegrated nuclear envelope and condensed nucleolei with dispersion of chromatic clumps. There was abundant of lipid droplets. However, lipid was never seen in the CA of normal *S. gregaria.*

At the highest tested concentration of abamectin (LC_{70}), the most pertinent feature of cells was the polymorphism of mitochondria, as well as the basic types, disc-shaped, cup-shaped, looped and filamentous mitochondria were frequent (Fig. 4). Several mitochondria may be seen stacked one inside the other (Fig. 4 a). Endoplasmic reticulum was broken into small pieces and autophagic vacuoles were filled with glycogen (Fig. 4 b). Abamectin treatment of CA was accompanied by obvious disorganization of endoplasmic reticulum (Fig 4 b and c). In the majority of these CA, few structures remained within the cells.

The data showed that damage in the 5th nymphal instar CA of *S. gregaria* was correlated with abamectin concentration. In other words, glands with the greatest ultrastructure damage were those treated with the highest abamectin dose (LC_{70}). The most important variations between normal and abamectin-treated CA concern the endoplasmic reticulum and mitochondrial system. The cells of the treated CA showed an increase of lytic structures. The distended intercellular spaces were invaded by phagocytic cells (Figs. 2 a and 3 c). None of the treated CA cells were similar in cytoarchitecture to those of control.

Similar results were reported by Ostlind *et al.* (1979) who studied the toxic effect of avermectins against *Triboluim confusum* and *Cuterebra* spp. Also, Wright (1984) reported that the inhibition of pupal-adult development of *Anthonomus grandis* was depended on the concentration of avermectin B_1. Similarly, Reed *et al.* (1985) reported that survival of 7-day-old codling moth larvae treated topically with avermectin B_1, was reduced by increasing dosages. Also, Hamouda and Dahi (2008) reported similar results in spinetoram-treated 4th instar larvae of *Spodoptera littoralis* (Boisd.). Dahi *et al.* (2009) reported that avermectin toxicity against *S. littoralis* larvae was increased by increasing avermectin concentration.

Abamectin exhibited a neurotoxic action in *S. gregaria.* Symptoms of abamectin poisoning were consistent with those previously described by Wright (1984) who treated boll weevil with avermectin B_1. The action of abamectin was similar to those induced by spinosad, a natural derived insecticidal

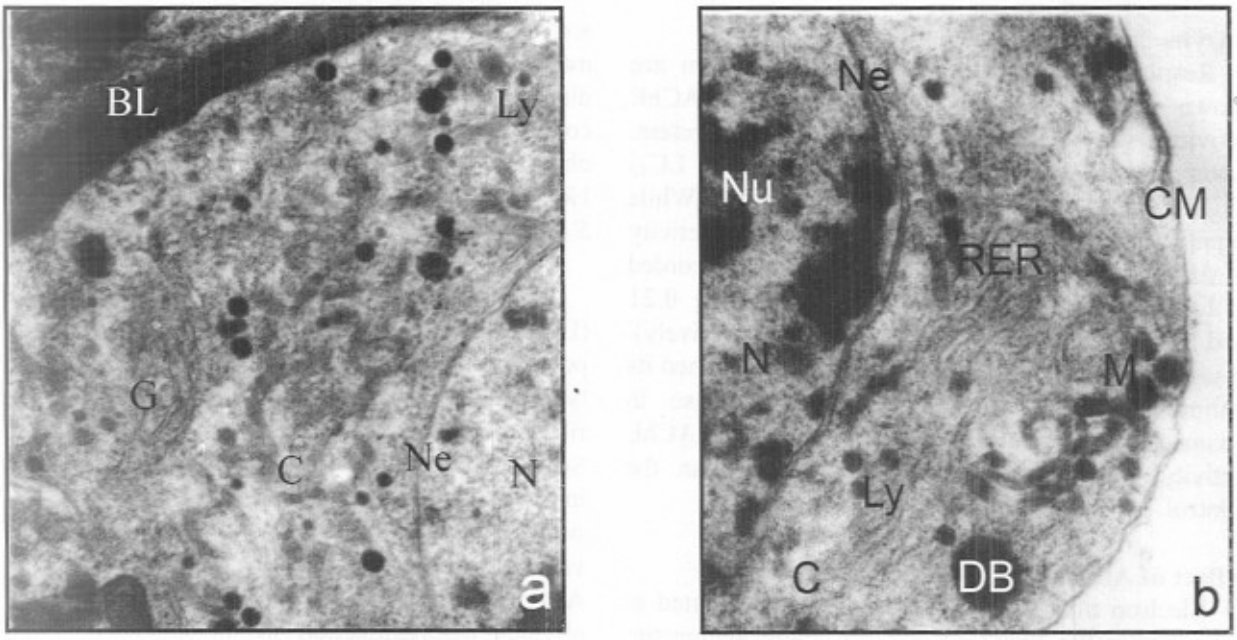


Fig. (1): Normal corpus allatum cells from 5th nymphal instar *Schistocera gregaria* showing well developed nuclei (N) with normal nucleoli (Nu) and double-layer nuclear envelop (Ne), Golgi complex (G), primary lysosomes (ly), vacuoles (V), rough endoplasmic reticulum (RER), and residual dense bodies(DB), Note the CA was surrounded by a basal lamina (BL). CM: cell membrane, C: cytoplasm. a) X=10000, b) X=15000.

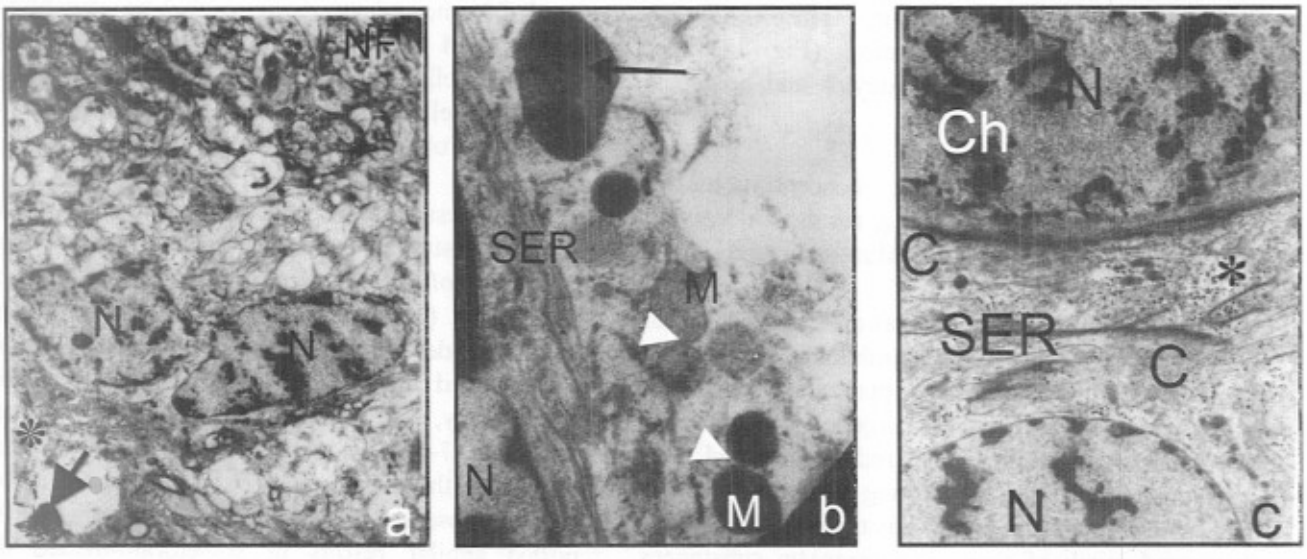
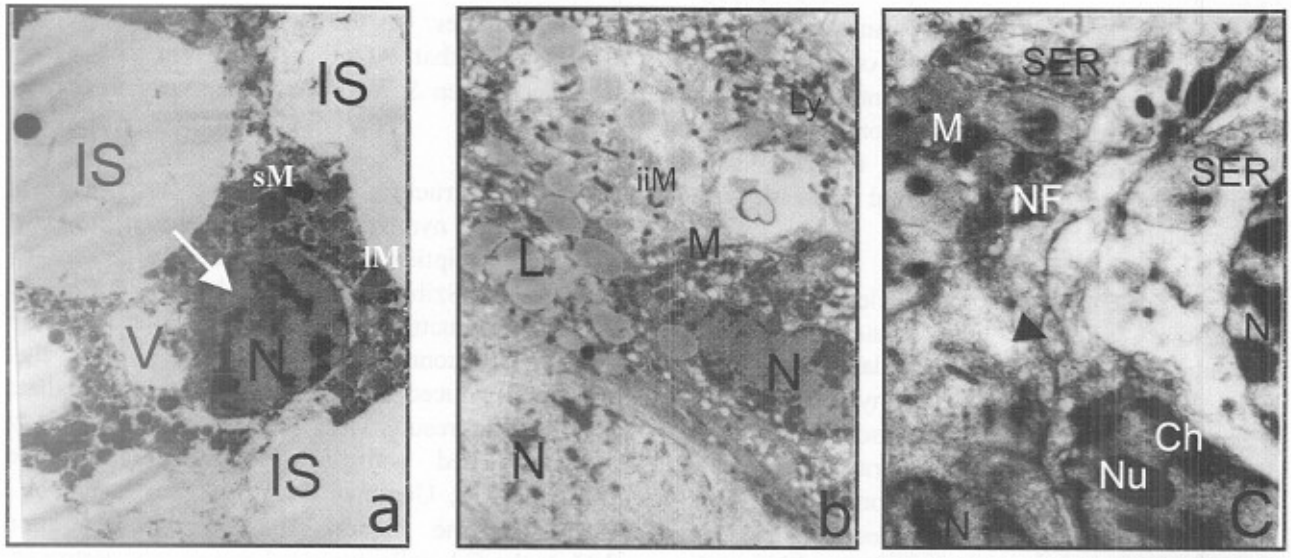


Fig. (2): Corpus allatum cells from nymphs treated with abamectin (LC_{10}). a) Two CA cells showing cytoplasm filled with scattered granules (*), degenerating nuclei (N) with wrinkling of nuclear membrane. Note discharge of granules (arrow), and absence of cell membrane. X=3000. b) A portion of CA cell showing aggregation of mitochondria (M) at a point (arrowheads) of fusion, smooth endoplasmic reticulum (SER). At point of arrow autophagic vacuole appears to be formed from endoplasmic reticulum and mitochondria. X=10000. c) Two CA cells showing absence of cell membrane and cytoplasm becomes very narrow. Smooth endoplasmic reticulum (SER) begins to degenerate. Nuclei (N) with clumps in chromatin (ch). X=6000. NF: nerve fibre, T: trachea.



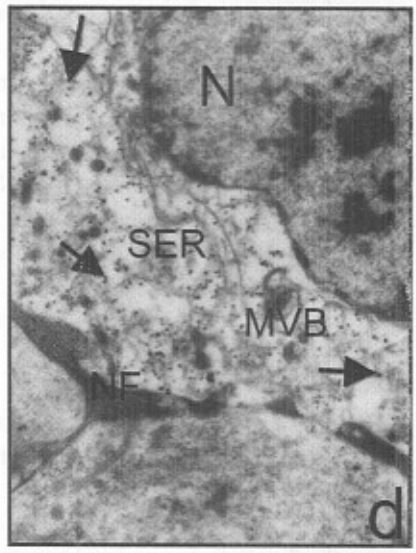


Fig. (3): Corpus allatum cells from nymphs treated with abamectin (LC_{30}). **a)** A part of CA showing degenerating nucleus (N) with disrupted nuclear envelope (white arrow), large vacuole (V) and intercellular spaces (IS) are very large. X=3000. **b)** Two CA cells showing absence of cell membrane, degenerated nucleus (N). Note presence of lipid droplets (L). X=4000. **c)** Two CA cells showing degenerating smooth endoplasmic reticulum (SER), clumps of chromatins (ch). Note cell junction (arrowhead) between two cells filled with secretary granules. X=7500.**d)** A part of CA showing formation of multivesicular bodies (MVB) and long profiles of endoplasmic reticulum (SER) with only few attached ribosomes. X=7500. Note the mitochondria (M) with different profiles (sM, swallen; lM, looped), Nu: nucleolus, ch: chromatin materials, NF: nerve fiber, Ly: lysosome.

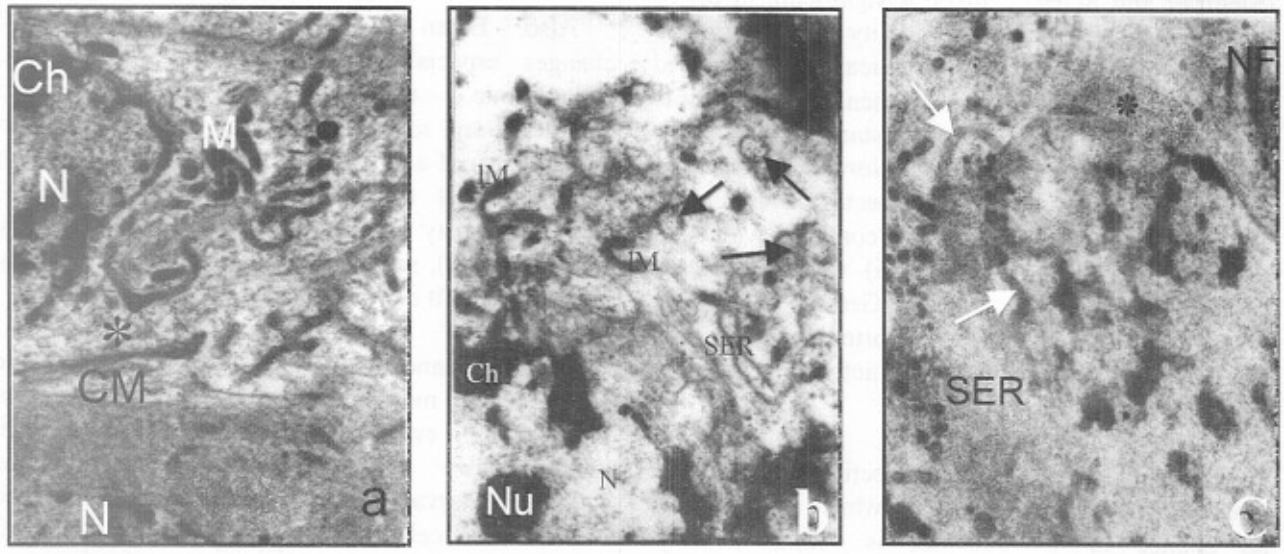


Fig. (4): Corpus allatum cells from nymphs treated with the lethal dose of abamectin (LC_{70}). **a)** Two CA cells showing mitochondria (M) stacked one inside the other, degenerating cell membrane (CM). X=7500. **b)** A portion of CA cell showing stack of endoplasmic reticulum (SER) appear to be in the process of degeneration. Auotophagic vacuoles (arrows) filled with glycogen. X=10000. **c)** A part of CA showing obvious degeneration of cells and most organelles present only as remnants. White arrows: filamentous mitochondria. X=15000. Note discharge of neurosecretory granules (*), nuclei (N) with clump of chromatins (ch).

macrocyclic lactone, which causes involuntary muscular contractions. Because of the prolonged hyperexcitation, insects eventually become paralyzed, apparently due to neuromuscular fatigue. Truner and Schaeffer (1989) reported similar symptoms, paralysis, in insects and nematode treated with avermectins.

In the present study, the data clearly showed that abamectin affected the haemolymph ion concentrations in a dose–dependant manner. The Na^+ / K^+ ratio in normal 5th nymphal instar *S. gregaria* equals to 8.4. A high sodium index is a distinct feature in order Orthoptera. The order, like other orders exhibits great variations with respect to ions content, among different species (Florkin and Jeuniaux 1974 and Amin, 2008). Phylogenetic position and food habits are the main factors that determine the feature of the haemolymph specially Na/K ratio (Sutcliff, 1963; Pichon, 1970 and Amin and Eid, 2002). Treatment with abamectin at LC_{10} and LC_{30} levels decreased the Na/K ratio by 2.2 times that of the control, while at LC_{70} abamctin treatment, the Na/K ratio decreased by 1.2 times that of the control. This, however, was due to the differences in the degree of reductions in Na and K ions concentrations.

AChE has a key role in neurotransmission by hydrolyzing the neurotransmitter acetylcholine in cholinergic synapses of nervous system which was the target site of several neurotoxic insecticides (Siegfrief and Scharf, 2001), a significant effect of the abamectin on the AChE activity which varied as function of the dose. Biochemical data revealed induction in AChE activities when abamectin was ingested by *S. gregaria* 5th instar nymphs. This confirms previous report on *S. littoralis* larvae when treated with methylamine avermectin (Dahi *et al.*, 2009). These results were in contrast with that reported by Habes *et al.* (2006) who studied the toxicity effect of boric acid on German cockroach. Also, Rabea *et al.* (2009) reported that spinosad significantly inhibited the AChE activity in different organs of honey bee workers.

This hyperactivity of abamectin was different than other insect control agents which caused either no change or a reduction is AChE activity. Abamectin may be works in a reversible manner, producing extra release of AChE which may prevent any message to be sent to the receptor and thus insects become without neural orientation.

Similarly, Salgado *et al.* (1998) reported that spinosad greatly prolonged ACh response duration and this indicated that spinosad and ACh can act simultaneously and therefore they must act at separate and distinct sites. On the other hand, Fahmy (2005) demonstrated that AChE activity remained nearly unchanged when *S. littoralis* larvae were treated by abamectin.

The fine structure of the normal corpora allata cells in the 5th nymphal instar of *S. gregaria* agrees with the description of the CA already reported by Dorn, 1973; Szibbo and Tobe, 1981 and Cassier, 1990. Ultrastructural examinations of the CA from treated and control insects clearly showed that abamectin induced degradation of the corpora allata cells. Similar results were reported in several insect species treated with neurotoxicants (Pratt and Bowers, 1977; Unnithan *et al.*, 1977; Pener *et al.*, 1981). At the highest abamectin concentration (LC_{70}), the CA cells appeared completely destroyed. Similarly, Schooneveld (1979) presented electron microscopic evidence for a rapid cytotoxic action of precocence on the corpora allata in nymphs of *Locusta migratoria*. Feyereisen *et al.* (1981) reported clumping and irregularity of the smooth endoplasmic reticulum which were the major changes found after treatment of *Diploptera punctata* with precocence, also all cytoplasm organelles have disintegrated into masses of tangled membranes. Alternations of the structure of CA cells were also reported by Unnithan *et al.* (1977) who observed autography changes in mitochondrial configurations and signs of disintegration in corpora allata of topically or contact precocence-treated *Oncopeltus fasciatus*.

Also, Ergen (2001) reported ultrastructural changes especially in the nuclei, mitochondria, endoplasmic reticulum membranes, Golgi complexes and some other intracellular organelles in the CA cells of adult females *Anacridium aegyptium* when topically treated with 500µg precocene II. Abamectin may exhibit growth-regulating activity (Wright, 1984), so it may interfere with the activity of the CA and it can affect the CA structure.

Hamouda and Dahi (2008) reported that the abundance of mitochondria and their accumulation were quite evident in nerve cells and all neurosecretory cells of the spinetoram–treated *S. littoralis* larvae. Also, vacuolization was observed in the nerve cells and all neurosecretory cells of *S. littoralis* larvae treated with LC_{50} of spinetoram reflecting cell destruction. Moreover, multivesicular bodies were observed in the cytoplasm of the neurosecretory cells. These bodies were a variety of heterolysosomes that behave as autolysosomes to digest endogenous material such as cell lysis products and secretory granules.

In conclusion, abamectin is fairly toxic compound

to the 5th nymphal instar of *S. gregaria*. It has a neurotoxic effect manifested as well defined histological changes in the corpora allata cells. Further studies are needed at the molecular level to detect the exact mode of action of this newly bioinsecticide which holds much promise to control insects in a novel mode of action.

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