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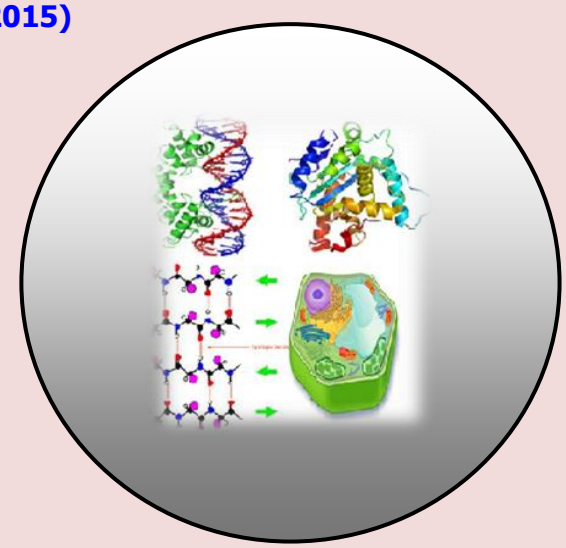

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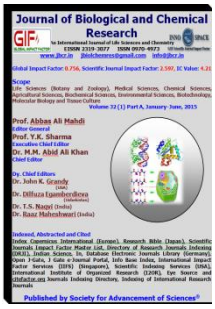

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RESEARCH PAPER

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Monitoring of Insecticides Resistance Level and Biochemical Changes in two Field Populations of Pink Bollworm, *Pectinophora gossypiella* (Saunders)

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ABSTRACT

The pink bollworm, Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechiidae), is an important pest of cotton crop in Egypt. The efficacy of five recommended insecticides, belonging to the organophosphate and synthetic pyrethroid groups, was evaluated under the laboratory conditions against two field populations of P. gossypiella collected from the Sharqia and Faiyum governorates in Egypt. Additionally, insecticide resistance level was monitored using the residual thin film technique. Results showed that, pyrethroid lambda cyhalothrin was the most effective insecticide with low to moderate resistance levels, whereas the organophosphate chlorpyrifos was the least toxic insecticide with high resistance level. Faiyum insects exhibited higher resistance levels to all tested insecticides compared to those from Sharqia. Biochemical assays revealed significant increase in protein content and acetyl cholinesterase, α-esterase & mixed function oxidase activities in Faiyum insects compared to the laboratory strain. A remarkable reduction in GST activity was also recorded in this population. Also insects from Sharqia, showed significant decrease in protein content and insignificant decrease in AChE and α esterase activities. While GST activity was significantly increased and slightly elevated in MFO activity, compared to the laboratory strain. Fractionation of total protein using PAGE revealed some variations in the two field populations in protein band number, their molecular weight, and staining intensity compared to the laboratory strain.

In conclusion, the elevation in protein content, α-esterase, and MFO, or GST activities may be involved in increased detoxification of the organophosphate and pyrethroid insecticides in the field populations, which may facilitate the development of insecticide resistance.

Keywords: Pectinophora gossypiella, Organophosphate and Pyrethroid insecticides, resistance, Metabolic enzymes, Protein profile.

INTRODUCTION

Cotton is attacked by many insect species during the different stages of its growth (Abd El – Mageed *et al.,* 2007). The pink bollworm, *Pectinophora gossypiella* (Saunders), is one of the most serious economic pests and is capable of causing considerable damage to cotton production (Korejo *et al*., 2000; Unlu, 2004 and El-Aswad & Aly, 2007). Protection of the cotton plant and mass production of harvested cotton fibers mainly depend on the efficient control of this pest. As part of the pest management program, many insecticides belonging to the organophosphate and pyrethroid groups have been used to effectively control pink bollworm infestation (Leonard *et al*., 1988; and Magdy *et al*., 2009). The continuous and intensive use of insecticides against this cotton pest has led to the emergence of strains that are more tolerant and resistant to these insecticides (Khurana & Verma 1990 andKranthi *et al*., 2002), and this insect has become difficult to control.

The most common mechanism of insecticide resistance in insect pests is attributable to an enhanced metabolic detoxification system that comprises esterases, glutathione-S-transferase (GST) (;Osman *et al.,* 2005; Muthusamy *et al.*, 2013 and Zibaee *et al*., 2016), and the cytochrome P450 monooxygenase (MFO) (Yu *et al*., 2003; Li *et al*., 2007; Eziah *et al.,* 2009). Metabolic enzyme activity significantly contributes to xenobiotic detoxification in many lepidopteran insects (Baek *et al.,* 2005). Thus, metabolic enzymes protect the insect by detoxifying the insecticide molecules, converting them to nontoxic compounds and/or into a form that is more suitable to rapid excretion from the insect body. Insensitivity of insecticide target sites is another important resistance mechanism (Brun-Barale *et al*. 2005 and Cassanelli *et al*., 2006). The insensitivity of the target site to organophosphates and pyrethroids is predominantly due to the activity of the acetylcholine esterase enzyme (AChE) (Baek *et al*., 2005 and Lee *et al*., 2007).

Conventional bioassay methods, which are used for making management decisions, considered the basic techniques used for continuous monitoring and resistance levels to various insecticides in field populations (Margaritopoulos *et al*., 2008). This information is essential for changing to a different insecticide class or to an alternative control strategy to avoid increasing selection pressure on populations (Prabhaker *et al*., 1996). However, early detection of resistance is very difficult using such conventional bioassay methods, especially when resistance is a recessive trait (Clark, 2010). Furthermore, it is difficult to collect large numbers of these insects.

Biochemical assays may be used along with bioassay methods as a useful technique to detect and monitoring insecticide resistance among field populations in early stages of development. Biochemical assays measure the activity of specific metabolic enzymes related to insecticide resistance and the changes in sensitivity at the insecticide target site (Roditakis *et al.*, 2009).

Therefore, the present work was undertaken to detect resistance to certain organophosphate and synthetic pyrethroid insecticides, which are regularly used in cotton fields in two field populations of *Pectinophora gossypiella* (Saund.) collected from the Sharqia and Faiyum governorates in Egypt. The changes in the activity of certain metabolic enzymes, as well as polymorphisms in the protein profile, were also determined to confirm their vital role in the development of resistance among these insects.

MATERIAL AND METHODS

Insect populations

Susceptible laboratory strain: The susceptible laboratory strain (lab-strain) of the pink bollworm, *P. gossypiella,* was obtained from the Bollworm Research Division, Plant Protection Research Institute, Agriculture Research Centre, Dokki, Giza, Egypt. This strain was reared on an artificial diet in the Central Agricultural Pesticides Laboratory (CAPL) as described by Rashad and Ammar (1984) under laboratory conditions (27 ±2 ^{o}C and 70 ±5% RH) for five years without any exposure to insecticides.

Field populations: The field populations of *P. gossypiella* were collected from infested cotton bolls obtained from cotton fields in the Sharqia and Faiyum governorates during the 2015. Full-grown larvae were released from the infested boll and kept under laboratory conditions until pupation, and the emerged moths were directly used for assays. Insecticides used

Organophosphate insecticides: Profenofos (Cord 72% EC) and Chlorpyrifos (Chlorzed 48% EC).

Synthetic pyrethroid insecticides: Lambda cyhalothrin (Agristar 5% EC), Esfenvalerate (Fenirate-S 5% EC), and Fenpropathrin (Fenthrin 30%). All insecticides used were supplied by CAPL (Dokki, Giza, Egypt).

Bioassay

Bioassays were performed to evaluate the toxicity of the above-listed insecticides against newly emerged moths of *P. gossypiella* using the residual thin film method as described by Plapp *et al.* (1987) with some modifications. Five concentrations of each insecticide were prepared and clean glass lamp chimney used as cages (9cm height and 6cm in diameter) were dipped in each concentration for 20 s and left to dry at room temperature. Ten newly emerged moths (0 day-old) were introduced into each individual treated and untreated glass chimney cages; cages dipped in water (untreated) were used as controls. Three replicates for each concentration and controls were maintained, and all cages were covered with a muslin cloth to allow air circulation. Mortality was recorded after 24 h of treatment, converted to percentage, and corrected as compared to control moths according to the Abbott formula (Abbott, 1925). The LC_{50} and slope values for each insecticide were calculated by Probit analysis using Ldp-line software according to Finney (1971), and the toxicity index (TI) was calculated using the following equation (Sun, 1950):

Toxicity index (TI) = LC_{50} of the most effective compound / LC_{50} of the other tested compound ×100

Additionally, resistance ratio (RR) was estimated using the following equation:

Resistance Ratio (RR) = LC_{50} of the field strain / LC_{50} of the laboratory strain.

According to these RRs that level of insecticide resistance was classified as reported by Torres-Vila *et al.* (2002). Low resistance (RR=2-10), moderate resistance (RR=11-30), high resistance (RR=31-100).

Biochemical assays

Enzyme Extract

Two hundred milligrams of laboratory and field moths of the pink bollworm were homogenized in Tris buffer pH 7.8 (1:5 w/v). The homogenates were centrifuged at 10,000 rpm for 20 min at 4 ^{o}C, the resulting supernatants transferred to new tubes, and preserved at –20^{o}C until biochemical analyses.

Determination of total protein content

Protein content was determined by the Biuret reaction (Henry 1964).

Metabolic enzyme activity

Acetylcholinesterase (AChE) activity

Acetylcholinesterase (AChE) activity was measured according to the method described by Simpson *et al.* (1964), using acetylcholine bromide (AChBr) as substrate. The reaction mixture contained 200 µL enzyme solution, 0.5 mL 0.067 M phosphate buffer (pH 7.0), and 0.5 mL AChBr (3 mM). The test tubes were incubated at 37 °C for exactly 30 min, 1 mL of alkaline hydroxylamine (equal volumes of 2 M hydroxylamine chloride and 3.5 M NaOH) added to the test tubes, followed by 0.5 mL of HCl (1 part of conc. HCl and 2 parts of distilled H_2O), the mixture shaken vigorously, and allowed to stand for 2 min. Ferric chloride solution (0.5 mL, 0.9 M $FeCl_3$ in 0.1M HCl) was added and mixed well. The decrease in AChBr concentration due to the hydrolysis by AChE was read at 515 nm using a double beam ultraviolet/visible spectrophotometer (Milton Roy Co. USA).

Non-specific esterase activity

Alpha esterase activity (α-esterase) was determined according to Van Asperen (I962) using α-naphthyl acetate as substrate.

The reaction mixture consisted of 5 mL substrate solution (3 x 10^{-4} M α-naphthylacetate, 1% acetone, and 0.1 M phosphate buffer, pH 7.0) and 20 µL of insect homogenate. The mixture was incubated for exactly 15 min at 27 °C, 1 mL of diazo blue color reagent (prepared by mixing 2 parts of 1% diazo blue B and 5 parts 5% sodium lauryl sulfate) added, and the developed color read at 600 nm for α-naphthol produced from hydrolysis of the substrate. The amount of α-napthyl acetate was calculated from a standard curve of α-naphthol.

Glutathione S- transferase (GST) activity

Glutathione S-transferase (GST) activity was assayed as described by Habing *et al.* (1974). Glutathione S-transferase (GST) catalyzes the conjugation of reduced glutathione (GSH) with 1-chloro 2,4-dinitrobenzene (CDNB) via the -SH group of glutathione. The reaction mixture consisted of 1 mL of potassium phosphate buffer (0.1 M, pH 6.5), 100 µL of GSH, and 200 µL of insect homogenate. The reaction was started by adding 25 µL substrate solution. The concentrations of GSH and CDNB were adjusted to 5 mM and 1mM, respectively. The reaction was incubated at 30 °C for 5 min. The increase in absorbance at 340 nm was recorded against an enzyme blank solution containing all components except the enzyme to determine the rate of the reaction (substrate conjugated per minute per insect) using a molar extinction coefficient of 9.6 mM^{-1} cm^{-1}.

Mixed function oxidases (MFO) activity

P-nitroanisole o-demethylation was estimated to determine mixed function oxidase activity according to the method of Hansen and Hodgson (1971), with slight modifications. The standard incubation mixture contained 1 mL sodium phosphate buffer (0.1 M, pH 7.6), 200 µL enzyme solution, 0.2 mL NADPH (final concentration 1 mM), 0.2 mL glucose-6-phosphate (G6P, 1 mM final concentration), and 50 µg glucose-6-phosphate dehydrogenase (G6PD). The reaction was initiated by the addition of p-nitroanisole in 10 µL of acetone to give a final concentration of 0.8 mM and incubated for 30 min at 37 °C. The reaction was terminated by adding 1 mL HCl (1N), p-nitrophenol was extracted with $CHCl_3$ and 0.5 N NaOH, and absorbance was measured at 405 nm. An extinction coefficient of 14.28 mM cm^{-1} was used to calculate 4-nitrophenol concentration.

Separation of total protein content

Proteins were separated by Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) technique according to the method of (Laemmli, 1970). Samples containing equal amount 30 µg of protein from whole body homogenates of laboratory and field *P. gossypiella* moths were denatured in sample buffer (1:1) for 5 min at 100 °C and analyzed on 10% (w/v) SDS-PAGE gel. After electrophoresis apparatus was prepared for run, the samples that will be observed are loaded into the wells. To determine the molecular weight of subunits of the refractionated sample protein, 15 µl of molecular weight marker proteins were loaded on the gel in the first well. Loading tips were used for loading the samples under the tank buffer in the wells. The gel sandwiches were removed from the casting stand and were inserted into the electrophoresis chamber. The upper and lower electrophoresis chambers were filled with tank buffer and refractionation process was carried out at room temperature (20 to 25 ºC), 45 mA constant current and terminated until the dye reached the bottom of the separating gel, then the gel was fixed and stained for ≥ 24 hrs in 0.1 Coomassie Brilliant Blue R-250 reagent with gentle shaking. It was destained for 1 hr in destaining solution until the gel between the protein bands become colorless. After destaining, the gel was photographed. Scanning molecular weight and the relative mobility (Rm), using phoretix 1D Quantifier program.

Statistical analysis

Data are presented as mean ±SE. Significant differences between the mean values of the laboratory strain and the two field populations were calculated by one-way analysis of variance (ANOVA), at $P \leq 0.05$.

RESULTS AND DISCUSSION

Bioassay

Efficacy of tested insecticides against laboratory and field populations of *P. gossypiella*

According to the LC_{50} values and toxicity index results revealed that profenofos was the most effective insecticide against the lab-strain moths of *P. gossypiella* (LC_{50} = 2.93 ppm and TI 100%), followed by lambda-cyhalothrin (LC_{50} = 3.32 ppm and TI 88.25%), and esfenvalerate (LC_{50} = 8 .22 ppm and TI 35.64%), while fenpropathrin (LC_{50} = 14.15 ppm and TI 21.27%) and chlorpyrifos (LC_{50} = 14.44 ppm and TI 20.29%) showed least efficacy (Table 1).

On the other hand, in the field moth populations from Sharqia and Faiyum, lambda-cyhalothrin (LC_{50} = 13.39 and 50.36 ppm, and TI = 100%) was the most effective insecticide, followed by esfenvalerate (LC_{50} = 51.84 and 110.21 ppm and TI 25.83 and 45.69 %), profenofos (LC_{50} = 58.89 and 118.74ppm, TI = 22.74 and 42.41%), fenpropathrin (LC_{50} = 79.66 and 157.73 ppm, and TI = 16.81 and 31.93 %). Chlorpyrifos showed the least toxicity (LC_{50}= 750 and 1349.50 ppm and TI=1.79 and 3.73 %) on moths from Sharqia and Faiyum, respectively.

Level of resistance in two field populations of *P. gossypiella*

Compared to the lab-strain, the field population collected from the Faiyum governorate exhibited higher resistance ratios to all tested insecticides at the LC_{50} level than that collected from Sharqia governorate (Table 1). The highest level of resistance (RR: 92.81- and 51.58-fold) were obtained with chlorpyrifos treatment in Faiyum and Sharqia population, respectively followed by profenofos (40.53-fold) in Faiyum moths. Whereas moderate resistance level (RR: 20.09-fold) was obtained with profenofos treatment in Sharqia moths. On the other hand, compared to the lab-strain moths, all tested pyrethroids showed low resistance levels that ranged from 4.03- to 6.31-fold in Sharqia moths and moderate resistance levels in Faiyum moths that ranged between 10.92- to 15.17-fold .

It is clear from these results that the lambda cyhalothrin was the most toxic compound with low or moderate resistance levels in Sharqia and Faiyum moths, respectively, whereas, the organophosphate chlorpyrifos was the least toxic compound with high resistance levels in both field populations.

These results are in accordance with those obtained by Radwan and El Malla (2015) who found that the pyrethroid lambda cyhalothrin was the most toxic to field moths of *P. gossypiella* and the insects exhibited low resistance level against it, while the organophosphate profenofos, although an effective toxicant was associated with the highest resistance.

The presence of resistance in the two field populations of *P. gossypiella* to some tested insecticides may be due to the extensive use of these insecticides in control programs for these governorates. To effectively counter this phenomenon, a different class of insecticides or alternative control strategies could be used to avoid increasing selection pressure on these populations (Prabhaker *et al*., 1996).

Similarly, Armes *et al*., 1996 found higher resistance levels *in H. armigera* in regions where the excessive application of chlorpyrifos is common. This observation explains the importance of the seasonal differences in chlorpyrifos resistance at locations under investigation (Kanga *et al*., 2003). Thus, the evaluation of resistance in the field to commonly used insecticides is important for both establishment and maintenance of a successful insect pest management strategy (Kristensen, 2005).

Resistance to insecticides in field populations may also appear in regions with low insecticides use due to the high migration rate of insects and the genetic mixing of populations, which can facilitate the rapid spread of resistance alleles among isolated populations.

Biochemical assays

Protein content of *P. gossypiella* populations

As shown in Table 2 protein content was significantly higher in Faiyum moths (60.96%) but lower in Sharqia moths (–15.5%) compared to lab-strain.

These results are in agreement with the findings of Nour El-Hoda *et al.,* (2012), who report that total protein concentration was higher in field population of the Pink bollworm than in susceptible strain. On the other hand, Nath *et al*., (1997) mentioned that protein depletion in tissues may constitute a physiological mechanism that might play a role in compensatory mechanisms under insecticidal stress to provide the Krebs cycle intermediates by retaining free amino acid content in the insect hemolymph.

Enzyme activity of *P. gossypiella* populations

As listed in Table 2, a significant elevation (14.84%) in AChE activity was recorded in Faiyum moths compared to the lab-strain. Further, both α-esterase and MFO activities were significantly higher (33.42% and 34.8%, respectively) compared to the lab-strain, while simultaneously, a remarkable reduction in GST activity (−58.57%) was recorded. These results are in confirmation with the increase in insecticide resistance in this population. In Sharqia field insects, AChE and α-esterase enzyme activity showed an insignificant decrease (–4.80% and –5.77%, respectively), compared to the lab-strain, while GST activity was significantly higher (43.2%) and MFO activity was only slightly elevated (6.61%). These results indicate that, in this governorate, GST may play an important role in insecticide degradation. The present investigation was conducted to assess insecticide resistance in insect field populations during early stages of development using biochemical assays as a rapid analytical method coupled with conventional bioassays to avert failure to control the infestation.

It is interesting to note that the variation in resistance ratios between these two field populations may be due to the detoxification of the tested insecticides by metabolic enzymes. In this context, we found a positive correlation between elevated AChE, α- esterase and MFO activities and the development of resistance to organophosphate and pyrethroid insecticides in the Faiyum insect population. Contrastingly, the Sharqia population exhibited a significant increase in GST activity and a mild increase in MFO activity, which may be associated with variable resistance levels to the tested insecticides.

These results are supported by the observations from previous studies, which report that insecticide resistance may be due to the enhanced activity of detoxifying enzymes (Baek *et al*., 2005; Haung and Han, 2007;Rodríguez *et al*., 2010 and Kshirsagar *et al.,* 2012) or the insensitivity of the acetylcholinesterase enzyme to organophosphates (Gunning *et al*., 2001; Osman *et al.,* 2005 and Nour El-Hoda *et al*., 2012).

Our results also agree with those obtained by (Byrne and Toscano, 2001)) who report that there is a positive correlation between AChE enzyme quantity and resistance to OP insecticides as AChE plays an important role in the detoxification of organophosphate and carbamate insecticides by overproduction of acetylcholine esterase in resistant insects.

Resistance to organophosphates (Chlorpyrifos) has been associated with elevated esterase activity in *P. gossypiella* (Abu-El Seoud *et al*., 2005), *Aedes albopictus* (Das and Dutta, 2014), *Plutella xylostella* (Gong *et al*., 2013), and *Tetranychus urticae* (Recep & Sibel Yorulmaz, 2010). Several studies have revealed that esterases play an important role in the metabolism of organophosphates and pyrethroids (Tang and Zhou 1993; Zhang *et al*., 2010)

Non-specific esterases are known to hydrolyze and catalyze the hydrolysis of a variety of insecticidal esters such as organophosphates, pyrethroids, and carbamate compounds into their corresponding acid and alcohol compounds (Devonshire, 1991). This transformation increases the polarity of the insecticidal metabolites, which can then be excreted more readily from the insect body. Esterases can also sequester insecticides such that these toxic molecules are no longer available for interactions with target proteins (Panini *et al.*, 2016).

Enhanced MFO activity in the two field populations used in this study may also be associated with an increase in resistance level to organophosphate insecticides, especially Chlorpyrifos, and could have contributed to the occurrence of moderate resistance to pyrethroids in the Faiyum insects.

Similarly, Huang and Han (2007) found that field strain of *Spodoptera. litura* generally exhibit higher microsomal monooxygenase activity than the laboratory susceptible strain. Previous studies have revealed that mixed function oxidases (MFOs) play an important role in the metabolism of many insecticide classes, including carbamates, organophosphates, pyrethroids, and neonicotinoids by catalyzing various reactions such as epoxidation, hydroxylation, N-dealkylation, O-dealkylation or desulfurization (Panini *et al.*, 2016).

Therefore, higher levels of metabolic enzyme activity (33.42 % and 34.8 % for esterase and MFO, respectively) in Faiyum moths may be due to the overexpression of esterase (Field *et al*., 2002) and cytochrome P450 genes (Li *et al.,* 2007).

In this study, we found no correlation between resistance to the tested insecticides and GST activity in the Faiyum field population, even though it is possible that the elevation in GST activity in the Sharqia field population contributed to organophosphate resistance (Das and Dutta, 2014 and Nehare *et al.*, 2010).

Table 1. The response of *P. gossypiella* moths collected from Sharqia and Faiyum Governorate against five insecticides belonging to organophosphate and synthetic pyrethroid groups under laboratory conditions.

Chemical group	Common name	Trade name	Laboratory strain			Sharqia Governorate				Faiyum Governorate			
			LC_{50} values in ppm (CL)	Slope ± SE	TI %	LC_{50} values in ppm (CL)	Slope ± SE	TI %	RR (Fold)	LC_{50} values in ppm (CL)	Slope ± SE	TI %	RR (Fold)
Organophosphate	Profenofos	Cord 72% EC	2.93 (2.008-3.897)	1.96 ±0.4246	100	58.89 (47.39-71.74)	2.58 ±0.5206	22.74	20.10	118.74 (86.88-180.15)	1.845 ±0.5382	42.41	40.53
	Chlorpyrifos	Chlorzed 48% EC	14.54 (10.35-21.58)	1.67 ±0.4157	20.15	749.99 (596.50-948.53)	2.2906 ±0.4396	1.79	51.58	1349.50 (1083.9 - 1943.4)	2.48 ±0.6848	3.73	92.81
Synthetic parathyroid	lambda cyhalothrin	Agristar 5 % EC	3.32 (2.67- 4.34)	2.27 ±0.4511	88.25	13.39 (10.84-17.32)	2.69 ±0.6302	100	4.03	50.36 (41.53-65.15)	2.55 ±0.5334	100	15.17
	Esfenvalerate	Fenirate-S 5% EC	8.22 (6.49-10.95)	2.11 ±0.4661	35.64	51.84 (43.05-66.80)	2.66 ±0.5404	25.83	6.31	110.21 (91.18-146.94)	2.59 ±0.5801	45.69	13.41
	Fenpropathrin	Fenthrin 30% EC	14.44 (11.43-17.67)	2.49 ±0.4727	20.29	79.66 (62.92-103.18)	2.17 ±0.3832	16.81	5.52	157.73 (125.44-210.69)	2.46 ±0.5842	31.93	10.92

CL : Confidence Limited
SE: Standard Error.

GSTs are the 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 is specifically involved in OP metabolism *via* glutathione conjugation reaction conjugation (Huang *et al*., 1998).

A higher level of GST has been associated with organophosphate and pyrethroid detoxification in several lepidopteran pests, including *Helicoverpa armigera* (Yu and Huang, 2000), *Spodoptera frugiperda* (Yu, 2002), *P. xylostella* (Wu *et al*., 2000) and *Spodoptera littoralis* (Osman *et al.,* 2005). Further, Kristensen (2005) reported that multi-resistant field populations of the housefly, *Musca domestica*, have significantly greater GST activity than laboratory strains.

On the other hand, significant elevation in GST activity and a slight increase in MFO activity in the Sharqia population may be the main reason for resistance to organophosphates. Analogously, previous studies have revealed that azinphos-methyl resistance in field populations of *C. pomonella* was attributable to GST activity (Fuentes-Contreras *et al.*, 2007) or both GST and MFO activity (Reyes *et al*., 2004), which was also associated with reduced esterase activity toward novel substrates.

Table 2. Total protein content and the activity of acetylcholinesterase, α-esterase, glutathione-S-transferase and, mixed function oxidase in whole body homogenates of the laboratory and field *P. gossypiella* moths.

Insect population	Total protein content (mg/100mg b.wt.)		Acetyl cholinesterase (nmole AchBr min^{-1} mg^{-1} protein)		α-esterase (nmole α *naphthol* min^{-1} mg^{-1} protein)		Glutathione-S-transferase (nmole min^{-1} mg^{-1} protein)		Mixed function oxidase (nmole min^{-1} mg^{-1} protein)	
	Mean ±SE	% Change	Mean ±SE	% Change	Mean ±SE	% Change	Mean ± SE	% Change	Mean ± SE	% Change
Sharqia	0.3853 ± 0.0128	(-) 15.5	64.27 ± 1.15	(-) 4.80	18.31 ± 0.1703	(-) 5.77	2949.67 ±41.30	(+) 43.21	24.2 ± 0.4359	(+) 6.61
Faiyum	0.734 ± 0.0055	(+) 60.96	77.53 ± 2.37	(+) 14.84	24.50 ± 0.6022	(+) 33.42	853.33 ±22.32	(-) 58.57	30.6 ± 0.7356	(+) 34.8
Laboratory	0.456 ± 0.0036	-	67.51 ± 2.23	-	18.62 ± 0.3407	-	2059.67 ±32.31	-	22.7 ± 0.2517	-

Data are presented as the means ±SE (standard error)
Enzyme activity expressed as nmole min^{-1} mg^{-1} protein.

Table 3. Relative mobility and molecular weight of protein bands in SDS PAGE detected in the whole body homogenates of the laboratory and field *P. gossypiella* moths.

Band No.	Relative mobility (Rm)	Molecular weight (KDa)	Presence of bands		
			Sharqia (Sh)	Faiyum (Fay)	Lab-strain (Lab)
1	0.287	85.88	+	+	+
2	0.329	72.05	+	+	+
3	0.386	58.70	+	+	–
4	0.409	55.22	+	+	+
5	0.450	50.89	–	+	+
6	0.494	46.86	+	–	+
7	0.562	41.22	+	+	+
8	0.596	34.58	+	+	+
9	0.628	31.26	+	+	–
10	0.713	26.93	+	+	+
Total number of bands			9	9	8

(+) present (–) absent

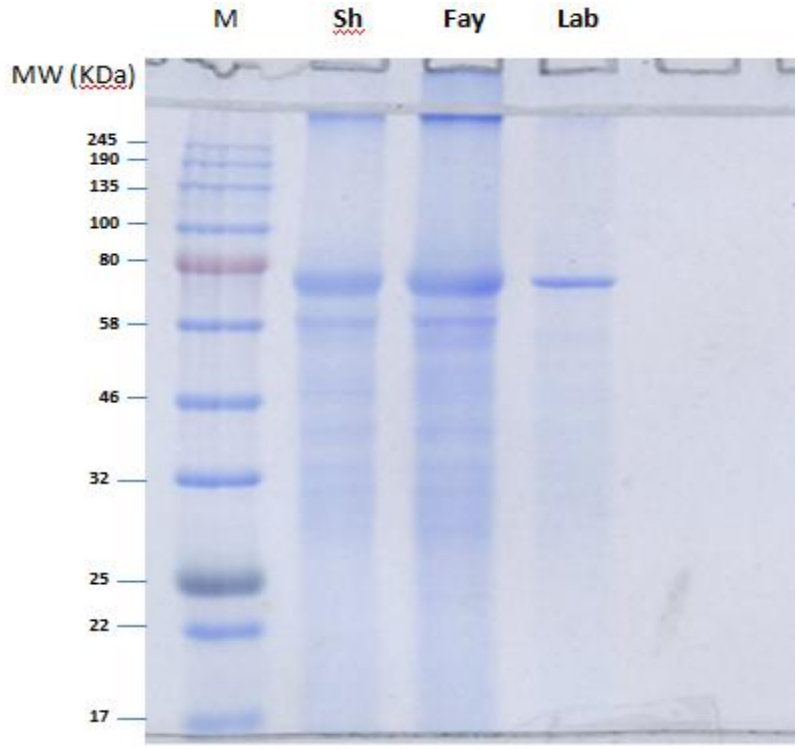

Figure 1. SDS polyacrylamide gel of denatured protein patterns in *P. gossypiella* moths. Lanes: Sh, Fay and Lab for insects from Sharqia, Faiyum Governorate and laboratory strain, respectively.

Separation of total protein content

The total protein content from whole body homogenate of laboratory and field *P. gossypiella* moths could be separated into ten bands, according to molecular weight, using 10% SDS-PAGE (Fig. 1 and Table 3). The total numbers of bands were 9, 9 and 8 in body tissue of Faiyum, Sharqia, and laboratory moths, respectively. There were six common bands (no. 1, 2, 4, 7, 8, and 10) in the field and laboratory insects. However, some differences were observed in the protein profiles of the tested insect populations. A protein band, no. 5 (0.450 Rm and 50.89 KDa MW) was detected in the Faiyum and laboratory insects only, whereas, band no. 6 (0.494 Rm and 46.86 KDa MW) was present in Sharqia and laboratory insects. Moreover, there were two specific bands, bands No. 3 and 9 with Rm 0.386 and 0.628, respectively, in Sharqia and Faiyum insects that were not common. It is clear from the separation profile that protein bands were more concentrated in the Faiyum moths than in the Sharqia or laboratory moths.

These findings are in harmony with the results obtained by Radwan and El Malla (2015) and Shekeban (2002) who reported that there are differences among protein profiles of laboratory and field strains of pink bollworm moths, both in concentration and molecular weight.

CONCLUSION

Results obtained in this study reveal that insects from the Faiyum governorate exhibit higher resistance levels to all tested insecticides than those from Sharqia. A positive correlation was found between the development of resistance to organophosphate and increased activity of metabolic enzymes in the Faiyum field population. Therefore, it could be suggested that increased activity of AChE, MFO, and α-esterases in Faiyum insects may be associated with the development of high resistance to organophosphates, especially Chlorpyrifos, and with moderate resistance to pyrethroid insecticides, while GST may only play a minor role. Thus, AChE, α-esterases and MFO enzymes may be used as biomarkers to detect organophosphate resistance in field populations of *P. gossypiella.*

Further, under certain conditions, GST may also be used as a biomarker to detect organophosphate resistance in other field populations. Hence, it is important to use biochemical assays in combination with conventional bioassay methods in *P. gossypiella* control programs to detect the occurrence of insecticide resistance in the early stages of development.

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