

# Pyrethroid Resistance and Its Mechanism in Field Populations of the Sand Termite, *Psammotermes hypostoma* Desneux

Mai. M. Toughan, Ahmed A. A. Sallam, Ashraf O. Abd El-Latif

**Abstract**—Termites are eusocial insects that are found on all continents except Antarctica. Termites have serious destructive impact, damaging local huts and crops of poor subsistence. The annual cost of termite damage and its control is determined in the billions globally. In Egypt, most of these damages are due to the subterranean termite species especially the sand termite, *P. hypostoma*. Pyrethroids became the primary weapon for subterranean termite control, after the use of chlorpyrifos as a soil termiticide was banned. Despite the important role of pyrethroids in termite control, its extensive use in pest control led to the eventual rise of insecticide resistance which may make many of the pyrethroids ineffective. The ability to diagnose the precise mechanism of pyrethroid resistance in any insect species would be the key component of its management at specified location for a specific population. In the present study, detailed toxicological and biochemical studies was conducted on the mechanism of pyrethroid resistance in *P. hypostoma*. The susceptibility of field populations of *P. hypostoma* against deltamethrin,  $\alpha$ -cypermethrin and  $\lambda$ -cyhalothrin was evaluated. The obtained results revealed that the workers of *P. hypostoma* have developed high resistance level against the tested pyrethroids. Studies carried out through estimation of detoxification enzyme activity indicated that enhanced esterase and cytochrome P450 activities were probably important mechanisms for pyrethroid resistance in field populations. Elevated esterase activity and also additional esterase isozyme were observed in the pyrethroid-resistant populations compared to the susceptible populations. Strong positive correlation between cytochrome P450 activity and pyrethroid resistance was also reported. Deltamethrin could be recommended as a resistance-breaking pyrethroid that is active against resistant populations of *P. hypostoma*.

**Keywords**—*Psammotermes hypostoma*, pyrethroid resistance, esterase, cytochrome P450.

## I. INTRODUCTION

TERMITES are serious pests that have a destructive impact, damaging local building and crops of poor farmers. Termites have destroyed villages in Egypt and India and forced the native people to move to other area [1]. In Egypt, most of these damages are due to the subterranean termite species [2]. The sand termite, *Psammotermes hypostoma*, was found mainly in Upper Egypt, fringes of Sahara and oasis within the desert, whereas the sandy soil supports some vegetation [3].

Mai. M. Toughan and Ahmed A. A. Sallam are with the Department of Plant Protection, Faculty of Agriculture, Sohag University, Sohag Egypt.

Ashraf O. Abd El-Latif is with the Department of Plant Protection, Faculty of Agriculture, Sohag University, Sohag Egypt (corresponding author, e-mail: ashrafoukasha@agr.sohag.edu.eg).

Chemical control is currently essential for management of termite and is likely to remain an important component of control strategies in the future. However, the most serious threat to their continued effectiveness in the field is the development of resistance. Knowledge of resistance mechanism determines the use of resistance-breaking compounds which is very important in resistance management. Chlorpyrifos as a soil termiticide was heavily used as soil treatment against *P. hypostoma* in Egypt. In the last few years, new compounds were introduced to control *P. hypostoma* in Egypt such as acetamiprid and thiamethoxam [4]. However, many unpublished reports revealed that termite has developed resistance to pyrethroid compounds which was not been used for termite control in Egypt. Thus, the current research was developed to study the status pyrethroid resistance and its mechanism in the Egyptian populations of *P. hypostoma*.

## II. MATERIAL AND METHODS

### A. Termite Collection and Maintenance

Three field populations of the sand termites, *P. hypostoma*, were collected from three different locations: (1) Infested grape shrubs stumps planted at the farm of the Faculty of Agriculture, Sohag University which is located at El-kawther City, Sohag Governorate, (2) manufacture houses wood collected from Dar El –Salaam city, Sohag Governorate, (3) underground termites collected from farm of the Faculty of Agriculture, South Valley University, Qena Governorate. The collected termites were brought back to the laboratory in their original wood and separated gently by tapping the wood and the ejected termites were maintained on moist filter papers (made of natural fiber to act as source of food) in plastic containers (30 x 15 x 10 cm) and the containers were covered with plastic top. Containers were placed in black cloth cover and were stored at controlled room conditions, 25±2 °C and approximately 70-80% RH. Termites were held for one week to allow for any mortality incurred during the separation process, and only healthy homogenous undifferentiated termite workers were used for the experiments.

### B. Insecticides Bioassay

Technical grades of three pyrethroid insecticides; deltamethrin (98.5%),  $\alpha$ -cypermethrin (97.8%), and  $\lambda$ -cyhalothrin (98%) were dissolved in acetone and at least 5-8 serially diluted concentrations were prepared for each insecticide. For each treatment, a Whatman filter paper of 90

mm diameters was treated with 1 ml of each concentration of the prepared insecticides and kept into the bottom of glass petri dish (9cm diameter). Control filter paper was treated with 1 ml acetone. The petri dishes were kept uncovered until the solvent was completely evaporated, and 20 healthy homogenous termite workers were released in each petri dish and three replications of each treatment were prepared. The dishes were covered and kept in a plastic container and maintained as described above (Section II.A).

Mortality was recorded up to three days. The  $LC_{50}$  was calculated according to Finney [5]. Resistance factors were calculated as the ratio of the  $LC_{50}$  of resistant population to the  $LC_{50}$  of the susceptible population

### C. Esterase Profile

#### 1. Sample Preparation for Esterase Purification

500 termite workers from each population were homogenized in 10 ml of sodium phosphate buffer (0.1 M, pH 7). Then, the homogenate was centrifuged for 20 min at 10,000 g and 4 °C and the supernatant was transferred to Eppendorf tubes and stored at -20 °C for further use.

#### 2. Determination of Esterase Activity

The total esterase activity was determined by measuring the rate of hydrolysis of the model substrates  $\alpha$ -naphthyl acetate and  $\beta$ -naphthyl acetate. In each reaction tube, an aliquot of 10  $\mu$ l enzyme stock was added to 188  $\mu$ l of 0.02 M sodium phosphate buffer (pH 7.0), 2  $\mu$ l of 25 mM substrate solution ( $\alpha$ -naphthyl acetate or  $\beta$ -naphthyl acetate dissolved in acetone) and was incubated for 20 min at 30 °C. Blank was prepared without adding enzyme. Three replications were used. Reactions were stopped with 50  $\mu$ l of 0.3% Fast Blue BB dissolved in distilled water containing 3.5% SDS, and then, the tubes were incubated at 30 °C for 15 min in dark. The absorbance was recorded at 600 nm using UV/VIS-Single beam spectrophotometer (Sigma), and the enzyme activity was calibrated from the  $\alpha$ -naphthol and  $\beta$ -naphthyl acetate standard curve.

The protein content of the sample was estimated following the method of Lowry et al. [6] using bovine serum albumin fraction V (Sigma) as the standard. The esterase specific activity in the three populations was calculated as the  $\mu$ mol of  $\alpha$ -naphthol produced/ mg protein/ min. The enzyme kinetics ( $V_{max}$  and  $K_m$ ) were calculated by measuring the enzyme activity at different concentration of the substrate. The inverse of the initial velocity was plotted against the inverse of the substrate concentration to develop the Lineweaver-Burk plot.

### D. Inhibition Studies

In order to confirm the role of esterase in the detoxification of selected pyrethroids, the binding of the selected pyrethroids to esterase was studied by measuring the inhibition activity of pyrethroids to esterase assuming that if a pyrethroid binds to esterase at its active site it will compete with the synthetic substrate ( $\beta$ -naphthyl acetate) that results in inhibition of esterase activity.

The pyrethroid inhibition activity was measured by

incubation of 500  $\mu$ l of esterase enzyme with different dilution of the tested insecticide for 20 min at 30 °C before adding the substrate and the esterase activity was measured as described above (C.2). Inhibitor activity for each insecticide was calculated by the amount of inhibitor required to inhibit 50% of esterase activity ( $I_{50}$ ).

### E. Electrophoretic Studies

In order to study the esterase isozyme profile and to find out the esterase(s) responsible for pyrethroid resistance, Non-denaturing PAGE assays were carried out on poly acrylamide gel. Electrophoresis (Native) was carried out on 12 x 10 cm mini vertical slab gel unit (LKB, Sweden) using 6.5% separating gel and 4% stacking gel with a continuous tris-glycine running buffer system (50 mM, pH 8.3). 100  $\mu$ l of the enzyme stock of each sample containing 20  $\mu$ g protein was diluted with 50  $\mu$ l of 2 x sample buffer (0.125 M Tris-HCl, 30% v/v glycerol, 0.02% bromophenol blue, pH 6.8) before loading. Electrophoresis was carried out at 75 V constant voltages and 25 milliamperes for approximately 30 minutes until the samples entered the resolving gel, later the current was gradually increased to 150 V and 50 mA, and the temperature was maintained at  $12 \pm 0.1$  °C using a circulating water bath. To visualize the esterase bands, the gels were incubated in 2%  $\alpha$ -naphthyl acetate (30 mM in phosphate buffer, pH 7.0) for 15 minutes at room temperature with continuous mild shaking before adding 0.04 % Fast blue RR. The gels were then incubated in the dark for 30 minutes until dark brown (greenish-black) coloured bands appeared. Two ml formaldehyde was added to maintain the transparency of the background gel matrix. After staining, the gels were examined for the presence or absence of esterase isozymes.

### F. Cytochrome P450 Monooxygenase Assay

In order to determine the possible role of cytochrome P450 monooxygenase in pyrethroid resistance in termite, the activity of cytochrome P450 monooxygenase was determined in a freshly workers homogenates (prepared as describes in C.1.) of the selected populations using the carbon monoxide differences spectra, following reaction with sodium dithionite as described by Omura and Sato [7], and six replications were used for each population.

## III. RESULTS AND DISCUSSION

### A. Insecticide Bioassay

The variation in concentration versus response and resistance ratio of three populations of *P. hypostoma*, viz El-kawther, Dar El-salaam, and Qena populations towards deltamethrin,  $\alpha$ -cypermethrin and  $\lambda$ -cyhalothrin are presented in Table I. Out of the three tested populations, El-kawther population displayed high susceptibility for the three tested pyrethroids compared to Dar El-salaam and Qena populations for which El-kawther population was considered as the "susceptible population". Qena population displayed moderate resistance to the tested pyrethroids compared to Dar El-salaam population.

Deltamethrin was found to be the least toxic against the

susceptible population (El-Kawther) with  $LC_{50}$  value of 0.0049 (Table I). The highest susceptibility and lowest  $LC_{50}$  were achieved in case of  $\alpha$ -cypermethrin with  $LC_{50}$  value of 0.00036  $\mu\text{g/ml}$  followed by  $\lambda$ -cyhalothrin with  $LC_{50}$  value of 0.00091  $\mu\text{g/ml}$ .

Compared to the susceptible population, workers of the resistant populations (Dar El-salaam and Qena) displayed high resistance ratio to  $\alpha$ -cypermethrin (661.11 and 121.39 folds for Dar El-salaam and Qena populations, respectively) with  $LC_{50}$  values of 0.238 and 0.0437  $\mu\text{g/ml}$  for Dar El-salaam and Qena populations, respectively. The lowest resistance ratio was achieved against deltamethrin (53.67 and 11.63 folds for Dar El-salaam and Qena populations, respectively) with  $LC_{50}$  values of 0.2630 and 0.0570  $\mu\text{g/ml}$  for Dar El-salaam and Qena populations, respectively. Dar El-salaam and Qena populations displayed moderate susceptibility towards  $\lambda$ -

cyhalothrin with a resistance ratio of (126.37 and 70.33 folds for Dar El-salaam and Qena populations, respectively) and  $LC_{50}$  values of 0.1150 and 0.0640  $\mu\text{g/ml}$  for Dar El-salaam and Qena populations, respectively (Table I).

Based on non-overlapping fiducial limits, it can be stated that there were significant variations in the  $LC_{50}$  values between the different populations, except in case of  $\lambda$ -cyhalothrin as the fiducial limits were overlapping between Dar El-salaam and Qena populations, and the  $LC_{50}$  values were not significantly different (Table I). The  $\chi^2$  test was used to estimate how well the data fits the assumption of the probit model. As the predicted values of the probit model did not differ significantly from the observed values (except in  $\alpha$ -cypermethrin – Qena combination), the probit model was suitable for the concentration-response analysis.

TABLE I  
TOXICITY OF SYNTHETIC PYRETHROIDS AGAINST DIFFERENT STRAINS OF *P. HYPOSTOMA* WORKERS

Pyrethroids	Strains	d.f	$\chi^2$	$LC_{50}$ ( $\mu\text{g/ml}$ )	Fiducial limits	Regression equation (Y)	Resistance ratio
Deltamethrin	Elkawther	5	4.7233	0.0049	0.0039- 0.0061	$Y=9.61647 + 1.99550X$	1
	Dar El salaam	6	11.9230	0.2630	0.1936 – 0.3130	$Y= 6.68800 + 1.800X$	53.67
	Qena	5	4.8400	0.0570	0.0229 – 0.1440	$Y=5.9386 + 0.7570X$	11.63
$\alpha$ -Cypermethrin	Elkawther	5	1.1218	0.00036	0.00027 – 0.00048	$Y=9.52810 + 1.31604X$	1
	Dar El salaam	5	4.0800	0.2380	0.0990 – 0.3500	$Y=5.4710 + 0.7550X$	661.11
	Qena	5	*26.600	0.0437	0.0423 – 0.1548	$Y=5.8960 + 0.6600X$	121.39
$\lambda$ -cyhalothrin	Elkawther	6	4.86603	0.00091	0.000528 – 0.0017	$Y=6.81144 + 0.59580X$	1
	Dar El salaam	5	9.032	0.1150	0.0131 – 0.0933	$Y=5.9780 + 1.0360X$	126.37
	Qena	5	7.8300	0.0640	0.0250 – 0.0132	$Y=5.9500 + 0.8800X$	70.33

\*Significantly heterogeneous at  $P= 0.05$ ,  $Y$ =Probit kill,  $X$ =Log dose

TABLE II  
ESTERASE ACTIVITY IN THE WORKERS OF DIFFERENT POPULATIONS OF *P. HYPOSTOMA* USING  $\alpha$ -NAPHTHYL ACETATE AS SUBSTRATE

Populations	Esterase specific activity ( $\mu\text{mol/mg protein/min}$ )	$V_{max}$ ( $\mu\text{mol/mg protein/min}$ )	$K_m$ ( $\mu\text{mol substrate}$ )	*Relative activity
El-kawther (Susceptible)	0.228 $\pm$ 0.023	0.322	0.235	1.00
Dar El-slaam	0.545 $\pm$ 0.013	0.610	0.180	2.39
Qena	0.573 $\pm$ 0.035	0.633	0.177	2.51

\* Relative activity = Esterase activity in resistant population/ Esterase activity in susceptible population

Note: Esterase specific activity values are means of three assays.

The pattern of pyrethroid resistance in this study is characterized by high  $LC_{50}$  values combined with low slopes for  $\text{ld-p}$  (Log-dose vs. probit mortality) lines that suggest a wide variation in the susceptibility of individuals of the same population.

This study supports the view that the cross-resistance between pyrethroids depends on their chemical structure [8]. Among the three pyrethroids tested, Dar El-salaam population and Qena population displayed higher resistance ratio against  $\alpha$ -cypermethrin followed by  $\lambda$ -cyhalothrin, while deltamethrin was found to be highly effective compound and poorly resisted by both the resistant populations. The structural comparison of these pyrethroids indicates that the substitution of chlorine by bromine in case of deltamethrin enhanced the insecticidal activity and reduced the resistance level in the resistant populations compared to  $\alpha$ -cypermethrin which is similar in its structure to deltamethrin. The differences in the chemical structure of these pyrethroids may affect the binding

ability of metabolic enzyme and hence affect the resistance. Our results support the assertion that it is possible and more practical to find resistance-breaking pyrethroids such as deltamethrin which are active against resistant populations than to increase the doses of pyrethroids that are highly resisted by field populations of *P. hypostoma*.

#### B. Esterase Profile

The specific activity and kinetic parameters of the esterase of the three populations of *P. hypostoma* using  $\alpha$ -naphthyl acetate are presented in Table II. The resistant populations, (Dar El-salaam and Qena) were found to have elevated esterase activity in comparison to the susceptible population (El-kawther). Dar El-salaam and Qena populations have 2.39 and 2.51-fold higher esterase activity, respectively, than that of the susceptible population. The kinetic parameters,  $K_m$  and  $V_{max}$ , indicate that Dar El-salaam and Qena populations had a higher active esterase than that of the susceptible strain characterized by high substrate binding affinity as the  $K_m$

values of both Dar El-salaam (0.180  $\mu\text{mol}$ ) and Qena (0.177  $\mu\text{mol}$ ) populations were lower than that of the susceptible population (El-kawther) (0.235  $\mu\text{mol}$ ). This also is supported by higher  $V_{\text{max}}$  values for Dar El-salaam (0.610) and Qena (0.633) populations compared to the susceptible population (0.322).

The same trend was obtained when the substrate,  $\beta$ -naphthyl acetate was used (Table III) as Dar El-salaam and Qena populations were found to have 3.46 and 2.46-fold higher esterase activity, respectively, than that of the

susceptible population (Table III). The esterase of the resistant populations has a higher substrate binding affinity compared to the susceptible population as the  $K_m$  values of both Dar El-salaam (0.212  $\mu\text{mol}$ ) and Qena populations (0.216  $\mu\text{mol}$ ) were lower than that of El-kawther susceptible population (0.298  $\mu\text{mol}$ ). The maximum velocity of the esterase of the resistant populations was higher than that of the susceptible population as the  $V_{\text{max}}$  values were 0.620, 0.583, and 0.213 for Dar El-salaam, Qena, and El-kawther populations, respectively.

TABLE III  
ESTERASE ACTIVITY IN THE WORKERS OF DIFFERENT POPULATIONS OF *P. HYPOSTOMA* USING  $\beta$ -NAPHTHYL ACETATE AS SUBSTRATE

Populations	Esterase specific activity ( $\mu\text{mol}/\text{mg protein}/\text{min}$ )	$V_{\text{max}}$ ( $\mu\text{mol}/\text{mg protein}/\text{min}$ )	$K_m$ ( $\mu\text{mol substrate}$ )	*Relative activity
El-kawther (Susceptible)	0.145 $\pm$ 0.030	0.213	0.298	1.00
Dar El-slaam	0.502 $\pm$ 0.011	0.620	0.212	3.46
Qena	0.357 $\pm$ 0.022	0.583	0.216	2.46

\* Relative activity = Esterase activity in resistant population/ Esterase activity in susceptible population

Note: Esterase specific activity values are means of three assays

It was observed that the esterase of three studied populations of *P. hypostoma* has higher affinity towards  $\alpha$ -naphthyl acetate than  $\beta$ -naphthyl acetate as the  $K_m$  values were lower when  $\alpha$ -naphthyl acetate was used as a substrate compared to  $\beta$ -naphthyl acetate (Tables II and III).

The correlation between esterase activity in the workers of different populations of *P. hypostoma* and pyrethroid resistance using both substrates,  $\alpha$ -naphthyl acetate and  $\beta$ -naphthyl acetate, was calculated. The highest esterase activity synchronized with highest  $\text{LC}_{50}$  values and hence highest resistance ratios. Correlation analysis showed positive correlation between esterase activity and pyrethroid resistance with correlation coefficient ( $r$ ) of 0.359, 0.340 and 0.740 for deltamethrin,  $\alpha$ -cypermethrin and  $\lambda$ -cyhalothrin, respectively when esterase activity was measured using  $\alpha$ -naphthyl acetate as substrate. Strong positive correlation between esterase activity and pyrethroid resistance was noticed with correlation coefficient ( $r$ ) of 0.819, 0.803, and 0.998 for deltamethrin,  $\alpha$ -cypermethrin and  $\lambda$ -cyhalothrin, respectively when esterase activity measured using  $\beta$ -naphthyl acetate as substrate.

Esterase isozymes of the workers of the three populations of the tested insect studied were identified using Native PAGE electrophoresis after incubation with  $\alpha$ -naphthyl acetate and visualized by Fast blue RR. Five esterase isozymes were identified and designated as E1-E5, from the slowest migrating esterase (highest molecular weight) E1, to the fastest (lowest molecular weight), E5 (Fig. 1). While four esterase bands were common to both susceptible and resistant populations, susceptible population (El-kawther) lacked one band, E4 that were found in the resistant populations (Dar El-salaam and Qena). Although E3, E4, and E5 were common for both susceptible and resistant populations, the intensity of these bands was more in Dar El-salaam and Qena populations, compared to that of the susceptible population (El-kawther).

Elevated esterase activity was reported to be a major mechanism of pyrethroid resistance in different insect species [9]-[11]. Abd El-latif and Subrahmanyam [9] demonstrated

that the pyrethroid resistant strains of *H. armigera* have elevated esterase activity and also additional esterase isozymes which was not observed in the susceptible strain.

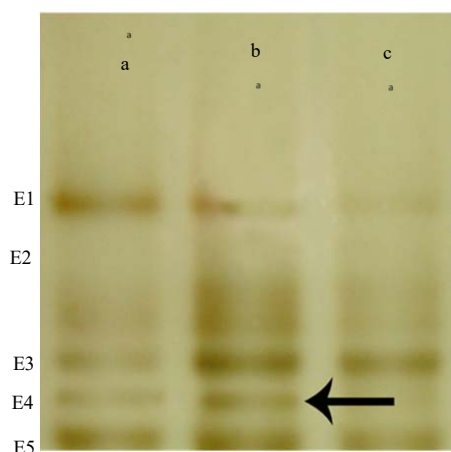


Fig. 1 Native PAGE profile of esterase isozyme from the workers of different population of *Pasmotermes hypostoma* (a) Dar El-Salaam, (b) Qena and (c) El-Kawther

### C. In vitro Inhibition of Esterase Activity

In order to confirm the role of esterase in the detoxification of the selected pyrethroids, the binding of affinity of the selected pyrethroids to esterase enzymes was studied by measuring the inhibition potency of pyrethroid to esterase activity.

The inhibition potency of the selected pyrethroids was studied and data displayed that the tested pyrethroids inhibited esterase activity in all termite populations. The inhibitor potency was estimated as the concentration of the pyrethroid that inhibits 50% of the esterase activity ( $\text{IC}_{50}$ ). The tested pyrethroids were found to be more potent (lower  $\text{IC}_{50}$  values) against esterase of the resistant populations (Dar El-salaam and Qena) compared with the esterase of the susceptible

population (El-kawther) (Table IV).  $\alpha$ -cypermethrin (to which high resistance factor was developed) was more potent as esterase inhibitor with lower  $IC_{50}$  value (14.031  $\mu\text{g/ml}$ ) against the esterase of the highly resistant population (Dar El-salaam) compared to deltamethrin ( $IC_{50}$ = 24.898  $\mu\text{g/ml}$ ) and  $\lambda$ -cyhalothrin ( $IC_{50}$ = 14.161  $\mu\text{g/ml}$ ).

TABLE IV  
IN VITRO INHIBITION OF ESTERASE ACTIVITY IN THE WORKERS OF DIFFERENT POPULATIONS OF *P. HYPOSTOMA* BY PYRETHROIDS

Pyrethroids	Strains	$IC_{50}$ ( $\mu\text{g/ml}$ )	$K_i$ ( $\mu\text{g/ml}$ )
	El-kawther	44.041	10.61
Deltamethrin	Dar El- salaam	24.898	1.30
	Qena	18.605	2.10
	El-kawther	31.362	7.98
$\alpha$ -Cypermethrin	Dar El- salaam	14.031	4.64
	Qena	27.594	5.50
	El-kawther	31.93	25.10
$\lambda$ -cyhalothrin	Dar El- salaam	14.61	0.14
	Qena	34.315	10.78

The  $K_i$  values (Table IV) revealed that the tested pyrethroids have higher esterase- binding affinity (lower  $K_i$  values) towards the esterase of the highly resistant population (Dar El-salaam) compared to the other selected populations with  $K_i$  values of 1.3, 4.64, and 0.14  $\mu\text{g/ml}$  towards deltamethrin,  $\alpha$ -cypermethrin and  $\lambda$ -cyhalothrin, respectively. On the other hand, the tested pyrethroids have lower esterase-binding affinity (higher  $K_i$  values) towards the esterase of the susceptible population (El-kawther) compared to the other selected populations with  $K_i$  values of 10.61, 7.98, and 25.01  $\mu\text{g/ml}$  towards deltamethrin,  $\alpha$ -cypermethrin and  $\lambda$ -cyhalothrin, respectively. A moderate esterase-binding affinity was obtained towards the esterase of the moderate resistant population (Qena) with  $K_i$  values of 2.10, 5.50, and 10.78  $\mu\text{g/ml}$  towards deltamethrin,  $\alpha$ -cypermethrin and  $\lambda$ -cyhalothrin, respectively.

Electrophoretic studies confirmed the presence of one or more DEF sensitive esterase that hydrolyzed  $\alpha$ -naphthyl acetate and  $\beta$ -naphthyl acetate and capable of hydrolyzing trans-cypermethrin [12]. Evidence that organophosphates (pyrethroid synergists) are esterase inhibitors was also reported in the Australian *H. armigera* [13]. Davis et al. [14] reported the presence of seven bands by Native polyacrylamide gel in *R. flavipes* which were named EI-E7 with molecular weight ranged from 71.5 to 97.2 kD. Out of them, four Bands (EI-E4) were determined to be cholinesterase based on physostigmine inhibition, while the other three bands were identified as carboxylesterases based on paraxon inhibition [14]. Our finding was considered as an acceptable proof to the role of esterases in pyrethroid resistance in *P. hypostoma*.

#### D. The Role of Cytochrome P450 Linked Monooxygenase

The total cytochrome P450 content was determined using the carbon monoxide differences spectra, following reaction with sodium dithionite (Table V). The resistant populations, i.e. Dar El-salaam population and Qena population, were

found to have elevated monooxygenase activity compared to the susceptible population (El-kawther). The highest monooxygenase activity was recorded in Dar El-salaam population (190.24 $\pm$ 1.99 pmol/mg protein) followed by Qena population (110.93 $\pm$ 2.86 pmol/mg protein), while monooxygenase activity was only 63.61 $\pm$ 2.3 pmol/ mg protein in the susceptible population. Dar El-salaam and Qena populations had 2.99 and 1.74 folds higher monooxygenase activity compared to the susceptible population.

Correlation studies revealed that the highest monooxygenase activity synchronized with highest  $LC_{50}$  values and hence highest resistance ratio. Correlation analysis revealed strongly positive correlation between monooxygenase activity and pyrethroid resistance with correlation coefficient ( $r$ ) of 0.96, 0.96, and 0.95 for deltamethrin,  $\alpha$ -cypermethrin and  $\lambda$ -cyhalothrin, respectively.

The purity of a reconstituted monooxygenase system can often be determined from a scan of cytochrome P450. Problems with preparations result in large amounts of a degradation product in the form of cytochrome P420. In this experiment, monooxygenase preparations were of a high purity because only minimal amounts (less than 0.02 AU) of P420 were detected in these assays.

TABLE V  
ACTIVITY OF CYTOCHROM P450 LINKED MONOOXYGENASE FROM THE WORKERS OF DIFFERENT POPULATION OF *P. HYPOSTOMA*

Population	monooxygenase activity (pmol/ mg protin)	*Relative activity
El - Kawther	63.61	1
Dar El - Salaam	190.24 $\pm$ 1.99	2.99
Qena	110.93 $\pm$ 2.86	1.74

\*Relative activity = monooxygenase activity resistant population / monooxygenase activity in susceptible population.

Elevated monooxygenase activity in pyrethroid-resistant insect species was reported by many authors, e.g. *H. virescens* [15], *Musca domestica* [16], [17], *Culex quinquefasciatus* [18] and *H. armigera* [19]. The evidences presented in this study strongly associate with specific increase in P450 monooxygenase activity with pyrethroid-resistance in *P. hypostoma*.

#### IV. CONCLUSION

The results of this study reveal that the Egyptian populations of *P. hypostoma* have developed high resistance level against pyrethroid insecticides. The data obtained in the current study revealed the important role of detoxification enzymes in pyrethroid resistance in the *P. hypostoma*. Two detoxification enzymes, viz esterase and monooxygenase, were found to be responsible for pyrethroid resistance. Strong positive correlation between esterase activity and pyrethroid resistance was reported. The pyrethroid-resistant populations of *P. hypostoma* were found to have elevated esterase activity and also additional esterase isozyme which were not observed in the susceptible population. The biochemical studies provide important evidence that the elevated cytochrome P450 monooxygenase activity observed in the pyrethroid-resistant

populations is associated with pyrethroids resistance *P. hypostoma*.

This study supports the structure-activity view that the cross-resistance between pyrethroids depends on their chemical structure. This provides evidence that it is possible and more practical to find resistance-breaking pyrethroids such as deltamethrin which are active against resistant populations than increase the doses of pyrethroids that are highly resisted by field populations of *P. hypostoma*.

#### REFERENCES

- [1] M. J. Pearce, Termites biology and pest management. CAB, International Wallingford, UK. 1997.
- [2] A. R. El-Bassiouny, and R. M. Abd El-Rahman. Susceptibility of Egyptian subterranean termite to some entomopathogenic nematodes. Egyptian Journal of Agricultural Research, 2011, 89: 121-135.
- [3] A. H. Kaschef, and L. S. El-Sherif. Distribution of four termite species in the A. R. Egypt. Insectes Sociaux, Paris, 1971, XVIII (4): 227-232. B. Smith, "An approach to graphs of linear forms (Unpublished work style)," unpublished.
- [4] M. A. I. Ahmed, E. A. Eraky, M. F. Mohamed, A. S. Soliman, Potential toxicity assessment of novel selected pesticides against sand termite, *Psammotermes hypostoma* Desneux workers (Isoptera: Rhinotermitidae) under field conditions in Egypt, Journal of Plant Protection Research, 2015, 55(2): 193-197.
- [5] D. J. Finney, Probit Analysis. (3rd Edition ed.), Cambridge University Press, Cambridge, 1971.
- [6] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, Protein Measurement with the Folin Phenol Reagent. Journal of Biological Chemistry, 1951, 193:265-275.
- [7] T. Omura and R. Sato, The carbon mono-oxide binding pigment of liver microsomes. II. Solubilisation, Purification and properties. Journal of Biological Chemistry, 1964, 239: 2379-2385.
- [8] J. Tan, and A. R. McCaffery, Efficacy of various pyrethroid structures against a highly metabolically resistant isogenic strain of *Helicoverpa armigera* (Lepidoptera: Noctuidae) from China. Pest Management Science, 2007, 63:960-968.
- [9] D. K. S. Goh, D. D. Anspaugh, N. Motoyama, G. C. Rock and R. M. R, Isolation and characterization of an insecticide-resistance associated esterase in the tobacco budworm, *Heliothis virescens* (F.). Pesticide Biochemistry and Physiology, 1995, 51: 192-204.
- [10] A. O. Abd El-Latif, B. Subrahmanyam, Pyrethroid synergists suppress esterase-mediated resistance in Indian strains of the cotton bollworm, *Helicoverpa armigera* (Hübner). Pesticide Biochemistry and Physiology, 2010, 97 (3): 279-288.
- [11] A. O. Abd El-Latif, B. Subrahmanyam, Pyrethroid resistance and esterase activity in three strains of the cotton bollworm, *Helicoverpa armigera* (Hübner). Pesticide Biochemistry and Physiology, 2010, 96 (3): 155-159.
- [12] K. A. Usmani, K. A. and C. O. Knowles, DEF Sensitive Esterases in Homogenates of Larval and Adult *Helicoverpa zea*, *Spodoptera frugiperda*, and *Agrotis ipsilon* (Lepidoptera: Noctuidae). Journal of Economic Entomology, 2001, 94(4): 884-891.
- [13] R. V. Gunning, G. D. Moores and A. L. Devonshire, Esterase inhibitors synergize the toxicity of pyrethroids in Australian *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Pesticide Biochemistry and Physiology, 1999, 63:50-62.
- [14] R. W. Davis, S. T. Kamble and S.K. Prabhakaran, Characterization of general esterases in workers of the eastern subterranean termite (Isoptera: Rhinotermitidae). Journal of Economic Entomology, 1995, 88(3): 574-578.
- [15] S. E. Clarck, C. H. Walker and A. R. McCaffery, A comparison of in vitro metabolism of cis-cypermethrin in a resistant and susceptible strain of *Heliothis virescens*. In Proc. Brighton Crop Protect. Conference: Pests and Diseases, 1990, PP. 1201-1206. Farnham, UK: The British Crop Protection council.
- [16] M. Zhang and J. G. Scott. Cytochrome b5 is essential for cytochrome P450 6D1-mediated cypermethrin resistance in LPR house flies. Pesticide Biochemistry and Physiology, 1996, 55: 150-156.
- [17] N. Liu, and J. G. Scott. Increased transcription of CYP6D1 causes cytochrome P450-mediated resistance in house fly. Insect Biochemistry and Molecular Biology 1998, 28: 531-535.
- [18] S. Kasai, I. S. Weerasinghe and T. Shono, P450 monooxygenases are an important mechanism of permethrin resistance in *Culex quinquefasciatus* Say larvae. Archive of Insect Biochemistry and Physiology, 1998, 37: 47-56.
- [19] A. O. Abd El-Latif, K. R. Kranthi, S. Kranthi, A. Sarwar and B. Subrahmanyam, Overexpression of cytochrome P450 CYP6B7 mediated pyrethroid resistance in Indian strains of the cotton bollworm, *Helicoverpa armigera* (Hübner). Journal of Plant Protection Research, 2014, 54(30): 10-15.