

Detoxification System in *Spodoptera littoralis* against Abamectin

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ABSTRACT: Biochemical and histological analysis were performed on 4th larval instar of the cotton leaf worm *Spodoptera littoralis* to determine the effect of abamectin (LC₅₀) on the biomarkers acetylcholinesterase (AChE), glutathione S-transferase (GST), α -, β - esterase and mixed function oxidase (MFO). Biochemical analysis showed a severe reduction in the enzymatic activities of GST and α -esterase, as compared to the control, reaching their minimum activity at 4-day post-treatment. Therefore, abamectin may be not detoxified by these enzymes. Abamectin increase the enzyme activity of β - esterase reaching its maximum activity at 4-day post-treatment. Abamectin treatment resulted in slightly reduction in MFO activity at 2- and 6-day post-treatment, while it has no effect on the enzyme activity of AChE at all-time intervals, as compared with control. The natural bio-pesticide is being tested here with reference to insect hemocyte count (THC) and ultrastructure. Two days after treatment THC increased by 1.7-fold, whereas decreased after 4 and 6 days of application by 1.3 and 1.6-fold, respectively, as compared to untreated controls.

Histological study on the *S. littoralis* larval hemocytes showed that abamectin induced pathological detrition. Electron dense granules, structured granules and desmosomes were synthesized. Also, large number of deformed mitochondria, autophagic lysosome and long pseudopodia were observed in hemocytes treated with abamectin. Finally, at late stage of infection cell organelles were completely lysed.

KEY WORDS: Detoxifying enzymes, Hemocytes, Ultrastructure, *Spodoptera littoralis*.

I. INTRODUCTION

The cottonleaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is one of the most polyphagous insects, whose larva is a serious pest of numerous kinds of cultivated crops, causes potential threat to cotton, corn, beet, tomato and many others in Egypt [1]. During the past few years, insecticides have been widely used for lepidopterous control. Because of its high reproductive potential and short life cycle, combined with frequent applications of pesticides usually required to maintain its population below economic thresholds, facilitate the development of resistance in insect species. This resistance is currently a serious and worldwide problem in agriculture production [2]; therefore, *S. littoralis* should be controlled in eco-friendly manner.

Insecticides application not only controls the target insect pests but may adversely affect the non-target organisms including biocontrol agents. It is, therefore, imperative to screen the insecticides for their selectivity for important biocontrol agents before they could be incorporated into IPM programs [3]. Successful screening of insecticides largely depends on a better understanding of their toxicological effects on target pests as well as on their natural enemies.

The insecticides with novel mode of action might herald a new era in pest control. They possess many desirable properties including high toxicity to insects and low toxicity to mammals. Therefore, they may be replacing the conventional chemical pesticides in controlling the resistant insect pests.

Abamectin is the major fermentation component of avermectins derived from a soil actinomycete (*Streptomyces avermitilis*) and has strong insecticidal, nematicidal, and acaricidal activity [4]. It has shown low toxicity to non-target

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beneficial arthropods that help its acceptance into integrated pest management (IPM) programs, besides supporting the safety to man and the environment [5]. The avermectins mode of action is blocking the transmittance of electrical activity in nerves and muscle cells, causing an influx of chloride into the cells, which lead to hyper polarization and subsequent paralysis of the neuromuscular systems and then death. This unique mode of action is effective on insect pests that are resistant to other synthetic insecticides [6].

Immunological studies are of crucial importance for insect toxicology. The immune system is divided into two major categories called innate and specific. The innate immune system classified into humoral and cellular defense responses. The innate immune response depends mainly on recognition and killing of invading xenobiotics. However innate immunity is the first line of defense which includes blood cells. Hemocytes play multifunctional roles such as phagocytosis, encapsulation, nodule formation, detoxification and secretion of humoral immunity factors may render them more sensitive than other cell towards insecticides and internal/ external factors [7]. The cellular defense is promoted through the interaction of different hemocytes [8].

Insecticide resistance in lepidopteran population involves mainly two mechanisms, enhanced detoxification and target site insensitivity. Various detoxifying enzymes, Glutathion *S*-transferase (GST; EC 2.5.1.18) and esterases (EC 3.1.1.7), are involved in the metabolism of a broad range of foreign and endogenous compounds in insects [9]. Acetylcholinesterase (AChE; EC 3.1.1.7.) is a key enzyme in the nervous system, terminating nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine (ACh). In insects, AChE is the only cholinesterase which known as the most defensive esterases as it is the major target for organophosphate and carbamate insecticides [10].

Mixed function oxygenases (also called mixed function oxidases, MFOs, cytochrome P-450s, polysubstrate multifunction oxygenases, PSMOs) are a family of membrane-bound enzymes which increase the water solubility of aromatic and lipophilic compounds by adding molecular oxygen to lipophilic compounds [11].

The goal of the present study is to understand the detoxification mechanism of *S. littoralis* toward abamectin. The impact of abamectin (LC₅₀) on the activity of glutathione *S*-transferase, mixed function oxidase, and α - and β -esterases in *S. littoralis* 4th instar larvae was assessed. In addition, total hemocyte count and ultrastructure of hemocytes were determined.

II. MATERIALS AND METHODS

2.1. Insects: A laboratory strain of *S. littoralis* larvae were purchased from Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt. It was maintained under constant conditions of 25 ± 2 °C and 70 ± 5 % RH and kept free of any contamination with chemicals to obtain a susceptible strain.

2.2. Chemicals: Abamectin (EC 1.8%) was purchased from Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt. The following chemicals were purchased from Sigma Chemical (St. Louis, MO): α -naphthyl acetate (α -NA), β -naphthyl acetate (β -NA), acetylthiocholine bromide (ATCh), 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB), reduced glutathione (GSH), serine, and 1-chloro-2,4-dinitrobenzene (CDNB).

2.3. Toxicity test: Bioassays were performed on 4th instar larvae using the leaf dip technique [12]. Castor-bean leaves were dipped for 30 sec in an aqueous solution of the prepared concentrations (1, 5, 10, 20, 50, 75 ppm) of abamectin, then left to dry for 1 h in room temperature before being offered to the 4th instar larvae and larvae treated with water served as control. Larvae were fed for 24 h on the treated leaves, and then transferred to fresh untreated leaves for three days. Knocking down effect was recorded 48 h after treatment. All tests were conducted at room temperature and bioassay consisted of six doses, with three replicates/dose and 30 individuals per replicate. The corrected mortality was calculated by using Abbott's formula [13], data were subjected to Probit analysis as described by Finney (1971) [14]. The lethal concentration (LC₅₀) value of abamectin was calculated. A similar technique was used to assess the sublethal effect of abamectin at the dosage of LC₅₀ level on the larval detoxifying enzymes activity, hemocytes count, and hemocytes structure of 4th instar *S. littoralis*.

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2.4. Quantification of enzyme activity:

2.4.1. Sample preparation

Insects (treated with abamectin at the LC₅₀ level) from selected bioassay experiments were weighed, the whole larval homogenate was prepared by grinding 4th instar larvae in ice-cold 50mM sodium phosphate buffer (pH 7.5). The homogenate was centrifuged using Eppendorf refrigerated 5415(Hamburg, Germany) at 10, 000 rpm for 20 min at 4°C. The supernatant were stored at -20°C and used as enzyme source[15]. The protein content of the enzyme extract was estimated by using the method of Lowry *et al.*(1951) [16], using bovine serum albumin as a standard protein to construct the standard curve.

2.4.2. Enzymes assay: The activity of α - and β -esterase, GST, AChE and MFO enzymes was analyzed in 4th *S. littoralis* larva treated with LC₅₀ abamectin at different time intervals (2, 4, 6 days post-treatment).

GST Assay: Activity of GST was carried using the method of Habig *et al.*(1974) [17]. Fifty microliters of 50mM CDNB and 150 μ l of GSH were added to 2.79 ml phosphate buffer (40mM pH 6.8). Ten microliters of enzyme stock was then added. The mixture were gently shaken and incubated for 2-3 minutes at 20°C and then transferred in the sample cuvette slot of a UV-1700 spectrophotometer. The change in absorbance was measured at 340nm and the enzyme activity in terms of μ mol of CDNB conjugated min⁻¹ mg of enzyme protein⁻¹ was calculated using the extinction coefficient of 9.6 mM⁻¹ cm⁻¹ [18].

AChE assay: AChE activity was measured using acetylcholine-bromide as a substrate according to Ellman *et al.* (1961) [19]. Two hundred microliters of enzyme stock and 100 μ l of 0.075 M acetylthiocholine-bromide, 240 μ l of 0.1M phosphate buffer (pH 7.4) were added and incubated for 15min at 27°C, and then 500 μ l of 0.1M serine DTNB was added and mixed. The change in absorbance was measured at 412 nm using a UV-1700 spectrophotometer.

MFO assay: Assays examining the *O*-demethoxylation of *p*-nitroanisole (PNOD) by cytochrome P450 monooxygenase were conducted using the procedures developed by Rose *et al.* (1995)[20]. The homogenate was centrifuged at 20,000 rpm for 20 min at 4 °C. Then 100 μ l of 2 mM *p*- nitroanisole solution, 45 μ l of enzyme, and 45 μ l of homogenization buffer were added to each well. The microplate was incubated for 5 min at 30 °C and the reaction initiated by the addition of 10 μ l of 9.6 mM NADPH. The absorbance was read at 405 nm and 30 °C for 15 min using a UV-1700 spectrophotometer.

α - and β - esterase assay: Determination of general esterase activities were evaluated colorimetrically according to the method of Van Asperen (1962)[21] and Chen *et al.* (2011)[15]. Briefly, 0.05 ml of extracted enzyme was incubated in a final reaction volume of 4.6 ml in 0.04 M phosphate buffer (pH 7.0) containing 0.03 M α -Na / β - Na and 10⁻⁴ M physostigmine at 30 C for 5 min. Reactions were stopped by adding 1 ml of DBLS solution (the mixture of 2 mL of 1% fast blue B and 5mL of 5% SDS). Absorbance was determined at 600 nm for α -Na and 555 nm for β - Na using a UV-1700 spectrophotometer. The activity was expressed as nmoles of hydrolyzed substrate/min/mg protein using α / β - naphthol as the standard [9].

2.5. Hemocytes study: The larvae (untreated control and treated at 2, 4, 6 days of treatment) were anesthetized and immobilized by low temperature (0°C, 5 min) and cleaned in 70% alcohol. The hemolymph was collected through delicate puncture with a needle in the abdominal region of the insect. The drops of hemolymph were collected with Pasteur pipettes, previously rinsed in anticoagulant solution for insect [22].

2.5.1. Hemocytes count: To determine the total hemocyte count, 20 μ L of the hemolymph was diluted 1:9 (v/v) in chilled saline (NaCl 7 g L⁻¹ KCl 0.2 g L⁻¹ CaCl₂ 0.2 g L⁻¹, MgCl₂ 0.1 g L⁻¹, NaHCO₃ 0.15 g L⁻¹, NaH₂PO₄ 0.2 g L⁻¹, Glucose 7.0 g L⁻¹), and aliquots were transferred to a Neubauer hemocytometer. The cells were counted using a light microscope and the number of total hemocytes per cubic millimeter (mm³) was calculated using the formula of Jones (1962) [23].

2.5.2. Ultrastructure of Hemocytes: For transmission electron microscopy (TEM), hemocytes were fixed in 4% glutaraldehyde in 0.2M cacodylate buffer, pH 7.2. After 30 min fixation the haemocytes were centrifuged for 10 min at

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3,000 rpm and the pellets were embedded in 2.5% agarose. They were washed in 5% sucrose solution in 0.2M cacodylate buffer, pH 7.2, and post-fixed in 1% osmium tetroxide in cacodylate buffer for 1 h. After dehydration in a graded alcohol series they were embedded in Epon-Araldite resin. Ultrathin sections were stained in uranyl acetate and lead citrate [24] and examined under a Philips EM-420 microscope at 70 kV. The hemocyte types were identified by using established morphological characters presented by Gupta (1985) [25].

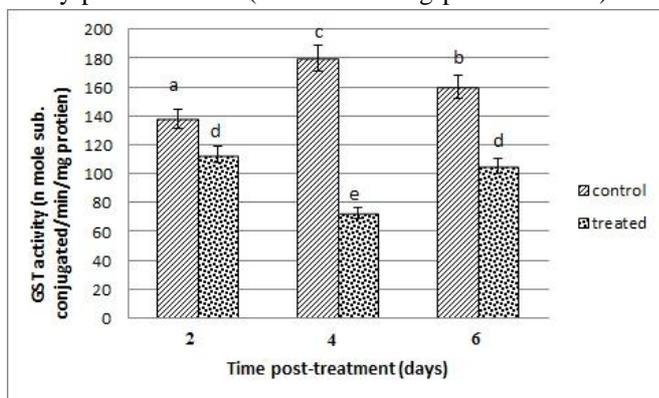
2.6. Statistical analysis: Results are presented as means \pm standard deviation (SD). Data were subjected to statistical analysis using the two-way analysis of variance (ANOVA) [26] and separated by the least significant difference (LSD) test and the probability level $P < 0.05$ was considered statistically significant by SPSS version 13.0 [27].

III. RESULTS and DISCUSSION

Bioassay: The toxicity of abamectin against the 4th instar *S. littoralis* was determined and the lethal concentration LC₅₀ was estimated as 44.7 ± 2.01 ppm [28].

Enzyme assays: Detoxification enzymes in insects are generally demonstrated as the enzymatic defense against foreign compounds and play a significant role in maintaining their normal physiological functions [9].

GST activity was increased significantly in abamectin treatment at both 2-day (113 ± 4.61 mM mg protein⁻¹ min⁻¹) and 6-day post-treatment (105 ± 7 mM mg protein⁻¹ min⁻¹) as compared with that at 4-day post-treatment (73 ± 5 mM mg



protein⁻¹ min⁻¹), however the

Fig. (1): Effect of abamectin (LC₅₀) on GST activity of 4th instar larvae *S. littoralis*.

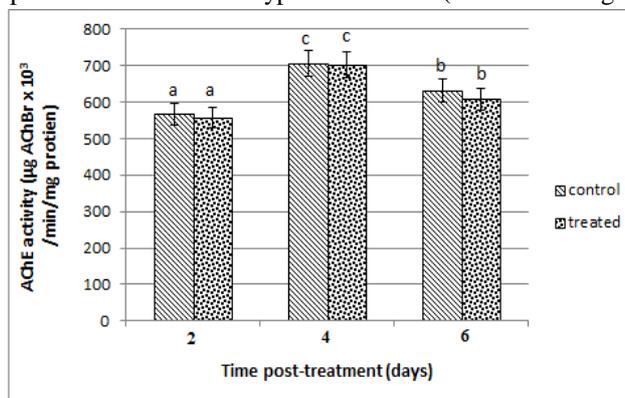


Fig. (2): Effect of

abamectin (LC₅₀) on AChE activity of 4th instar larvae *S. littoralis*.

enzyme activity decreased in all treatments (2-, 4-, 6-day post-treatment) as compared to control (138 ± 4.35 , 180 ± 10 , 160 ± 5.13 mM mg protein⁻¹ min⁻¹, respectively) (Fig.1). At 4-day post-treatment, GST activity recorded maximal depression where it decreased by 59.44% lower than control. On the other hand, the enzyme depressions were 18.12 and 34.38% lower than control at 2- and 6-day post-treatment, respectively. This depression in the GST activity due to the effect of abamectin may be due to reaction of insecticide with essential residues on the enzyme related to GST binding [29]. On the contrary, a significant enhancement in the activity of GST was reported when 3rd instar of *S. litura* larvae treated with LC₁₀ and LC₃₀ methoxyfenozide [30].

The results obtained in figure (2) showed that abamectin (LC₅₀) has no effect on the activity of AChE in *S. littoralis* 4th instar as compared with control. The enzyme activities were 558 ± 12 , 703 ± 24 and 607 ± 20 μ g mg protein⁻¹ min⁻¹ at 2-day, 4-day and 6-day post-treatment, respectively, while in control the enzyme activity recorded 567 ± 39 , 705 ± 17 and 631 ± 16 μ g mg protein⁻¹ min⁻¹, respectively. However, Rashwan (2013) [31] and Fahmy and Dahi (2009) [32] recorded a significant changes and increase in AChE activity, when *S. littoralis* larvae treated with spinetoram. Similarly, the change of response to spinosad could be associated with the decrease in AChE activity [1].

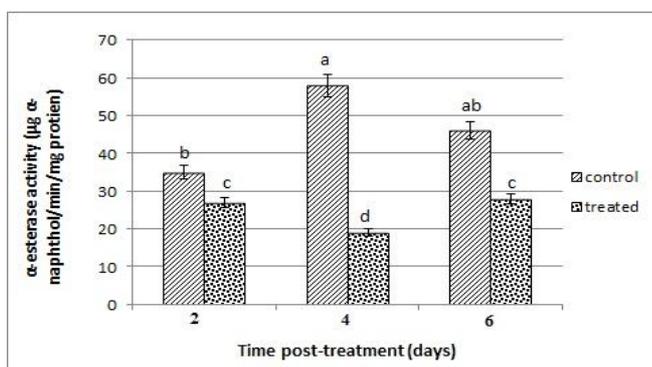


Fig. (3): Effect of abamectin(LC₅₀) on α-esterase activity of 4th instar larvae *S.littoralis*.

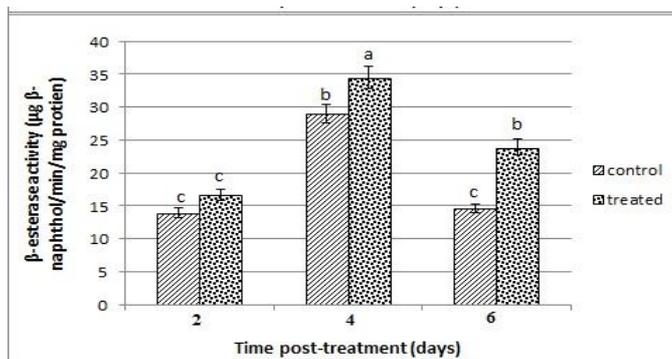


Fig. (4): Effect of abamectin(LC₅₀) on β-esterase activity of 4th instar larvae *S.littoralis*.

Dealing with α-esterase and its correlation with abamectin treatment, our data represented in figure (3) showed an inhibitory effect in the enzyme activity. The enzyme activities were 27 ± 3.9, 19 ± 2, 28 ± 1.7 µg α-naphsol mg protein⁻¹ min⁻¹ at 2-day, 4-day and 6-day post-treatment, respectively, while it recorded 35 ± 1, 58 ± 3, 46 ± 5 µg α-naphsol mg protein⁻¹ min⁻¹ at 2-day, 4-day and 6-day post-treatment, respectively in untreated insects. In control, enzyme activity reached its maximum activity at 4-day post-treatment (58 ± 3µg α-naphsol mg protein⁻¹ min⁻¹) as compared with all other treatments, while at the same time interval, α-esterase activity was at its minimum level (19 ± 2 µg α-naphsol mg protein⁻¹ min⁻¹) in abamectin treatment as compared with all other treatments. In other words, the change in activity it was obvious that abamectin exhibited remarkably significant reduction (P<0.05) by 22.86, 67.24 and 39.13% at 2-, 4-, 6-day post-treatment, respectively as relative to control. This decreased activity of esterases occurred by bio-pesticide may be increased the insect susceptibility to insecticides have an ester bond [9]. So far, esterases are known to be involved in the detoxification of the major groups of insecticides [33]. As shown in figure (4) a significant effect (P<0.05) of time was recorded in β-esterase activity. In control, the enzyme activity increase from 14 ± 0.9 at 2-day to 29.1 ± 0.9 at 4-day, while at 6-day post-treatment the activity recorded 14.6 ± 1.6 µmol/min/mg protein. As compared with control, abamectin (LC₅₀) showed a significant increase in the β-esterase activity at 4- and 6-day post-treatment, 34.5 ± 2.2 and 23.9 ± 1.7 µmol/min/mg protein, respectively. While, at 2-day post-treatment abamectin has slightly effect on the enzyme activity (16.7 ± 1.3 µmol/min/mg protein). However, a remarkable reduction in β-esterase activity was investigated as *S. littoralis* 5th instar larvae treated with LC₅₀ spinetoram and rynaxypyr insecticides [31]. Also, a reduction in the activity of α- and β- esterases was reported in the 2nd and 4th *S. littoralis* larvae treated with 2 different IGRs as time post-treatment increase and as doses increase [34]. Moreover, a reduction in the enzyme activity in the *S. littoralis* larvae affected by buprofezin, diafenthuran and triflumuran [35].

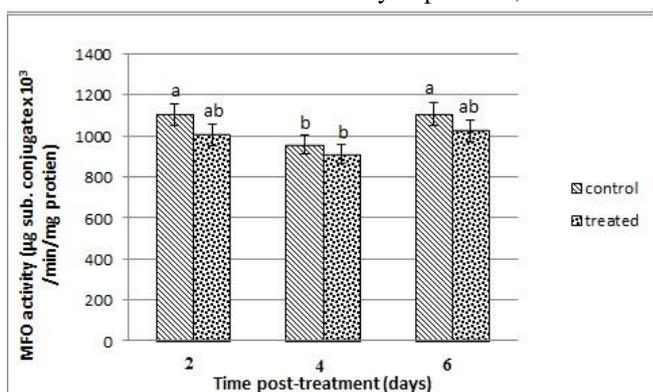


Fig. (5): Effect of abamectin(LC₅₀) on MFO activity of 4th instar larvae *S.littoralis*.

Time post-treatment (days)	THC (x10 ³ m/L)	
	Control	Treated
2	4.7±0.8 ^d	8.01±1.12 ^c
4	10.02±2.83 ^b	7.89±0.89 ^c
6	13.23±1.99 ^a	8.12±1.08 ^c

Table (1): Effect of abamectin (LC₅₀) on total hemocyte count of 4th instar larvae of *S.littoralis*. (Values in each

time intervals followed by the same letters are not significantly different ($P < 0.05$).

Data recorded in figure (5) showed effect of abamectin (LC_{50}) treatment in *S. littoralis* 4th instar on MFO activity. In the controls, there is no significant change ($P < 0.05$) in MFO activity at 2- and 6-day post-treatment enzyme activity recorded 1104 ± 53 and $1105 \pm 45 \mu\text{g sub-conj} \times 10^3 \text{ mg protein}^{-1} \text{ min}^{-1}$, respectively, while, at 4-day post-treatment enzyme activity decreased to reach $958 \pm 38 \mu\text{g sub-conj} \times 10^3 \text{ mg protein}^{-1} \text{ min}^{-1}$. Similarly, at the same time intervals, abamectin showed insignificant differences in *S. littoralis* 4th instar (1005 ± 10 and $1025 \pm 11 \mu\text{g sub-conj} \times 10^3 \text{ mg protein}^{-1} \text{ min}^{-1}$ at 2- and 6-day post-treatment, respectively and decreases to reach $911 \pm 11 \mu\text{g sub-conj} \times 10^3 \text{ mg protein}^{-1} \text{ min}^{-1}$ at 4-day. Generally, abamectin has no effect on the MFO enzyme activity in *S. littoralis* 4th instar. On the other hand, several studies investigated that abamectin resistance might be due to increased activities of microsomal oxidases [36], [37].

The suppression of detoxification enzymes (α -esterase and GST) indicated that these enzymes play no role in the detoxification of tested compounds and may be increase the susceptibility of insect pest to these insecticides [38].

Effect of abamectin on larval hemocytes:

Four well-defined hemocyte types could be distinguished in the hemolymph of the *S. littoralis* 4th instar larvae: prohemocyte (PR), plasmatocyte (PL), granulocyte (GR), and spherulocyte (SP) (Fig. 6a). Similarly, Gelbic *et al.* (2006) [39] investigated four types of hemocytes in the hemolymph of *S. littoralis* PLs, GRs, SPs and oenocytoides (OEs). While, 5 types (PRs, PLs, GRs, SPs, OEs) of hemocytes were recorded in *S. littoralis* 4th instar [40].

Total hemocytes count (THC): The total hemocyte count in the 4th instar *S. littoralis* was affected by the LC_{50} abamectin treatment. As shown in table (1), at 2 days after treatment, THC significantly increased with abamectin treatment ($8.01 \pm 1.12 \times 10^3$ cells m/l) as compared to control ($4.7 \pm 0.8 \times 10^3$ cells m/l) indicating that hemocytes in the larvae exhibited positive stress immunity in response to the bioinsecticide [40,41]. At 4-day and 6-day, THC in the control significantly increased to $10.02 \pm 2.83 \times 10^3$ and $13.23 \pm 1.99 \times 10^3$ cells mL^{-1} , respectively; whereas, THC in the treated larvae were still maintained the same low level ($7.89 \pm 0.89 \times 10^3$ and $8.12 \pm 1.08 \times 10^3$ cells m/l at 4-day and 6-day, respectively) as the 2-day post-treatment, indicating that the LC_{50} abamectin concentration inhibited the normal proliferation of hemocytes at 4-day and 6-day post-treatment. These changes in the THC might be attributed to the release of hemocytes that adhered on surfaces within the hemocoel, and the inhibition of larval hematopoietic function and/or the cell proliferation [41].

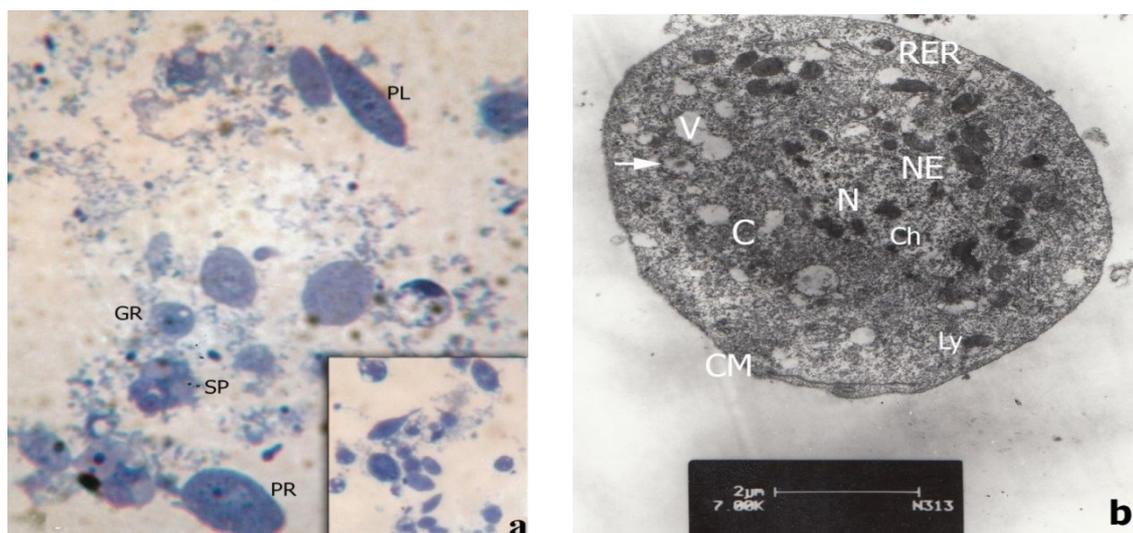


Fig. (6): Normal hemocytes of 4th instar *S. littoralis* larvae. **a)** Semi-thin section showing Prohemocyte (PR), Plasmatocyte (PL), Granulocyte (GR), Spherulocytes (SP). (X: 1000). **b)** TEM micrograph showing normal plasmatocytes.

The increase in THC by abamectin at 2 days post-treatment may be correlated to the degree of the defensive action of haemocytes involved in detoxification [42]. The increase in the THC after treatment may indicate whether the host defensive system has activated or deactivated [43], while, the decrease in the number of hemocytes in the treated larvae may be due to nodulation and encapsulation as well as degranulation of some cell types or the inhibition of the brain hormone secretion [44].

Ultrastructure of Hemocytes: The normal hemocytes show well developed cell membrane (CM) with cytoplasmic processes, rough endoplasmic reticulum (RER), mitochondria (M), nucleus (N) and primary lysosomes (Ly). The nucleoli have dense chromatin (Ch) dispersed in clumps with only occasional batches near the nuclear membrane (NE). Free ribosomes (arrow) are scattered in the cytoplasm (C) (Fig.6 b). The infection of *S. littoralis* 4th instar larvae with LC₅₀ abamectin induced several pathological detritions. Figure (7) shows 2-day post-treatment hemocytes of abamectin treated 4th instar *S. littoralis*. Chromatin material (Ch) begins to migrate toward nuclear envelop (NE), large number of swollen (sM) and looped (IM) mitochondria were observed in the cytoplasm of the infected cell (Fig 7 a). Endoplasmic reticulum filled with fibrous materials in its cisternae. Granulocytes synthesized electron dense granules (DG) containing homogenous materials and cell membrane (CM) with long pseudopodia (Ps) in order to form capsule and nodule (Fig.7a). Well-developed rough endoplasmic reticulum (RER) and autophagy lysosome (Ly) were found (Fig 7 b) [44].

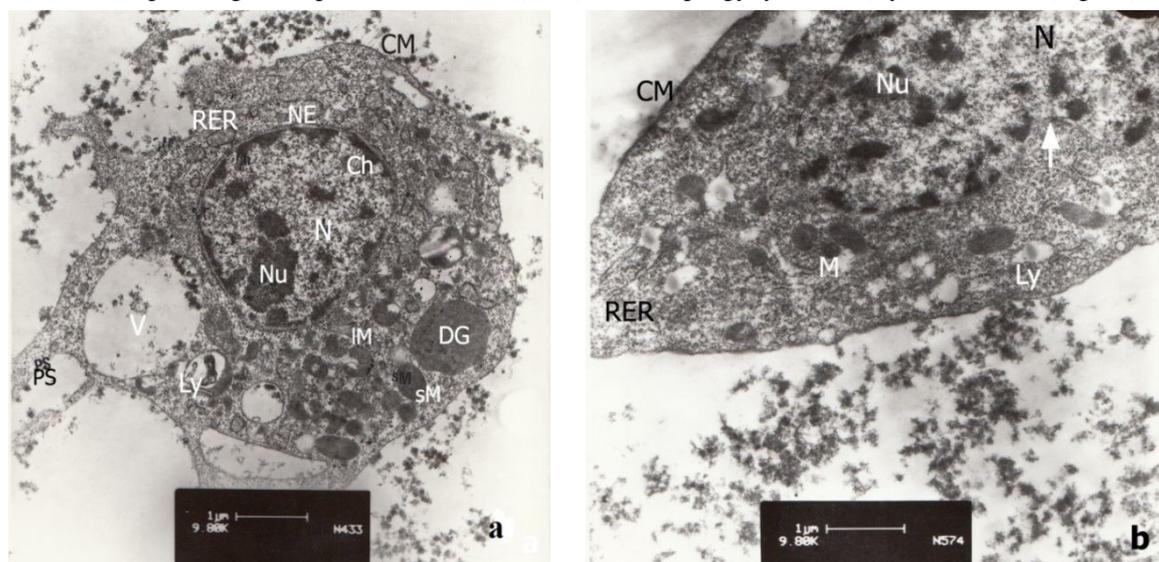


Fig. (7): TEM micrographs showing ultrastructural changes in the hemocytes of the 4th larval instar of *S. littoralis* 2-day post-treatment with LC₅₀ abamectin. **a)** Granulocyte. **b)** A portion of plasmatocyte.

Figure (8) exhibits abamectin-treated hemocytes (4-day post-treatment) of *S. littoralis* fourth instar. The contents of the cytoplasm (C) seemed to swell giving the cells an extremely vacuolated (V) appearance. Nucleus was observed with ruptured envelop (arrow). Two types of membrane bound granules were observed, dense granules (DG) which contain electron-dense homogenous contents and structured granules (SG) with crystalloid contents (Fig 8 a). Figure (8b) showed cells tend to adhere to one another to contact, and to form aggregations. These unstructured aggregations may later be encapsulated by other haemocytes, or by cells that may be released from the aggregations [44, 45].

In the late stage of abamectin treatment (After 6 days), the cell membrane was completely lysed and nucleus (DN) was severely distorted. Hemocytes synthesized numerous desmosomes (D) containing large amounts of microtubule and large nuclei (DN) occupying the entire volume of cells (Fig. 9 a). Also, cell organelles were completely lysed; the

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chromatin had dispersed into the peripheral area and the nucleoli disappeared and generally, cell structure becomes unformed (Fig. 9 b). At 6 days of treatment, more destructive effects were observed in the haemocytes of *S.littoralis* infected with LC₅₀ abamectin.

Ultrastructural alternations and malformations have been observed in circulating haemocytes of *Agrotisipsilon* fourth larval instar treated with dimilin and *Bacillus thuringiensis* [44]. Also, abnormalities such as change in shape, extensive vacuolization, and necrosis were reported in hemocytes of *S. littoralis* larvae fed on endophyte infected cauliflower plant leaves, and the percentage of abnormal hemocytes increased with increase in feeding duration. This cytotoxic effect of endophytic fungi on the hemocytes ultimately leads to adverse effects on survival and fitness of the insect [46]. The present results are consistent with that investigated by Shaurubet *al.* (2014) [40] when the fourth instar of *S. littoralis* was treated with azadirachtin (LC₅₀). Granulocytes and plasmatocytes were the main affected cell types, where the most common symptoms were the presence of rough endoplasmic reticulum filled with fibrous materials in their cisternae, disorganization of mitochondria, and the cytoplasm was vacuolated with the appearance of autophagic lysosomes. Similar extreme pathological symptoms in hemocytes structure of several insect species treated with different insecticides [44, 45, 47].

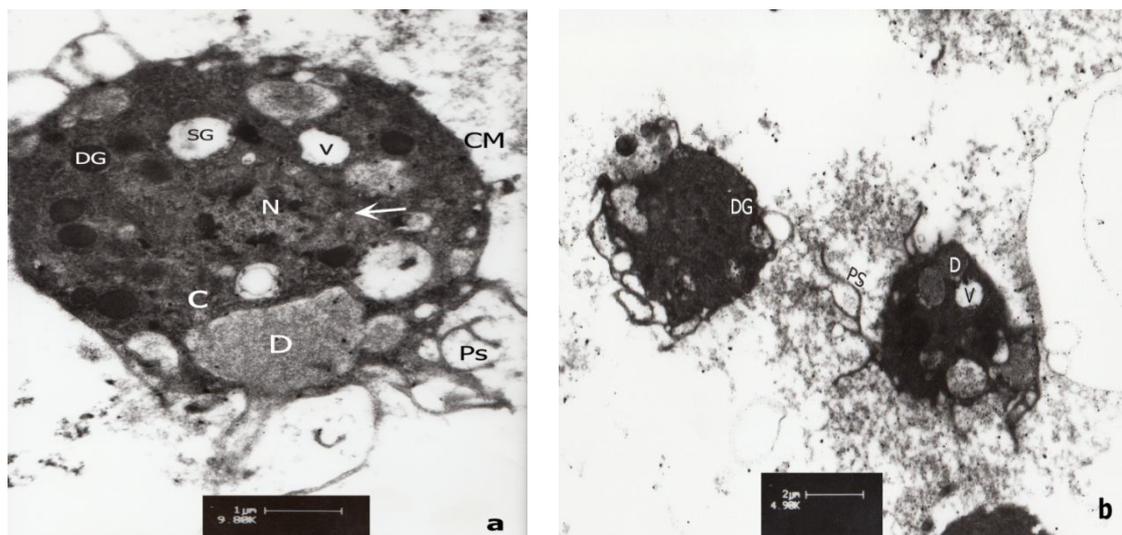


Fig. (8): TEM micrographs showing ultrastructural changes in the hemocytes of the 4th larval instar of *S. littoralis* 4-day post-treatment with LC₅₀ abamectin. **a)** A plasmatocyte. **b)** Granulocytes.

IV. CONCLUSION

In conclusion, this study was conducted to understand the possible mechanisms of abamectin resistance in *S. littoralis* by determining which detoxification enzymes were associated with the resistance. The present results indicated that the tested bio-pesticide abamectin induced a reduction in the activities of GST and α -esterase. Therefore, the tested abamectin, may be not detoxify by these enzymes. β -esterase may be playing a role in the resistance mechanism of *S. littoralis* against abamectin biopesticide. Also, results of the present work may help in the investigation of the role played by the hemocyte cells on the defense mechanisms of the cotton leaf worm against abamectin. Generally, abamectin is toxic to *S. littoralis* and should be enclosed in the IPM systems.

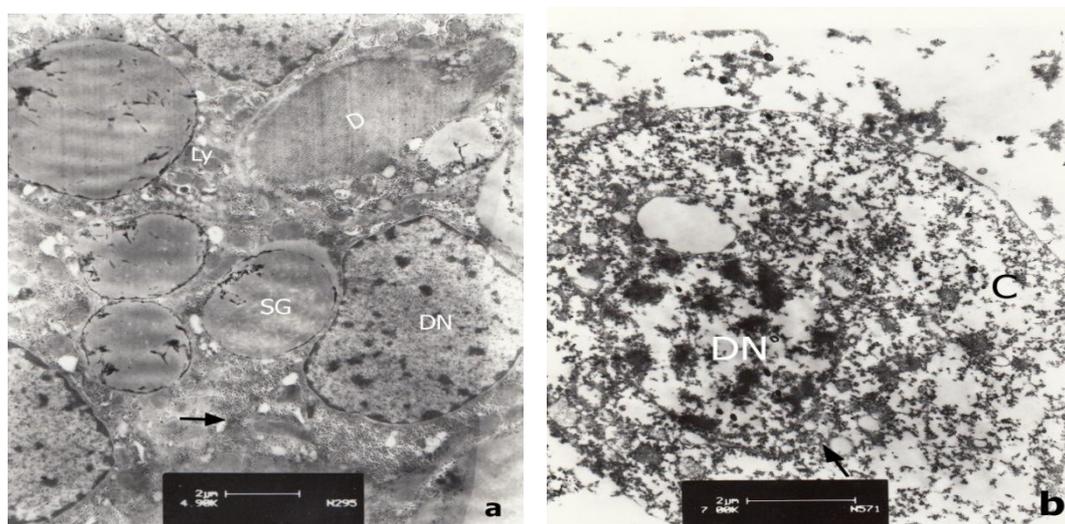


Fig. (9): TEM micrograph (s) showing ultrastructural changes in the hemocytes of the 4th larval instar of *S. littoralis* 6-day post-treatment with LC₅₀ abamectin. **a)** A group of hemocytes attached to one another. Note disappearance of cell membrane. **b)** Granulocyte begins to lysis. Note curved nuclear envelope (arrow).

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