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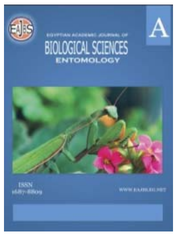

Pesticides Liability For Enzymatic Activity Variance In Field Pink Boll Worm, *Pectinophora gossypiella* (Saunders)

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ABSTRACT

Toxic influence of Chlorpyrifos, Lambda-Cyhalothrin, Methomyl and Spinosad against lab and field moths of the pink bollworm, Pectinophora gossypiella (Saund.) were tested under Laboratory conditions. Methomyl had the superior toxic effect followed by Lambda-Cyhalothrin, Chlorpyrifos and Spinosad (LC50 = 1.16, 2.45, 4.01 and 20.75 ppm respectively) to Lab insects. The field insects from Menoufia Governorate were more tolerant to toxic effect of the tested insecticides especially chlorpyrifos than Bani-Suif Governorate insects. A high significant increase of enzyme activity was determined in Glutathione-S-Transferase (GST) than MFO Cytochrome P450 (PCMAN-demethylase) of Field moths than lab ones. While the depletion in enzyme activity was presented in Acetylcholinesterase enzyme of field insects. These changes in enzymes activity of the field insects may be correlated with their tolerance ability to toxic effect of the tested insecticides.

INTRODUCTION

Several pests infest Cotton, the pink bollworm (PBW), *Pectinophora gossypiella* (Saund.) is the most destructive pest causes extreme cotton yield losses in Egypt and worldwide (El-Wakeil, 2010). In fact; insecticides are the most elective management tool and often provide the only possible method of declining insect pest populations or falling them to acceptable levels (Harein and Davis, 1992). Successfully used of Organophosphate and Pyrethroid caused reduction in population of the pink bollworm (Leonard *et al.*, 1988). Synthetic pyrethroids showed greatest fall in bollworms infestation associated with highest extent of seed cotton yield (Abd-Elrahman *et al*., 2007). Spinosad shown to be an effective pest control agent (Brickle et al., 2001) chiefly for control of lepidopteran insect pests (Wanner *et al*., 2000, Aydin and Gurkan 2006 and Al-Shannaf, 2007).

Insecticides continual application leads to development of insect resistance in different Egyptian Governorates (Abo-Sholoa *et al*., 1998). In insect; esterases together with Glutathione-S-transferase (GST) are focused on the metabolic detoxification of insecticides which results in insecticides resistance (Hou *et al*., 2008). Esterase enzyme exhibit more polymorphism than other enzymes because they act on a class of molecules; many of which come directly from external environment (Kojima *et al*., 1970). Glutathione-S-transferase (GSTs); are members of a large family of multifunctional intracellular enzymes involved in the detoxification of endogenous and xenobiotic compounds via glutathione conjugation, dehydrochlorination and glutathione peroxidase activity (Yang *et al*., 2001). Glutathione-S-transferase (GST) and cytochrome P450 (general oxidase) are two important groups of multifunctional detoxifying enzymes responsible for metabolizing an array of xenobiotic compounds as well as endogenous compounds (Booth *et al*., 1961, Feyereisen 1999, Scott and Wen 2001, Yang *et al*., 2007 and Gui *et al*., 2009). Cytochrome P450 is known to have a wide range of substrates and therefore has a greater role in the metabolism and detoxification of various compounds. The cytochrome P450 enzyme complex is often noted as the most important group of enzymes responsible for causing metabolism-based insecticide resistance (Scott, 1999).

The present study aimed to evaluate the resistance levels of in the pink bollworm moths collected from Bani-Suif and Menofeia Governorates to Chlorpyrifos, lambda- cyhalothrin, Methomyl and Spinosad insecticides compared with lab insects. Also, the activity of Acetylcholinesterase (AchE) enzyme and two detoxification enzymes; Glutathione-S-transferase (GST), and Cytochrome P450 (PCMAN-demethylase), were determined in these insects.

MATERIALS AND METHODS

Insect:

Laboratory strain of the pink bollworm (PBW), *Pectinophora gossypiella* (Saund.) was obtained from the Bollworms Research Department, Plant Protection Research Institute and reared for two years without any exposure to insecticides, in Central Agricultural Pesticides Laboratory, Agricultural Research Centre on artificial diet according to Rashed and Ammar (1985), under constant conditions (27±2 ^{o}C and 70 ± 5R.H.).

The full-grown larvae of field strains were collected from infested cotton bolls of Menofiea and Bani-Suif Governorates; during 2015 and 2016 and kept under lab constant conditions until pupation. The emerged moths were used in bioassay.

Insecticides:

Chlorpyrifos: (Pestban 48% EC, from Dow AgroSciences company) an organophosphorus compound, Non-systemic with contact and stomach effect which acts as inhibitor of Acetylcholinesterase enzyme in insects (e-pesticides manual V5-2011).

Methomyl: (Lannate 90% SP from DuPont company) a carbamate compound used as a systemic insecticide-nematicide which killing by contact and stomach effect which inhibit the activity of pest Acetylcholinesterase enzyme (e-pesticides manual V5-2011).

Lambda-Cyhalothrin: (Pilarmada 2.5% EC, from Samtrade company**)** a synthetic pyrethroid, non-systemic, with contact and stomach effect. This insecticide interacts with Sodium channels of insect nervous system neurons (e-pesticides manual V5-2011).

Spinosad: (Traser 24% SC from Dow AgroSciences company) a biochemical insecticide produced from fermentation of the soil actinomycete *Saccharopolyspora spinosa* which acts as contact and stomach poison which activates the nicotinic Acetylcholine receptor of insect nervous system (e-pesticides manual V5-2011).

Bioassay:

Residual thin film method (Vial method according to Plapp *et al.*, 1987 and Snodgrees, 1996) was used to evaluate the toxic effect of tested insecticides against PBW moths. Seven aqueous serial concentrations from each insecticide were prepared then three glass chimneys (9D×10H) were dipped in each pesticide concentration and control (treated with tap water only). Five Moths were added to each treated and control chimney. Mortality was recorded after 24hrs. of treatment, then correct with Abbot's formula (Abbot, 1925). LC_{50}, LC_{95} and slope were determined according to Finney (1971). Toxicity index (TI) (Sun, 1950), resistance ratio (RR) and resistance coefficient (RC) (Wegorek, 2011) values for each insecticide were determined.

Biochemical assay:

Enzyme extract:

For Acetylcholinesterase (AchE) and Glutathione-S-Transferase (GST) enzymes activity, 500mg of control and field insects were homogenized in 1ml sodium phosphate buffer (0.1M, pH 7) using Teflon glass homogenizer and centrifuged at 10,000g for 15 min at 4^oC (five replicates of each sample). The supernatant was used as a source of enzyme.

For PCMAN-demethylase activity, 100 mg of control and treated insects were homogenized in 0.2 ml sod. Phosphate buffer (0.1M, pH 7.8) containing 10% glycerol, 1mM DDT (1,4-dithiothreitol) 1mM EDTA (ethylenediamine tetra acetic acid), 1mM PMSF (phenylmethanesulfonylfluoride) and 1mM PTU (N-phenyl thiourea) (five replicates of each sample). The samples were centrifuged at 10,000g for 10 min at 4^oC. The supernatants were centrifuged at 18,000 for 30 min at 4^oC. The produced supernatants were collected and used as enzyme resource (Chen *et al.*, 2011) with some modification. The total protein of all moth samples was determined as Biuret reaction kit (Henry, 1964).

Enzyme Activity:

Activity of AChE enzyme was measured spectrophotometrically as Ellman *et al.*, (1961). The reaction mixture consists of 50µl of sample enzyme 10µl of 100mM AChI, (acetylthiocholine iodide), 10µI 9.2mM DTNB (5,5-dithio-bis (2-nitrobenzoic acid and potassium phosphate buffer (0.1M, pH 7.2) up to 1ml (five replicates for each sample). The increment in absorbance at 405 nm & 25^oC was recorded during 5min. The activity was expressed as nanomoles of acetylthiocholine hydrolyzed/mg $protein^{-1}/min^{-1}$. GST activity was measured based on the method of Habig *et al.*, (1974). The assay was conducted to incubating 50mM of CDNB(1-chloro-2,4-dinitrobenzen) as a substrate with 50mM GSH (reduced glutathione) and 50ul of sample enzyme in 0.1M phosphate buffer (pH7) for 5min. at 27^oC (five replicates for each sample). The activity monitored at 340 nm and expressed as nmoles of CDNB conjugated/mg $protein^{-1}/min^{-1}$. Demethylation of the model substrate P-chloro-N-methylaniline was quantified following the method of Kupfer and Bruggerman (1966). The reaction mixture contained 10µM p-chloro-N-methylaniline, 2.5mM glucose-6-phsphate (G6P), 0.4 unit of glucose-6-phosphate dehydrogenase (G6P-dh), 0.5mM nicotinamide adenine dinucleotide phosphate (NADP+) and 7.5mM magnesium chloride ($MgC1_2$). Five replicates for each sample, each replicate contained 50µl of sample enzyme and 400µl of reaction mixture the reaction proceeded at 37^oC for 10 min in a water bath and stopped with the addition of 750µl of p-dimethylaminobenzaldehyde (PDAB) solution, then centrifuged. The product p-chloroaniline was quantified by comparing absorbance at 445 nm to simultaneously determined standard curve (0-50nmol). the activity of enzyme was represented as nmoles of p-chloroaniline/mg $protein^{-1}/min^{-1}$.

Data analysis:

The total protein content and enzyme activities of Lab and field moths were statistically analyzed as Means ± SE (standard error) by using SPSS program V25. Differences were considered by significant of P less than 0.05.

RESULTS

1- Bioassay:

The obtained results showed that, Methomyl was the most toxic insecticide (LC_{50}= 1.16 ppm and TI= 100%), followed by Lambda-Cyhalothrin (2.45ppm and 28.9%), Chlorpyrifos (4.01ppm and 47.4%) and Spinosad (20.65ppm and 5.6%), Table 1.

Table 1. Toxic effect of tested insecticides on lab moths of the pink bollworm, *P. gossypiella*.

Insecticides	LC_{50} (ppm) C. l.	Slope ± S. E	T. I
Methomyl	1.16 (0.87-1.76)	1.07 ± 0.15	100
Lambda-Cyhalothrin	2.45 (2.07-2.99)	1.78 ± 0.21	28.9
Chlorpyrifos	4.01 (3.41-4.69)	1.80 ± 0.16	47.4
Spinosad	20.65 (19.92-35.91)	1.15± 0.15	5.6

C. l. = Confidence limits

Table 2. Illustrated response of the field moths from Bani-Suif governorate to toxic effect of Lambda-Cyhalothrin (LC_{50}= 9.68 ppm and T. I = 100%), Methomyl (10.36 ppm and 93.41%), Spinosad (25.75 ppm and 37.6%) and Chlorpyrifos (586.28 ppm and 1.7%) respectively. In comparing $LC_{50's}$ values of Lab and field moths, the resistance ratio (RR) was produced. In Ban-Suif insects, RR reached to 4, 8.9, 1.3 and 146.2 folds respectively. Resistance coefficient (RC) of Bani-Suif moths was resulted from comparing $LC_{95s'}$ values of field insects with recommended field concentration of tested insecticides which reached to 1.1, 0.06, 2.2 and 3.4 folds, respectively.

Table 2. Toxic effect of tested insecticides on Bani-Suif moths of the pink bollworm, *P. gossypiella*.

Insecticide	LC_{50} (ppm) C. l.	LC_{95} (ppm) C. l.	Slope ± S. E	T. I	RR Folds	RC Folds
Lambda-Cyhalothrin	9.68 (8.08:11.93)	105.66 (65.62:210.83)	1.58 ± 0.16	100	4.0	1.1
Methomyl	10.36 (8.86:12.08)	81.01 (58.61:126.13)	1.84 ± 0.16	93.4	8.9	0.06
Spinosad	25.75 (17.84:24.26)	156.47 (112.074:246.92)	1.88 ± 0.16	37.6	1.3	2.2
Chlorpyrifos	586.28 (479.82:707.17)	8033.11 (5136.21:15471.65)	1.45 ± 0.15	1.7	146.2	3.4

C. l. = Confidence limits

Table 3. Shown; Lambda-Cyhalothrin had the most toxic effect (LC_{50}=10.79 ppm and T.I = 100%) on Menofeia insects and Chlorpyrifos was the least toxic insecticide (566.58 ppm and 1.9%). RR and RC of Menofeia insects reached to 4.4 and 1.7 folds for Lambda-Cyhalothrin, 12.9 and 0.07 folds for Methomyl, 2.0 and 4.9 folds for Spinosad and 141.3 and 26.2 folds for Chlorpyrifos, respectively.

Table 3. Toxic effect of tested insecticides on Menofeia Moths of the Pink Bollworm, *P. gossypiella*.

Insecticide	LC_{50} (ppm) C. l.	LC_{95}(ppm) C. l.	Slope ± S. E	T. I	RR Folds	RC Folds
Lambda-Cyhalothrin	10.79 (8.77:13.89)	160.48 (89.50:386.59)	1.40 ± 0.15	100	4.4	1.7
Methomyl	14.96 (12.98 :17.42)	99.21 (73.44:146.78)	2.002 ± 0.15	72.1	12.9	0.07
Spinosad	41.71 (34.42:53.84)	349.37 (204.28:828.23)	1.78 ± 0.22	25.9	2.0	4.9
Chlorpyrifos	566.58 (417.43:805.58)	62785.32 (24275.33:264753.7)	0.81 ± 0.09	1.9	141.3	26.2

C. l. = Confidence limits

Biochemical assay:

Evaluation of the total protein contents indicated that, lab moths had a low protein concentration (3.31 ± 0.662 mg/100mg body weight) in their bodies. This content increased with high significant value (84.1%) in Menofeia moths and very high value (118.1 %) in Bani-Suif moths compared with lab insects Table 4 and Figs. 1 and 5.

The specific AchE activity was 96.37 ± 9.2 × 10^{-7}, 47.33± 14.43. ×10^{-7} and 84.61 ± 3.15 × 10^{-7} nmole of acetylcholine hydrolyzed/min/mg protein in lab, Bani-Suif and Menofeia moths. The high significant depletion in enzyme activity (50.9%) was recorded in Bani-Suif moths, while a slight one (12.2%) was presented in Menofeia insects than lab insects Table 4 and Figs. 2 and 5. Also, data in Table 4 and Figs. 3, 4 and 5. Show a highly significant increase (119.6 and 116.6%) in GST activity was recorded in Menofeia and Bani-Suif insects than lab ones. The insignificant increase (11.5.and 1.3 %) in cytochrome P450 (PCMAN-demethylase) activity was presented in Menofeia and Bani-Suif insects, when than lab insects

Table 4. The total protein concentration and activity of acetylcholinesterase (AchE), Glutathione-S-transferase (GST) and Cytochrome P450 (PCMAN-demethylase) enzymes in the whole-body homogenate of lab and field moths of the pink boll worm, *P. gossypiella*.

Insect population	**Total Protein concentration**		**AchE**		**GST**		**PCMAN-demethylase**	
	Mean ± SE (mg/100mg B.W.)	Change %	Mean activity ±SE nmole/min/mg Pt.	Change %	Mean activity ±SE nmole/min/mg Pt.	Change %	Mean activity ±SE nmole/min/mg Pt.	Change %
Laboratory	3.31 ± 0.665^{C}	0	96.37 ± 9.206 $*10^{-7a}$	0	12.60 ± 0.923^{b}	0	0.078 ± 0.016^{a}	0
Bani-Suif	7.22 ± 0.213^{a}	(+) 118.1	47.33 ± 14.430 * 10^{-7b}	(-) 50.9	27.29 ± 1.036^{a}	(+) 116.6	0.079 ± 0.020^{a}	(+) 1.3
Menofeia	6.15 ± 1.246^{b}	(+) 84.8	84.61 ± 3.158* 10^{-7a}	(-) 12.2	27.67 ± 2.125^{a}	(+) 119.6	0.087 ± 0.008^{a}	(+) 11.5

(+) increase (-) decrease

Change % = Mean activity of field – Mean activity of lab/ Mean activity of lab X 100

Means followed by the same letter at the same column are not significantly difference ($P< 0.05$).

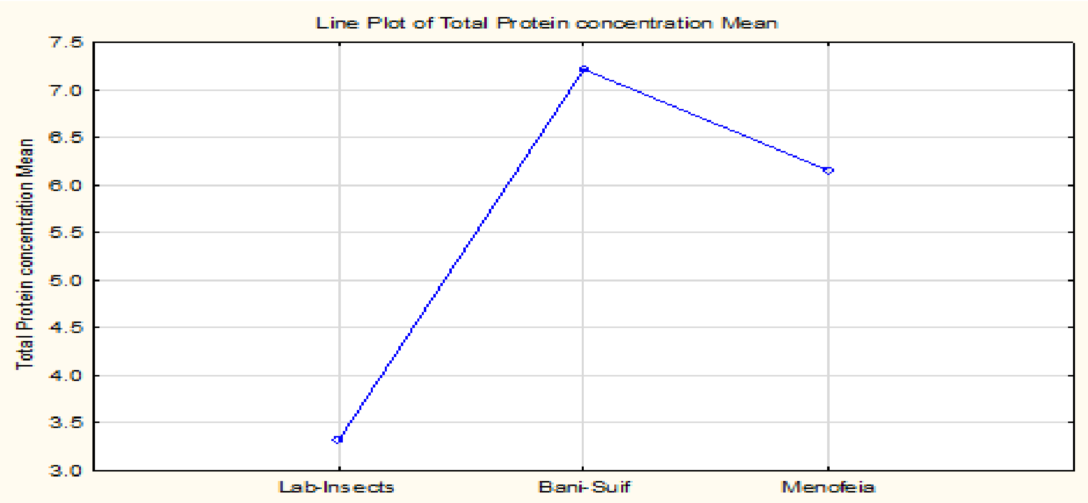

Fig. 1. The total protein concentration in whole-body homogenate of lab and field moths of the pink boll worm, *P. gossypiella*.

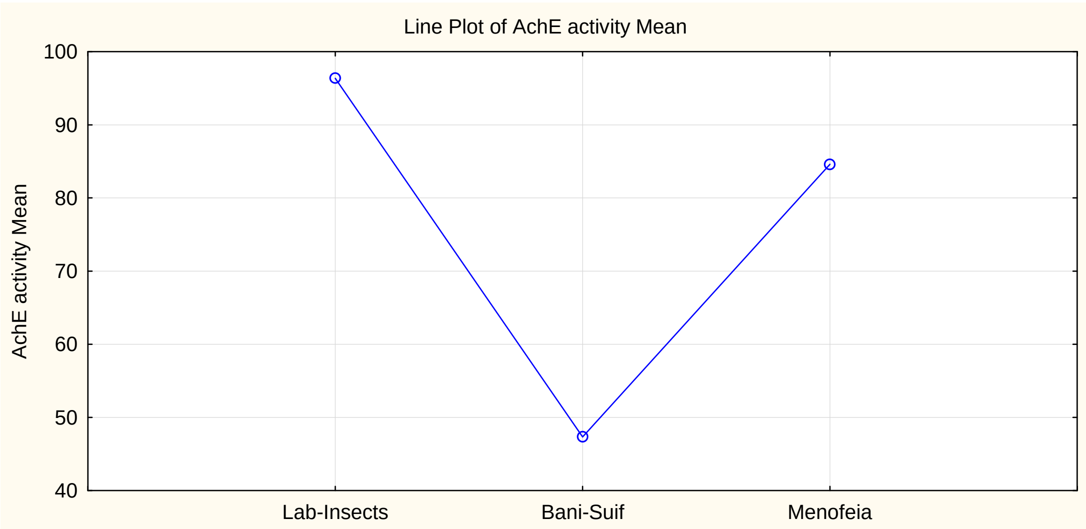

Fig. 2. Activity of acetylcholinesterase (AchE) enzyme in whole-body homogenate of lab and field moths of the pink boll worm, *P. gossypiella*.

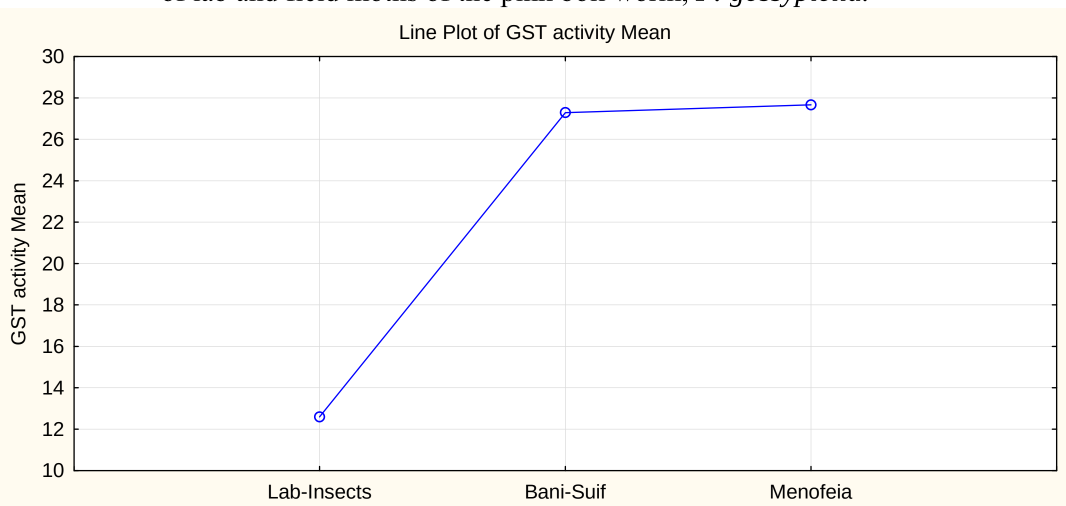

Fig. 3. Glutathione-S-transferase (GST) enzyme in whole-body homogenate of lab and field moths of the pink boll worm, *P. gossypiella*.

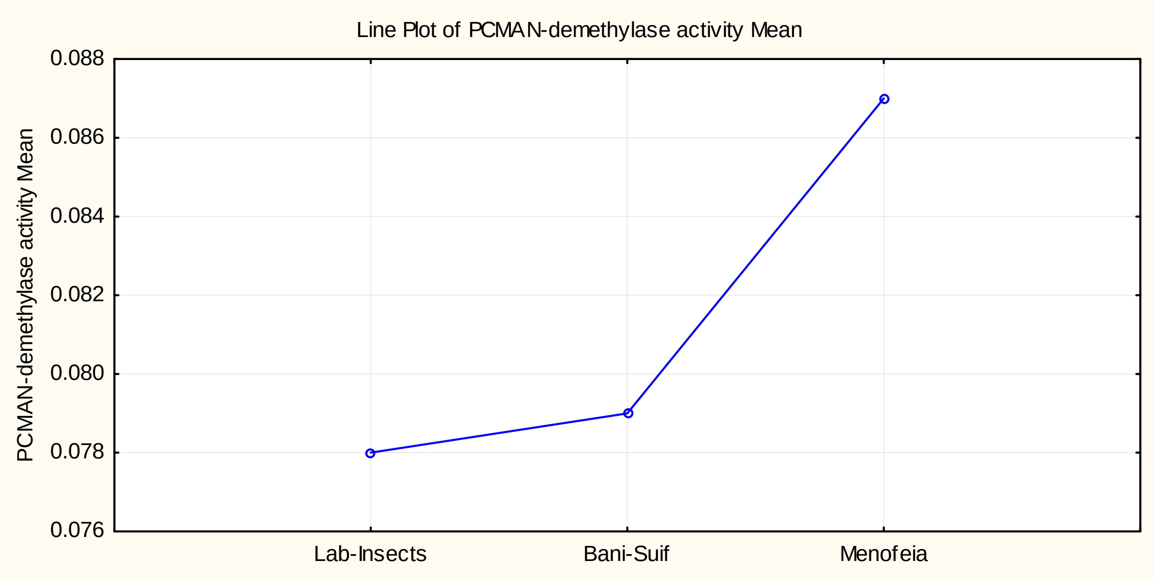

Fig. 4. Cytochrome P450 (PCMAN-demethylase) enzyme in whole-body homogenate of lab and field moths of the pink boll worm, *P. gossypiella.*

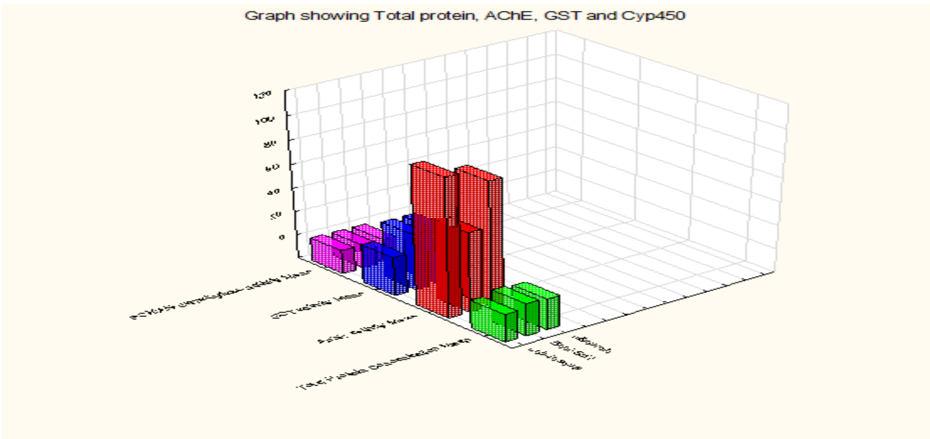

Fig. 5. The total protein concentration and activity of acetylcholinesterase (AchE), Glutathione-S-transferase (GST) and Cytochrome P450 (PCMAN-demethylase) enzymes in whole-body homogenate of lab and field moths of the pink boll worm, *P. gossypiella.*

DISCUSSION

The result of toxicological experiment points to presence of difference in response of the field pink bollworm, *P. gossypiella* against tested insecticides. The high levels of resistance ratio of the two field strains were observed in Chlorpyrifos treatment compared with those of Lambda-Cyhalothrin, Methomyl and Spinosad. The highly resistance coefficient was presented in Menofeia insects to Chlorpyrifos insecticide. These results agree with those of Abo-Elghar *et al.* (2005) who mentioned that current levels of resistance to selected insecticides in field locations were moderated to high which due to intensive use of these insecticides. Field populations insect's susceptibility to insecticides was positively correlated with the number of chemical sprays in the field (Reyes *et al.*, 2012). The evaluation of resistance in the field to commonly used insecticides is important in the establishment and maintenance of a successful insect pest management strategy (Kristensen, 2005). the pyrethroid Lambda-Cyhalothrin was the most effective insecticide and the field moths of *P. gossypiella* built up low resistance level against it, while organophosphate profenofos was good toxicant with the highest level of resistance (Radwan and El-Malla, 2010).

Bioassay and biochemical methods are the primary means of testing for insecticides resistance. biochemical techniques are advantageous because they test for activity that is directly linked to a resistance mechanism (Miyata, 1986). If the biochemical bases of resistance can be determined highly sensitive monitoring techniques can be devised; these techniques are key factors in developing successful resistance management programs (Ffench- Constant and Roush, 1990). The examination of esterase activity and intensive acetylcholinesterase, which limits the use of organophosphate and carbamate pesticides are the important biochemical techniques. (Brewer and Trumble, 1989). In our study the field moths had high significant increase in protein concentration and activity of glutathione-S-transferase enzyme with a slight increase in activity of MFO Cytochrome p450 enzyme comparing with lab moths. The activity of acetylcholinesterase was high decreased in Bani-Suif moths, while this reduction in enzyme viability was slightly in Menofeia moths compared with lab insects. These low levels of acetylcholinesterase in field insects were correlated with their high levels of resistance to Chlorpyrifos. These results agree with those of (Magana *et al*., 2008) who reported that the correlation observed between reduced AchE activity and reduced sensitivity to Malathion in wild strain, may be a very important fact indicating possible fitness costs associated with AchE activity. The resistance to organophosphorus insecticides can be due to mutation on the target site, the acetylcholinesterase (Walsh *et al*., 2001). GST activity in *Spodoptera littoralis* (Boisd.) exposed to lindane for 8 h showed a 1.5-fold elevation in enzyme activity over control (Lagadic *et al*., 1993). The evaluating mechanism would be involved in insecticide resistance of populations of *P. gossypiella*, presenting an increased MFO activity in populations (Reyes *et al*., 2012). It appears that enhanced oxidative metabolism mediated by Cytochrome P450 monooxygenase was a major mechanism for insecticide resistance in the western flower thrips (Chen *et al*., 2011).

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ARABIC SUMMARY

علاقة مبيدات الآفات بالنشاط الإنزيمي في ديدان اللوز القرنفلية الحقلية، بكتينوفورا جوسبيلا (سويندرز)

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تم اختبار التأثير السام لكل من مبيدات كلوربيريفوس، لامبدا-سيهالوثرين، ميثوميل وسبينوساد ضد فراشات دودة اللوز القرنفلية، بكتينوفورا جوسيبيلا (سوند.) المعملية والحقلية تحت الظروف المعملية، كان مبيد ميثوميل هو الأكثر فاعلية يليه مبيدات لامبدا-سيهالوثرين وكلوربيريفوس وسبينوساد وكان التركيز النصفي المميت (1.16، 2.45، 4.01 و20.75 جزء بالمليون على التوالي) للحشرات المعملية. وكانت الحشرات الحقلية من محافظة المنوفية أكثر تحملا للأثر السام للمبيدات الحشرية التي تم اختبارها وخاصة مبيد الكلوربيريفوس عن حشرات بني سويف. وعند قياس النشاط الإنزيمي للحشرات وجدت زيادة معنوية مرتفعة في إنزيم جلوتاثيون-إس-ترانسفيريز عن إنزيمات السيتوكروم في الحشرات الحقلية عنه في الحشرات المعملية. في حين أن انزيم الأسيتيل كولين استريز كان أقل في الحشرات الحقلية عنه في الحشرات المعملية. وهذه التغيرات في نشاط إنزيمات الحشرات الحقلية ربما يكون مرتبط بقدرتها على تحمل التأثير السام للمبيدات المختبرة.