Esterases and Glutathione-S-Transferase Activities Related Responses in Cotton Leaf Worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) After Insecticides Exposure

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Abstract:- Toxicities of some traditional insecticides; Rider®, Reldan®, Biolarve®, Speedo®, Roxy® and Grand® were examined on 2nd and 4th instars of both laboratory and field strain of *Spodoptera littoralis* (Boisd.) under laboratory conditions. Examined insecticides exhibited toxicities on laboratory strain greater than field strain. LC₅₀ values on 2nd instar of laboratory strain were 0.224, 0.885, 1.033, 12.017, 24.723 and 35.171 ppm for Speedo®, Rider®, Biolarve®, Grand®, Roxy®, and Reldan®, respectively. Regarding field strain, the values were 0.391, 2.891, 7.076, 28.262, 30.675 and 45.565 ppm in the same manner. All treatments decrease activities of acetylcholine esterase (AChE) compared with control. Similarly, they exhibited a decrease in α and β-esterases (CE) of larval homogenates lower than control. Glutathione-S-transferase (GST) displayed activities in field strain and laboratory strains greater than control. In field strain, the activities were 22.50, 15.00, 16.50, 15.50, 14.00 and 20.50 nM/mg/min for Rider®, Reldan®, Biolarve®, Speedo®, Roxy®, and Grand®, respectively. In the same manner, Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of treated insects displayed activities greater than control. Esterases and GST enzymes may provide a primary investigation concern insect resistance and/or susceptibility to insecticide mixtures.

Keywords:- Insecticides; Spodoptera littoralis; AChE; Esterases; GST.

I. INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd.) is considered one of the most harmful insectal pests in Egypt. It causes major damage to cotton plants as well as more 29 hosts from other crops and vegetables. The environmental hazards of conventional insecticides necessitate introducing of alternative groups that are effective, safe to human and ecosystem (Karrot et al., 2012).

The mode of action of the examined compounds were previously demonstrated. For example, organophosphates have an effect on acetylcholinesterase (AChE) enzyme. Emamectin benzoate cause inhibition in muscle contraction, resulting in continuous flow of chlorine ions in the Gama Amino Butyric Acid (GABA) and H-Glutamate receptor sites (Fanigliulo and Sacchetti, 2008). Group of benzoyl phenyl urea is a class of Insect Growth Regulators (IGRs) that interferes with insect growth and development through inhibiting of chitin synthesis in insect (Post and Vincent, 1973). They are highly desirable in integrated and resistance, pest management programs. They are advantageous, because they are allowing to low persistence in the environment, rapid biodegradable and less impacted on non-target organisms (Zibaee et al., 2011). For example, Novaluron® is a relatively new benzoyl phenyleneurea with low potential toxic effects on mammals. Its residues tend its disappearance with half-life 2.08 days (Eshaay et al., 2001, 2002). It acts by ingestion and contact pathways on several insect pests such as *Spodoptera spp.*, *Tuta absoluta*, *Heliothis armigera* and *Liriomyza huidobrensis* (Kim et al., 2000). Ghoneim et al. (2015) recorded various degrees of inhibited growth and retarded development of *S. littoralis* by Novaluron®. Treatment of the last instar larvae of the same insect with Novaluron® caused some features of impaired adult morphogenesis (Hamadah et al., 2015).

The major reports of insecticide resistance implicate either enzymatic detoxication by microsomal mono-oxygenases and carboxylesterases, or non-metabolic changes in target-site insensitivity. As stated previously, esterases are classified as hydrolases, where a large and diverse group of enzymes catalyze the hydrolysis of a wide range of aliphatic and aromatic esters, choline esterase and organophosphate (OP) compounds (Moharil et al., 2008).

The role of glutathione-S-transferase (GST, EC2-5.1.18) is catalyzing the conjugation of a wide variety of electrophilic compounds with the thiol group of glutathione (GSH). The role of this enzyme has been suggested to protect physiological nucleophiles by conjugated electrophilic foreign compounds such as pesticides, drugs, and carcinogens (Boyland and Chasseaud, 1969; Fukami, 1980). Subsequently, the highly activities GST in the fat body and guts were confirmed in various insects, such as American cockroach, *periplaneta american* (Shishido et al., 1972), the horn battle, *Hylotrups dichotomus*, the silky warm, *Bombyx mori* (Fukami and Shishido, 1966) and the sheep blowfly, *Lucilla cuprina* (Kotze and Rose, 1987). Organophosphates and others are raised as great interest in insect esterases. They strongly induce inhibition of most esterases of large number of insects (Asperen and
Oppenorth, 1960). Their mode of action is the inhibition of the cholinesterase present in the nervous system and genetically modified esterases are capable to hydrolyze OP-compounds and thus confer resistance to these substances in several strains of insects (Dauterman et al., 1962). The study of insecticide effects on esterases and GST is an essential goal to obtain metabolic resistance, where general esterases and GST have been detected to cause a metabolic resistance in numerous insect pests (Hemingway, 2000; Chen et al., 2007).

Extensive consumption of insecticides, multiple generations of the cotton leaf warm per annum, and the availability of host crops have contribution to the development of resistance in the host against different insecticidal groups (Abou-Elghar et al., 2005). Theoretically, under certain conditions, mixtures, sequences or rotation can delay development of resistance (Roush, 1993). The objective of the present study aims to investigate the toxicity of Grand, Roxy, speedo, Biolarve®, Rider®, and Rildan® on 2nd and 4th larval instars of cotton leaf warm, Spodoptera littoralis (Boisd.) and their effects on certain enzymes in the warm under laboratory conditions.

II. MATERIAL AND METHODS

A. Insecticides and Chemicals.

Rider® [Indoxacarb; (S)-methyl 7-chloro-2, 5-dihydro-2-[(methoxycarbonyl) [4- (trifluoromethoxy) phenyl] amino] carbonyl] indeno [1,2-e][1,3,4] oxadiazine-4a (3H)-carboxylate], Reldane® [Chlorpyrifos-methyl; O,O-dimethyl O-3, 5, 6-trichloro-2-pyridyl phosphorothiolate], Biolarve® and Speedo® [Emamectin benzoate; 4’-deoxy-4”-epi-methylamino-avermetin B1; Epi-methylamino-4”-deoxy-avermetin], Roxy® [Novluron; 1-[3-chloro-4(1, 1, 2-trifluoro-2-trifluoromethoxy) phenyl]-3-(2, 6-difluorobenzoyl) urea], Grand® 5% EC [Lufenuron; -{N}-[[2,5-dichloro-4-(1, 1, 2, 3, 3, 3-hexafluoropropoxy) phenyl] carbamoyl]-2, 6-difluorobenzamide] were supplied by Dibo, Dow Agro Scinces, Chemvet, Starchem , United phosphorus Ltd and Sand Valley Co. Egypt, respectively. The chemicals such as potassium phosphate dibase and monobase and ethanol (analytical grade) were supplied by J. T. Baker Chem. Co., Phillipsburg, N. J. 08865. 1-Chloro, 2,4-dinitrobenzene (CDNB), reduced glutathione (GSH), α-naphthyl acetate, β-naphthyl acetate, Fast blue RR, 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide (ASCII) and bovine serum albumin (BSA) were supplied by Sigma Chem. Co., P. O. Box 14508 St. Louis Mo 63178 USA.

B. Insect Rearing

Laboratory Strain

Individuals of S. littoralis (Biosed.) were reared in the laboratory of Etay El-Baroud Research station, Egypt on castor bean leaves. The egg masses were daily taken and allowed to hatch on oleander leaves. The generated larvae were transferred to fresh castor leaves and kept at a condition [25±2 °C and RH of 65%] (El-Defrawi et al., 1964). The newly molted 2nd and 4th instar larvae were used for this study. The emerged adults were kept in glass jars that were provided with Oleander leaves and allowed for oviposition. They were fed on 10% sugar solution in a soaked cotton wool ball.

Field Strain

Egg masses of S. littoralis were collected from cultivated Soybean plant in the farm of Research Station early 2017, before insecticide applications. They were transferred to the laboratory and the new hatches were reared as described above.

C. Leaf Dip Bioassay

Leaf dip technique was used for larval bioassay. Series of concentrations for each formulation were prepared in tap water. Castor bean leaves were dipped into the solutions for 30 sec and allowed to dry in a dark place. Other leaves were dipped into tap water and served as a control. Five concentrations with three replicates were used to estimate each insecticide-mortality line. Ten larval instars were transferred into a petri dish containing treated leaves. All treatment was kept in the rearing chamber at [25±2 °C, 65% RH, and 12 D/L cycle]. After 24 hr, dead individuals were counted, the data were corrected by using Abbott's formula (Abott, 1925), and LC50 values were estimated using Ldp line software program (Finney, 1971).

D. Biochemical Quantifications

Acute toxicity

LC50 values of examined insecticides were used as described above for 4th instar of S. littoralis after 24 hr for insecticides (Rider® and Reldan®) and 48 hr for IGRs and bio-insecticides (Roxy®, Grand®, Speedo®, and Biolarve®). The life individuals were kept in vails and stored at -20 °C until used.

Sample preparation.

Half g of mild gut tissue of larvae was homogenized in potassium phosphate buffer pH 6.5 (1:10 w/v) by using ceramic motor placed on ice. The homogenate was centrifuged at 13,200 rpm for 10 min at 4 °C and the supernatant was taken for assays.

Glutathione-S-transferase (GST) assay

The activity was determined according to the spectrophotometric method of Habig et al. (1974) by using 1-chloro, 2,4 dinitrobenzene (CDNB) as a substrate. In each cuvette, enzyme source (250 µl) was mixed with 500 µl of 50 mM of potassium phosphate buffer pH 6.5. The cuvette was incubated at 25 °C for 5 min. Ethanol–dissolved CDNB (10 µl; 0.2 M) and reduced glutathione (GSH) (150 µl; 19 mM), were added. After 1min, the absorbance was recorded at 340 nm. For blank sample, cuvette contained all reactions except substrate. The assay was done in triplicate. GST specific activity was calculated as nm/min/mg protein.

Esterase’s activities

Evaluation of esterase's (CEs) activities was performed based on the method of Van Asperen (1962) with little modification. The substrates; α-naphthyl acetate (30 mM) or

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β-naphthyl acetate (30 mM) were diluted in phosphate buffer 0.02 M (1: 99 v/v) before used. An aliquot (15 µl) of α-naphthyl acetate or 10 µl for β-naphthyl acetate plus 200 µl of corresponding substrate and 50 µl of Fast blue RR (dissolved in dist. H2O ratio 10: 1 v/v) were poured in cuvette. The absorbance was measured at 450 nm for α-CE and 540 nm for β-CE every 2 min until 10 min.

**Acetylcholinesterase (AChE) assay**

The enzyme activity was determined according to the method of Ellman et al. (1961). In each test tube, 2.5 ml of 0.1M phosphate buffer, pH 8.0, 0.1 ml of ten times dilutes DTNB reagent solution [39.5 mg of 5,5 dithiobis (2-nitrobenzoic acid)] and 15 mg of sodium bicarbonate in 10 ml of 0.1M phosphate buffer pH 7.0, and 20 µl of the enzyme were mixed. An aliquot (0.02 ml) of the substrate [acetylthiocholine iodide; (ASChI); 0.075 M] was added. The optical density of the developed yellow color was recorded after 10 min against the blank, which contained all the reagents except the enzyme source at 412 nm, by using Spectronic 20. The activity of the enzyme were mixed. An aliquot (0.02 ml) of the substrate [acetylthiocholine iodide; (ASChI); 0.075 M] was added. The optical density of the developed yellow color was recorded after 10 min against the blank, which contained all the reagents except the enzyme source at 412 nm, by using Spectronic 20. The activity of acetyl cholinesterase was calculated as µM of ASChI hydrolyzed per mg protein per min.

**AST/ALT**

The activity these enzymes was measured according to the method of Gello et al. (1985) by using specific kits (Biosystem kits, Spain). It was expressed as U/L.

**Total protein**

Assay of total protein was done according to the method of Lowry et al. (1951). Bovine Serum Albumin (BSA) was used as a standard.

**E. Statistical Analysis**

LC50 value was expressed as µg/ml with confidence limit (CL) and slope for each insecticide which computed using probit analysis. All data were viewed as mean±SE and subjected for analysis of variance (ANOVA). The means compared to significance by student-Newman Keuls at the probability of 0.05 (Cohort software Inc.,1985).

### III. 3 RESULTS

#### A. Insecticide Efficacy

The toxicities of examined insecticides on 2nd instar larvae of *S. litoralis* are listed in Table (1) and illustrated in Figure (1). For laboratory and field strain, insecticide; Speedo® exhibited the greatest toxic effect, followed by Rider®. In addition, examined insecticides exhibited toxicities on laboratory strain greater than field strain. The LC50 values were 0.224, 0.885, 1.033, 12.017, 24.723 and 35.171 ppm for Speedo®, Rider®, Biolarve®, Grand®, Roxy® and Reldane®, respectively, on laboratory strain. Compared with Speedo® as the highest toxic, it was greater than others with 3.82, 4.61, 53.65, 110.37, 157.01-folds on laboratory strain and 3.25, 7.94, 31.72, 34.43, 51.14-folds on field strain for Rider®, Biolarve®, Grand®, Roxy®, and Reldane®, respectively.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Line No.</th>
<th>LC50 (ppm)</th>
<th>Lower limit</th>
<th>Upper Limit</th>
<th>Slope</th>
<th>Index%</th>
<th>Folds</th>
<th>LC90 (ppm)</th>
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<td>0.19</td>
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<td>1.881</td>
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<td>1.81</td>
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<td>1.955</td>
<td>51.14</td>
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Table 1: Relative toxicities of examined insecticides on 2nd instar larvae of *S. litoralis* under laboratory conditions.

- The folds were estimated depending on Speedo as the highest toxic insecticide.
- Line No. was used as cited on dPLines of examined insecticides.
Fig 1: Relative toxicities of examined insecticides on 2nd instar larvae of S. litorallis (a) lab. Strain and (b) field strain in laboratory conditions.

The toxicities of examined insecticides on 4th instar larvae of S. litoralis are listed in Table (2) and illustrated in Figure (2). On laboratory strain, LC$_{50}$ values were 0.411, 1.508, 1.983, 33.853, 35.884 and 42.604 ppm for Speedo®, Rider®, Biolarve®, Reldan®, Grand®, and Roxy®, respectively. Regarding field strain, LC$_{50}$ values were 1.712, 4.697, 7.763, 73.012, 88.77, and 134.243 ppm for insecticides at the same manner. Insecticide; Speedo® was the greatest toxic against S. litoralis compared with others arising 3.67, 4.83, 82.32, 87.31 and 103.66-folds for Rider®, Biolarve®, Reldan®, Grand® and Roxy® on laboratory strain. The toxicity increased to 2.74, 4.53, 42.65, 51.86 and 87.38-folds of insecticides at the above manner on field strain.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Line No.</th>
<th>LC$_{50}$ (ppm)</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
<th>Slope</th>
<th>Index %</th>
<th>Folds</th>
<th>LC$_{90}$ (ppm)</th>
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<td>1.275</td>
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Table 2: Relative toxicities of examined insecticides on 4th instar larvae of S. litoralis in laboratory conditions.

- The folds were estimated depending on Speedo as the highest toxic insecticide.
- Line No. was used as cited on dPLines of examined insecticides
Fig 2:- Relative toxicities of examined insecticides on 4th instar larvae of S. litorallis (a) Lab. Strain and (b) field strain in laboratory conditions.

B. Biochemical Responses

- **AChE**

  The examined insecticides induced decrease in AChE activity in the homogenate of the two strains of S. littoralis compared with control (Figure 3). Reldan® induced the greatest decrease in enzyme activity; 0.28 and 0.34 µM/mg/min on field and laboratory strain, respectively, followed by Biolarve® (0.39 and 0.40 µM/mg/min), and Speedo® (0.43 and 0.39 µM/mg/min). Roxy® was the least potent to decrease enzyme activity; 0.57 µM/mg/min compared with control; 60 µM/mg/min on field strain.

- **Esterases**

  The examined insecticides induced alterations in esterase's activities in the two strains of S. littoralis as illustrated in Figure 4a and b. α-CE significantly increased in laboratory strain than in field strain. All insecticides exhibited values lower than control (1.43 µM/mg/min) as observed in the order: 0.14, 0.38, 0.90, 0.94, 0.84, and 0.95 µM/mg/min in laboratory strain, for Rider®, Reldan®, Biolarve®, Speedo®, Roxy®, and Grand®, respectively. Insecticide; Speedo® induced the greatest inhibition in larval homogenate of field strain (0.04 µM/mg/min) compared with control; 0.19 µM/mg/min. However, Rider® induced the greatest inhibition; 0.14 µM/mg/min in the larval homogenate of laboratory strain. Regarding β-CE, all insecticides induced inhibition in larval homogenate of field strain greater than control (0.42 µM/mg/min) arising the values; 0.25, 0.08, 0.005, 0.10, 0.08 and 0.15 µM/mg/min for Rider®, Reldan®, Biolarve®, Speedo®, Roxy® and Grand®, respectively. However, samples of laboratory strain showed activities greater than control (0.16 µM/mg/min), except Rider® and Grand® induced inhibition in larval homogenate lower than control with activities 0.03 and 0.15 µM/mg/min, respectively. The least effect was recorded for Biolarve with value; 0.43 µM/mg/min.
Fig 4: Activity of esterases (µM/mg/min) (a) α-CE and (b) β-CE in the 4th instar of larval homogenate of *S. littoralis* treated with different insecticides under laboratory conditions. Each value is the mean of three replicates±SE. No significant different obtained for the same letters at 0.05 levels.

**GST**

The examined insecticides induced changes in enzyme activity in the larval homogenate of laboratory and field strain of *S. littoralis* (Figure 5). The activities in field strain were greater than control (51.50 nM/mg/min) with values; 22.50, 15.00, 16.50, 15.50, 14.00 and 20.50 nM/mg/min for Rider®, Reldan®, Biolarve®, Speedo®, Roxy®, and Grand®, respectively. Regarding laboratory strain, insecticides induced an increase in enzyme activity greater than control (31.50 nM/mg/min), except Rider® and Grand® (9.00 and 14.50 nM/mg/min). Reldan® induced the greatest activity; 42.00 nM/mg/min.

Fig 5: Activity of GST (nM/mg/min) in 4th instar larval homogenate of *S. littoralis* treated with different insecticides under laboratory conditions. Each value is the mean of three replicates±SE. No significant different obtained for the same letters at 0.05 levels.

**ALT/AST**

The activities in the larval homogenate of laboratory and field strain are illustrated in Figure 6a and b. In field strain, ALT activities were greater than control (66.80 U/L), with values; 74.97, 38.93, and 71.93 U/L for Rider®, and Grand®, respectively. Regarding laboratory strain, the activities were 41.18, 40.68, 30.89, 19.58, 10.03 U/L for Rider®, Reldan®, Biolarve®, Speedo®, Roxy®, and Grand®, respectively. Biolarve® induced the least activity; 61.00 U/L compared with control which did not exceed 46.13 U/L. Activities of AST enzyme in laboratory and field strains were lower than control (Figure 6 b). The activities were in the following order: 16.72, 11.22, 11.44, 19.11, 30.58 and 26.81 U/L for Rider®, Reldan®, Biolarve®, Speedo®, Roxy®, and Grand®.
respectively, in the larval homogenate of field strain. While samples of laboratory strain achieved the following order: 27.11, 10.56, 26.32, 33.96 and 24.86 U/L at the same manner, respectively.

Fig 6: Activity of aminotransferases (U/L) (a) ALT and (b) AST in the 4th instar of larval homogenate of S. littoralis treated with different insecticides under laboratory conditions. Each value is the mean of three replicates±SE. No significant different obtained for the same letters at 0.05 levels.

➢ Total Protein
Protein contents in the larval homogenate of laboratory and field strain treated with insecticides are illustrated in Figure 7. In field strain, protein contents were lower than control with values: 8.23, 7.90, 8.43, 7.17, 7.04 and 6.07 mg/g biomass for Rider®, Reldane®, Biolarve®, Speedo®, Roxy®. and Grand®, respectively. Regarding laboratory strain, protein contents were in the following order: 12.97, 10.70, 6.63, 10.83, 11.97, 9.53 mg/g biomass at the same manner. The control group did not exceed 9.83 mg/g biomass.
Metabolic resistance is one of the important insecticide resistance mechanisms. General esterases and glutathione-S-transferase have been detected to cause metabolic resistance in numerous insect pests (Hemingway, 2000; Chen et al., 2007). The present work indicates that, GST and esterases in field strain were lower than the laboratory strain. This concept is in contrast with Yu et al. (2003), where activities of detoxication enzymes e.g. microsomal oxidases, GST, and hydrolases in field strains of *S. frugiperda* were higher than in the susceptible strain. On the other hand, some IGRs induced decreased in GST activity. The present findings are in accordance with Badawy et al. (2013). They reported a decrease in GST activity of earthworms *Aporrectodea caliginosa* as urgent of exposure to lufenuron.

Generally, an increase of activity of detoxication enzymes in the most universal resistant mechanism in insects, for example, esterase-based resistance to organophosphorus and carbamate insecticides is common in a range of different insect pests (Field et al., 1988; Hemingway and Karunaratne, 1998). The esterases either produce broad-spectrum insecticide resistance through rapid-binding and slow turnover of insecticide, i.e. sequestration, or narrow spectrum resistance through the metabolism of a very restricted range of insecticides containing a common ester bond (Herath et al., 1987). The majority of esterases which function by sequestration are elevated through gene amplification, (Vaughan and Hemingway, 1995). Since, enhanced metabolism is an important mechanism, thus oxidative, hydrolytic and conjugative detoxication enzyme activities toward universal substrates were measured for insecticides (Abo Elghar et al., 2005).

An exception of our findings was detected during the evaluation of AChE, showing a marked enhancement in the production of this enzyme as well as its substrate, ACh after being treated with examined insecticides. AChE has an important role in neurotransmission through hydrolyzing the neurotransmitter acetylcholine in cholinergic synapses of the nervous system and is the target site of several neurotoxic insecticides. Decrease in AChE activity in the tested larvae is attributed to inhibitory potential of examined insecticides compared with control. In contrast, previous studies stated that overproduction of ACh may be explained according to Salgado et al. (1998) who have demonstrated that spinosad could attack the nicotinic acetylcholine receptor (nAChR) with acetylcholine ACh simultaneously, as well as acting on a new site differing from the site on which ACh acts. They gave a hypothesis that there were two special sites on nAChR for Spinetoram and ACh individually. When both
Spinetoram and ACh are absent, the receptor channel will keep closed. When either of them is present or both of them are present, the channel will open up and subsequently the receptor will be activated. This assumption may be able to explain the overproduction of both AChE and ACh. However, there is no evidence to demonstrate that spinosad directly links to a site on nAChR, and it probably means that Spinetoram indirectly regulates the nAChR. Furthermore, Watson (2001) indicated that Spinetoram could also act on γ-aminobutyric acid (GABA) receptor and increase the neural activity of pest in excess and subsequently make the pest fall into a decline and be dead eventually.

All examined insecticides induced a decrease in AChE activity. This concept is in contrast reason with that obtained by phosphorus and carbamate insecticides caused high activities of enzyme in the treated larvae of *S. littoralis* compared with untreated one. It may be attributed to reflect gaining of the tested pests of resistance phenomena.

Some studies indicated that, AST and ALT activities significantly increased in *Eurygaster integriceps* after exposure to pyroxenyl (Zibaee *et al.*, 2011). Similary, Abou-Taleb *et al.* (2015) mentioned that ALT and AST activities increased in larvae of *S. littoralis* after exposure to lufenuron and chlorfluazuron compared with control. Also, an increase was recorded after exposure to emamectin benzoate (Abou-Taleb *et al.*, 2009). The changes in ALT and AST activities may be attributed to probably aiding gluconeogenesis through transamination of glucogenic amino acids to provide the emergy demand under lufenuron and chlorfluazuron toxicity. These alterations of AST and ALT may be attributed to the re-generation of hemocytes by hematopoietic organs and fat bodies that definitely needs to different amino acids prepared by transamination process (Abou-Taleb *et al.*, 2015).

**V. CONCLUSION**

The current study compared the efficiency of examined insecticides on *S. littoralis* (Bosid.). Esterases and GST enzymes quantification provide a primary investigation concern insect resistance or susceptibility to insecticide mixtures or individual application to make good control. On the other hand, changes in ALT and AST provide another mechanism to induce the toxic effect of this insecticide.

**REFERENCES**

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