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Detection of glutathione S-transferase and activity levels of the esterases associated with insecticide resistance in field collected mosquito *Culex pipiens* (Diptera: Culicidae) from Egypt

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Abstract
Chemical insecticides are still the main part of vector mosquitoes control program. The effectiveness is eventually reduces as mosquitoes become resistant to them with repeated use. Therefore, understanding the mechanisms of resistance will be required. The present study was carried out to evaluate the potential biochemical mechanism in contributing toward insecticide resistance in *Culex pipiens* field populations in Egypt. Natural populations of *Culex pipiens* were collected from Giza, Beheira, Sohag and Monufia Governorates. The present finding showed difference in enzyme activity in *Culex pipiens* natural populations compared with laboratory colony. Monufia populations show the highest level of beta and alpha esterases (9.2 and 5.7 times higher activity than S-lab respectively). The most significant values of GST were in Sohag and Giza populations (3.8 and 2.4 times higher activity than S-lab respectively). A significant increase in specific carboxylesterases was detected only in Beheira populations (1.8 times higher activity than S-lab). Mechanisms of resistance are discussed in relation to biochemical assay results.

Keywords: *Culex pipiens*, resistance, esterases, GST, Egypt

1. Introduction
Mosquitoes are the most important vectors of human and animal diseases. In Egypt, the mosquito *Culex pipiens* is a main vector of lymphatic filariasis (Harb *et al*. 1993) [17]. It is also capable of transmitting Rift Valley fever Virus (Hoogstraal *et al*.1979) [22] and West Nile Virus (Taylor *et al*. 1956 and Soluman *et al*. 2010) [53, 49]. Its control is essential to prevent disease transmission.
The use of insecticides on adult and immature stages is efficient strategy for mosquito - borne disease control. Mosquitoes control big problem with regard to developing insecticide resistance. The most common mechanisms of resistance are the increase in metabolic detoxification of insecticides before it reaches its target site and changes in sensitivity of the target site (Hemingway *et al*. 1986 and Price 1991) [20, 37].
Three major enzyme families, esterases, glutathione S-transferase (GST) and Mixed Function Oxidases (MFO) such as cytochrome P450 oxidases are mainly involved in insecticides metabolism. Each of these enzymes catalyzes a wide range of detoxification reaction (Raymond *et el*. 1998; Scott 1999; Wheelock *et al*. 2005; Ranson and Hemingway 2005) [43, 46, 56, 40]. Measuring the activity of these enzymes in natural population is an important step in monitoring insecticide resistance. Therefore, the aim of the present work was to evaluate the levels of detoxification enzymes (non specific esterases, glutathione S – transferase and specific carboxylesterases) in natural populations and laboratory strain.

2. Materials and Methods
Mosquito samples
Natural populations of *Culex pipiens* were collected as larval stage from breeding sites of four Governorates, Giza, Beheira, Sohag and Monufia. Laboratory strain was obtained from Namru (S- lab).

Biochemical assay
Batches of 50 third instar larvae were homogenized in 1.5ml of ice-cold phosphate buffer, the homogenates were centrifuged at 4000 rpm for 30 min at 4◦C using cooling centrifuge. The supernatant was used as enzyme samples.
Total proteins were determined by the method of Bradford (1976) [6]. Protein reagent was prepared by dissolving 100mg of Coomassie Brilliant blue G-250 in 50 ml 95% ethanol. To this solution 100ml 85% (W/V) phosphoric acid were added. The resulting solution was diluted to a final volume of 1 liter. Sample solution (50µl) or for preparation of standard curve 50µl of serial concentrations containing 10 to 100µg bovine serum albumin (standard protein) were pipetted into test tubes. The volume in the test tube was adjusted to 1 ml with phosphate buffer (0.1 M, PH 6.6). Five millimeters of protein reagent were added to test tube and the contents were mixed either by inversion or vortexing. The absorbance at 595 nm was measured after 2 min. and before 1 hr. against blank prepare from 1 ml of phosphate buffer and 5 ml protein reagent
Nonspecific esterases (alpha and beta esterases) were determined according to Van Asperen (1962) [54] using α-naphthyl acetate or β-naphthyl acetate as substrates, respectively. The reaction mixture consisted of 5ml substrate solution ($3x10^{-4}$ M α or β-naphthyl acetate, 1% acetone and 0.1 M phosphate buffer, PH 7) and 20µl of larval homogenate. The mixture was incubated for exactly 15 min at 27◦C, then 1ml of diazoblue color reagent (prepared by mixing 2 parts of 1% diazoblue B and 5 parts of 5% sodium lauryl sulphate) was added. The developed color was read at 600 or 555 nm for α- and β-naphthol produced from hydrolysis of the substrate, respectively. α-and β-naphthol standard curves were prepared by dissolving 20 mg α- or β-naphthol in 100ml phosphate buffer, PH7 (stock solution). Ten milliliters of stock solution were diluted up to100ml by the buffer. Aliquots of 0.1, 0.2, 0.4, 0.8 and 1.6 ml of diluted

solution (equal to 2, 4, 8, 16 and 32µg naphthol) were pipetted into test tubes and were completed to 5ml by phosphate buffer. One milliliter of diazoblue reagent was added and the developed color was measured as mentioned before.

Glutathione S-transferase (GST) catalyzed the conjugation of reduced glutathione (GSH) with 1-chloro 2, 4-dinitrobenzene (CDNB) via the –SH group of glutathione. The conjugate, S-(2, 4-dinitro-phenyl)-L-glutathione could be detected as described by the method of Habig *et al.* (*1974)* [15]. The reaction mixture consisted of 1ml of the potassium salt of phosphate buffer (PH 6.5), 100µl of GSH and 200 µl of larval homogenate. The reaction started by the addition of 25µl of the substrate CDNB solution. The concentration of both GSH and CDNB was adjusted to be 5mM and 1mM, respectively. Enzyme and reagents were incubated at 30◦c for 5 min. The increment in absorbance at 340 nm was recorded against blank containing everything except the enzyme to determine the nanomole substrate conjugated / min. / larvae using a molar extinction coefficient of 9.6 /mM /cm.

Carboxylesterase activity was measured according to the method described by simpson *et al.* (1964) [48], using methyl n butyrate (MeB) as substrate. The reaction mixture contained 200µl enzyme solution, 0.5ml 0.067 M phosphate buffer (PH7) and 0.5ml MeB (4mM). The test tubes were incubated at 37◦c for exactly 30 min. 1ml of alkaline hydroxylamine (equal volume of 2M hydroxylamine chloride and 3.5 M NaOH) was added to the test tubes. Then o.5 ml of HCL (1 part of conc. HCL and 2parts of ΔH2O) was added. The mixture was shaken vigorously and allowed to stand for 2 min. 0.5ml of ferric chloride solution (0.9M Fecl3 in 0.1M Hcl) was added and mixed well. The decrease in MeB resulting from hydrolysis by carboxylesterases was read at 515nm.

Statistical analysis

One - way ANOVA revealed that the mean of all tested enzyme activities ($P<0.001$). The data were subjected to analysis of variance (ANOVA) with at least three repeats (Duncan 1955) [14].

3. Results

In non-specific esterases (NSE) activity by using α or β naphthyl acetate, Monufia populations showed the highest level of beta and alpha esterases (9.2 and 5.7 times higher activity than S-lab strain respectively). Giza and Sohag populations exhibited a significant increase in α-esterases (1.7 and 1.3 times higher activity than S-lab strain respectively) and non-significant with β-esterases. Populations of Beheira did not show any significant elevation of alpha and beta esterases (Tables and Fig. 1 and 2).

Table 1: Alpha esterases activity in 3rd instar larvae of *Culex pipiens* in comparison with laboratory strain.

Natural populations	Alpha esterases ($\mu g\ \alpha -$ naphthol/min/mg protein)	
	Mean ± SD	Enzyme level
Giza	44 ± 3.2^{b}	1.7
Beheira	14 ± 1.07^{d}	0.5
Sohag	33 ± 1.05^{bc}	1.3
Monufia	149 ± 12.99^{a}	5.7
laboratory strain	26 ± 1.08^{cd}	0.0

Mean values followed by different litters are significantly different

$$\text{Enzyme level} = \frac{\text{Enzymatic activity in field populations}}{\text{Enzymatic activity in laboratory colony}}$$

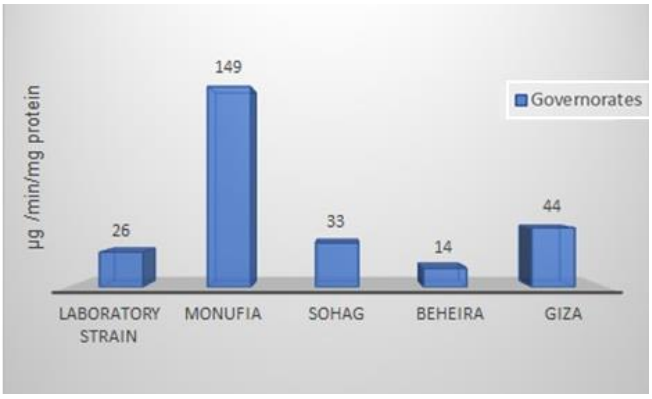

Fig 1: Alpha esterases activity for larvae of *Culex pipiens* populations.

Table 2: Beta esterases activity in 3rd instar larvae of *Culex pipiens* in comparison with laboratory strain.

Natural populations	Beta esterases ($\mu g\ \beta -$ naphthol/min/mg protein)	
	Mean ± SD	Enzyme level
Giza	24.4 ± 1.6^{b}	1.5
Beheira	12.04 ± 1.5^{b}	0.8
Sohag	15.3 ± 0.87^{b}	0.96
Monufia	147 ± 13.6^{a}	9.2
laboratory strain	15.9 ± 1.04^{b}	0.0

Fig 2: Beta esterases activity for larvae of *Culex pipiens* populations.

In regard to glutathione S-transferases (GST), all populations revealed a significant increase as compared to S-lab except in Behrira populations did not show significant elevation. Sohag, Giza and Monufia populations showed 3.8, 2.4 and 1.4 times higher activity than S-lab strain respectively (Table and Fig. 3).

Table 3: Glutathione S-transferase activity in 3rd instar larvae of *Culex pipiens* in comparison with laboratory strain.

Natural populations	GST activity ($\mu mol\ substrate\ conjucated/min/mg\ protein$)	
	Mean ± SD	Enzyme level
Giza	621 ± 72^{b}	2.4
Beheira	245 ± 14^{d}	0.96
Sohag	968 ± 21^{a}	3.8
Monufia	361 ± 14^{c}	1.4
laboratory strain	255 ± 8^{d}	0.0

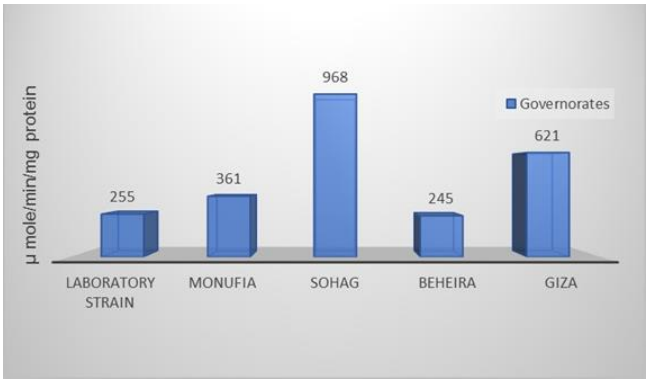

Fig 3: GST activity for larvae of *Culex pipiens* populations.

The activity level of specific carboxylesterases (CarE) was significantly increased in Beheira populations, 1.8 times higher than S-lab strain. Populations of Giza, Sohog and Monufia were nearly in the same range, the enzyme levels were 1.1, 1 and 0.98 respectively and did not show any significant differences from one to another compared to S-lab strain (Table and Fig. 4).

Table 4: Carboxylesterases activity in 3rd instar larvae of *Culex pipiens* in comparison with laboratory strain.

Natural populations	Carboxylesterases (μgMeb$/min/mg protein$)	
	Mean ± SD	Enzyme level
Giza	131 ± 6.2^c	1.1
Beheira	218± 11.02^b	1.8
Sohag	120± 4^c	1
Monufia	114 ± 3.6^c	0.98
laboratory strain	120 ± 4.5^c	0.0

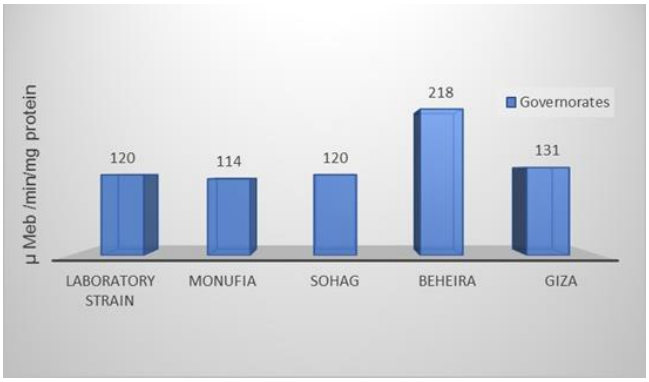

Fig 4: Carboxylesterases activity for larvae of *Culex pipiens* populations.

4. Discussion

Many organophosphate (OP) insecticides including, temephos, chlorpyrifos, pirimiphos-methyl and fenthion are most widely used in *Culex pipiens* control in Egypt, they are effective as larvicide for mosquitoes. In order to understand the resistance of *C. pipiens* to OP insecticides, We evaluated the levels of detoxification enzymes (non- specific esterases, glutathione S-transferase and specific carboxylesterases) in natural populations.

The major mechanism of OP insecticide resistance in *Culex* mosquitoes involves the elevation of one or more esterases. The esterases which preferentially hydrolyses α and β-naphthyl acetate are called A and B esterases respectively. Esterases A and B are coded by two closely linked gene Est-3 and Est-2 respectively (Pasteur *et al*. 1981) [34].

The current study indicated that, the highest level of β and α-esterases in Monufia populations. The association between the activity of α and β esterases has been documented previously such as A2 and B2, A4-B4, A5-B5 and A8-B8 (Rivet *et al*.1993; Wirth *et al*. 1995; Qiao *et al*. 1998 and Wirth 1998) [44, 58, 38, 59]. A3-B3 has been described in *CX. Tarsalis* (Pasteur 1987) [36]. The occurrence of incidence might be due to co-amplification of two esterase genes (Est-2 and Est3 loci) which commonly found in organophosphate resistance (Rooker *et al.1996* and Hemingway *et al.* 2004) [45,21].

Many previous studies observed the association between the alpha and beta esterases involved in the OP resistance in several *Culex* species. Resistance to fenthion in *C. pipiens* judged by its hydrolysis of alpha and beta-naphthyl acetates (Stone and Brown 1969) [51]. Two sets of over-produced esterases (A2-B2 and A4-B4), known to be involved in resistance to temephos in *C. pipiens* from Tunisia (Ben Cheikh and Pasteur 1993) [5]. In Martinique esterases A2 and B2 are known to confer a 100 fold resistance to temephos in *C. p. quinquefasciatus* (Yebakima *et al*. 1995) [60].The A5 plus B5 demonstrated a significant level of temephos resistant in *C.pipiens* from Italy (Severini 1997) [47] and Cyprus (Wirth and Georghiou 1996) [57]. A moderate level of temephos resistance was observed in *CX. quinquefasciatus* from Barazil and biochemical profile of detoxification enzymes showed increased activity for α-esterase and β-esterase in 30 and 17% respectively in strain resistance to temephos compared to susceptible colony (Amorim *et al* 2013)[3]. The increase of detoxification by esterases was also involved in primiphos methyl resistance, overproduced esterases A and B among the Tunisia populations of *C. pipiens* resistance (Tabbabi *et al* 2017) [52].

The present investigation has also revealed that Giza and Sohag populations exhibited a significant increase in alpha esterase and non-significant with beta esterase. Similar observation by Pasteur *et al* 1981[35] who mentioned that ethanol formulating from chlorpyrifos hydrolysis could be demonstrated from esterase A but not for esterse B. Chlopyrifos resistance in *C. quinquefasciatus* from Californa and *C. pipiens* from France associated with a highly active detoxifying esterases encoded by the Est-3A gene (Raymond *et al*. 1986 and Raymond *et al* 1987) [41, 42]. In Egypt resistant strain of *Pectinophora gossypiella* to chlorpyrifos showed significant elevation in the alpha-esterase (Abo-El Seoud *et al*. 2005) [2]. Also the level of non-specific esterases(α-naphthol) significantly increase in both field populations and chlorpyrifos resistant strain of *C. pipiens* compared with laboratory colony in Egypt (Abd El-Samie and Abd El-Basset 2012) [1].

Glutathione S-trasferases (GST) enzymes are particularly important in the metabolism of OP insecticides, in which either the alkyl or aryl substituents on phosphorus can be cleaved. In O-dealkylation (methyl or ethyl groups) the GSH is conjugation with the alkyl portion of OP insecticides, while in the O-dearylation the GSH reacts with leaving group (Wei *et al*. 2001) [55]. Many workers have reported the involvement of GST in OP insecticides (Hayes *et al* 2005 and Che-Mendoza *et al*. 2009) [18, 8].

The present investigation with regared to GST, all populations revealed a significant increase as compared to S-lab except in Beheira populations which did not show significant elevation. Results confirmed by previous studies, GST mediated detoxification in the chlorpyrifos resistance in

Cx quinquefasciatus (Liu *et al.* 2005) [29]. Also Daaboub *et al.* (2017) [11] showed that the increased detoxification by Est (and or GST) had a minor role in the chlorpyrifos resistance. The increased detoxification by Est (and or GST) was also involved in pirimphos methy resistance in *C. pipiens* of field populations from Tanisia (Tabbabi *et al* 2017) [52]. Although glutation greatly increased the demethylation of methyl termed O-dealkylation, or specifically demethylation, deethylation and involved the removal of only one of the alkyl groups, thus destroying the toxicity of fenthion in *C. pipiens* strain (Stone 1969) [50]. In *CX quinquefasciatus*, GST activity increased in temephos at low dose, whereas decreased significantly at high concentration (Muthusamy and Shivakumar 2015) [30]. Amorim *et al*. (2013) [3] suggested that the activity of GST was not significant between moderate level of resistance to temephos strain and susceptible colony of *CX.quinquefasciatus*. Similar observation for resistance strain of *Ae*. *Aegypti* to temephos showed increased for α and β esterases whereas the activity of GST was not significantly different from susceptible strain (Dhang *et al*. 2008; Muthusamy and Shirakumar 2015) [12, 31].

The most commonly observed change that has been linked to resistance development was the increase in the activity of carboxylesterases (Hemingway and Karunaratne 1998) [19]. Carboxylesterases were able to detoxify insecticides by catalyzing the hydrolysis of carboxyl ester, carboxy amide ester and carboxy thioesters and also certain phosphoric acid esters (Heymann and Jakoby 1980) [22]. For *Culex* mosquito this enzyme was referred to as A2 because of its preferential hydrolysis of α- over β- naphthyl acetat (Katterman *et al.* 1992) [27].

Our study detected the activity level of specific carboxylesterase, which was significantly increased only in Beheira populations. Many previous studies observed that *C. pipiens, C. quinquefasciatus* and other Culicidae, their OP-resistance was due to esterase isozymes which can break down OP compounds by phosphatas-type hydrolysis (Pasteur and Georghiou 1980) [33]. The alkaline phosphatases activites might be used as biomarkers in chlorpyrifos resistance in *C.pipiens* in Egypt (Abd El-Samie and Abd El- Basset 2012) [1]. Thionase activity was more important in the mechanism of resistance strain of *CX. P. fatigans* resistance to fenthion (Stone and Brown1969) [51]. Biochemical assay suggested a role for increased carboxylesterases activities in conferring temephos resistance in *Ae*. *Aegypti* larvae in Thailand (Pardin *et al*. 2014) [32].

The juvenods (JH) (such as methoprene and pyriproxyfen) with the benzoylphenyl urea chitin synthesis inhibitor (diflobenzuron) introduced a group of insecticides termed insect growth regulators (IGR). This IGR and Bti (*Bucillus thuringiesis*) are used as larvecidal in mosquitoes control in Egypt.

Specific and non specific esterases have been shown to hydrolyze JH to JH –acid in several tissues of different insects (Yu and Terriere 1978) [61]. The most abundant primary metabolite of JH in *Culex* and *Aedes* larvae was the hydroxyl ester (Quistad *et al.* 1975) [39]. Juvenile hormones analogues such as mothoprene have been identified as that possess an α and β unsaturated methyl ester at one end of the molecule and an epoxide at other. The methyl ester of JH is hydrolyzed by a JH –specific esterases (JHE) and the epoxid by JH epoxid hydrolas (JHEh) (Kamita and Hammock 2010) [26]. Hooper (1976) [24] concluded that esterases hydrolysing malathion and α- NA were similar to each other and were distinct from those hydrolyzing JH. Downer *et al* (1975) [13] mentioned that the juvenoid methoprene could depress the activity of a non – specific esterase in *Aedes aegypti*.

On the other hand *Ae. aegypt* populations with resistance to the organophosphate temephos were less susceptible to pyriproxyfen, indicating a possible cross resistance in mosquitoes between these two insecticides families (Andrighetti *et al*. 2008) [4]. In Malaysia, *Aedes* larvae tolerance against temephos was reported and exhibited moderate resistance toward pyriproxyfen (Chen 2005) [9] but susceptible to diflobenzuron (Lau *et al*. 2015) [28]. Also Braga *et al.* (2005) [7] indicated a potential cross resistance when larvae were exposed to temephos and methoprene. Resistance to methoprene associated with activation of the esterases and mixed function oxidases were responsible for the metabolic resistance to conventional chemical insecticides, including OP insecticide. *C.pipiens* selected for mothoprene showed cross resistance to other juvenoids but not to diflubenzuron (Ishaay 2009) [25].

The effectiveness of Bti as a larvicide is an agent that does not cross resistance with motheprene (Cornel *et al.* 2002) [10]. The enzymatic activities of GST and carboxylesterases could be affected by Bti treatment. The activity of GST and carboxylesterases were increased after Bti treatment, but recovered to normal level quickly (Han *et al.* 2015) [16].

5. Conclusion

Our studies suggested that the difference in enzyme activity in *C.pipiens* natural populations could pose a threat for future control strategies. In addition, the early detection of resistance is essential for the successful implementation of insecticide resistance management strategies. These data will help monitoring and resistance management as a part of any program of vector control.

6. References

1. Abd El, Samie EM, Abd El –Basset TT. Efficacy of some insecticides on field populations of *Culex pipiens* (Linnaeus) from Egypt. J Basic and Applied zoology. 2012; 65:62-73.
2. Abu-El Seoud S, Abd-El Samie EM, Abdel Basset TT. Toxicological and biochemical characteristics of certain insecticides on chlorpyrifos resistant strains of the pink boll worm, *Pectinophora gossypiella* (Saunders) in Egypt. J. Egypt. Acard. Soc. Environ. Dev. 2005; 6:63-89.
3. Amorim LB, Helvecio E, de Oliveira CMF, Ayres CFJ. Susceptibility status of *Culex quinquefasciatus* (Diptera: Culicidae) populations to the chemical insecticide temephos in Pernambuco, Brazil. Pest Manag Sci, 2013, 1-9.
4. Andrighetti MTM, Cerone F, Rigueti M, Galvani K C, Macori M. Effect of pyriproxyfen in Aedes aegypti populations with different levels of susceptibility to the organophosphate temephos. Dengue. Bulletin, 2008, 186-198.
5. Ben Cheikh H, Pasteur N. Resistance to temephos: An organophosphate insecticide in *Culex pipiens* from Tunisia, North Africa. J. Am. Mosq. Control Assoc. 1993; 9:335.
6. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal. Biochem. 1976; 2:248-254.

7. Braga I, Mello CB, Montella IR, Lima JB, Martins AJ, *et al*. Effectiveness of methoprene, an insect growth regulator, against temephos-resistant *Aedes aegypti* population from different Brazilian localities, under laboratory condition. J. Med. Entomol. 2005; 43:830-7.
8. Che-Mendoza A, Penilla RP, Rodriguez DA. Insecticide resistance and glutathione S-transferases in mosquitoes: A review. African. J. Botechnology. 2009; 8(8):1386-1397.
9. Chen CD, Nazni WA, Lee HL, Sofian-Azirun M. Susceptibility of *Aedes aegypti* and *Aedes albopictus* to temephos in four sites in Kuala Lumpur City Center and Selangor State, Malaysia. Trop. Bio-med. 2005; 22:207-16.
10. Cornel AJ, Stanich MA, McAbee RD, Mulligan FS. High level methoprene resistance in the mosquito *Ochlerotatus nigromaculis* (Ludlow) in Central California. Pest. Manag Sci. 2002; 58:791-798.
11. Daaboub J, Tabbabi A, Lamari A, Ben Cheikh R, Ben Jha I, Ben Cheikh H. Evaluation of chlorpyrifos resistance and biochemical mechanisms of *Culex pipiens* in five localities of Grand Tunis area, northeast Tunisia. Hereditary Genet. 2017; 6(2):1-3.
12. Dhang CC, Ahmad NW, Lim LH, Benjamin S, Azirun MS. Biochemical detection of temephos resistance in *Aedes* (Stegomyia) *aegypti* (Linnaeus) from dengue-endemic areas of Salangor state, Malaysia. Trop. Med. Parasitol. 2008; 3:6-20.
13. Downer NGH, Wiegand M, Smith SM. Suppression of pupal eserrase activity in *Aedes aegypti* (Diptera-Culicidae) by an insec growth regulator. Experientia. 1975; 31:1239-1240.
14. Duncan DB. Multiple range and multiple F- test. Bometrics.1955; 11:1-42.
15. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferase, the first enzymatic step in mercapturic acid formation. J. Bio. Chem. 1974; 249:7130-7139.
16. Han GJ, Li CM, Sun J, Liu Q, Zhao S, Qi JH, Xu J. Effect of *Bucillus thuringiensis* Var. israelensis (Bti) on detoxification enzyme of larvae of *Culex pipiens* Pallens and *Aedes aegypti*. Zhongguo. Xue. xi. Chong. Bing. Fiang Zhi. Za. Zhi. 2015; 27(4):385-389.
17. Harb M, Faris R, Gad AM, Hafez ON, Ramz R, Buck AA. The resurgence of lymphatic Filariasis in the Nile Delta. Blletin. WHO. 1993; 71:49-54.
18. Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. Annu. Aev. Pharmacol. Toxicol. 2005; 45:51-88.
19. Hemingway J, Karunaratne SH. Mosquito carboxylesterases. A review of molecular biology and biochemistry of a major insecticide resistance mechanism. Med. Veterinary Entomol. 1998; 12:1-12.
20. Hemingway J, Smith C, Jayawardena KGI, Herath PRJ. Field and laboratory detection of the altered acetylcholinesterase resistance genes which confer organophosphate and carbamate resistance in mosquitoes (Diptera: Culicidae). Bull. Entmol. Resea. 1986; 76:559-565.
21. Hemingway J, Hawkes NJ, Mc Carroll L, Ranson H. The molecular basis of insecticides resistance in mosquitoes. Insect Bioche and Molec Biol. 2004; 34:653-665.
22. Heymann E, Jakoby WB. Carboxylesterases and amidases. Enzymatic basis of detoxification. 11.2980; 291-323.
23. Hoogstraal H, Meegan JM, Khalil GM, Adham FK. The Rift Valley fever epizootic in Egypt 1977-1978. Ecological and entomological stdudies. Trans. R. Soc. Trop. Med. Hyg. 1979; 73:624-629.
24. Hooper GHS. Esterases mediated hydrolysis of naphyhyl ester, malathion, methoprene and cecropia juvenile hormone in *Culex pipiens*. Insect. Biochem. 1976; (6):255-266.
25. Ishaay I. Insect resistance to benzoylphenylureas and other insec growth regulators. American. Chemical. Society. 2009; (505):231-246.
26. Kamita SG, Hammock BD. Juvenile hormones esterase: biochemistry and structure. J. Pestic. Sci. 2010; 35:265-274.
27. Ketterman AJ, Jayawardene KG I, Hemingway J. Purification and characterization of a carboxylesterase involved in insecticide resistance from the mosquito *Culex quinquefasciatus* Biochemical Journal. 1992; 287:355-360.
28. Lau KW, Chen CD, Lee HL, Norma-Rashid Y, Sofian-Azirun M. Evaluation of insect growth regulators against field- collected *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) from Malaysia. J. Med.Entomol. 2015; 52:199-206.
29. Liu H, Xu Q, Zhang L, Liu N. Chlorpyrifos resistance in *Culex quiquefasclatus*. J. Med. Entomol. 2005; 42:815-820.
30. Muthusamy R, Shivakumar MS. Effect of lambda cyhalothrin and temephos on detoxification enzymes in *Culex quinquefasciatus* (Diptera: Culicidae). J. Environ. Biol. 2015; 36:235-239.
31. Muthusamy R, Shirakumar MS. Susceptibility stutus of *Aedes aegypti* (L) (Diptera: Culicidae) to temephos from three districts of Tamil Nadu, India. J. Vector Borne. Dis. 2015; 5:159 -165.
32. pardin R, Srisukorat W, Yunta C, Ranson H. Identification of carboxyl esterase genes implicated in temephos resistance in the dengue vector *Aedes aegypti*. Plos. Neglected. Tropica. Diseases. 2014; 8(3):1-11.
33. Pasteur N, Georghiou GP. Analysis of esterases as a means of determining organophosphate resistance in field populations of *Culex pipiens* mosquitoes. Proc. Calif. Mosq, Vector Control Assoc. 1980; 48:74-77.
34. Pasteur N, Iseki A, Georghiou GP. Genetic and biochemical studies on the highly active esterases A and B associated with organophsphate resistance in *Culex pipiens* complex. Biochem. Genet. 1981; 19:909-919.
35. Pasteur N, Sinegre G, Gabinaud A. Est-2 and Est-3 polymorphism in *Culex pipiens* L. from southern France in relation to organophosphate resistance. Biochem. Genet. 1981; 19:499.
36. Pasteur N. Genetic association between highly active esterases and organophosphate resistance in *Culex tarsalis*. J.Am. Mosq.Control. Assoc. 1987; 3:473-475.
37. Price NR. Insect resistance to insecticides: mechanism and diagnosis. Comp. Biochem. Physiol. 1991; 100:319-326.
38. Qiao CL, Marquine M, Pasteur N, Raymond M. A new esterase gene amplification involved In OP resistance in *Culex pipiens* Mosquitoes from China. Biochemical. Genetics. 1998; 36:417-426.
39. Quistad GB, Staiger LE, Schooley DA. Environmental degradation of the insect growth regulator methoprene: V. Metabolism by houseflies and mosquitoes. Pest.

Bioch and Physio. 1975; 5:233-241.
40. Ranson H, Hemingway J. Mosquito glutathione transferases. Methods in Enzymology. 2005; 401:226-241.
41. Raymond M, Fournier D, Bride JM, Cuany A, Berge, J, Magnin M, Pasteur N. Identification of resistance mechanisms in *Culex pipiens* (Diptera: Culicidae) from southern France: insensitive acetylcholinesterase and detoxifying oxidases J. Econ. Entomol. 1986; 79:1452-1458.
42. Raymond M, Pasteur N, Georghiou GP, Mellon RB, Wirth MC, Hawley MK. Detoxification esterases new to California in organophosphate-resistant *Culex quinquefasciatus* (Diptera:Culicidae). J. Med. Entomol. 1987; 24: 24-27.
43. Raymond M, Chevillon C, Guillemand T, Lenormond T, Pasteur N. An overview of the evaluation of overproduced esterases in the mosquito *Culex ppiens* Philos. Trans. R. Soc. Land. Biol. Sci. 1998; 353:1707-1711.
44. Rivet Y, Marquine M, Ramond M. French mosquito populations invaded by A2- B2 esterases causing insecticide resistance. Biol. J. Linn. Soc. 1993; 49:249.
45. Rooker S, Guillemoud T, Berge J, Pasteur N, Raymond M. Coamplification of esterase A and B genes as a single unit in the mosquito, *Culex pipiens*. Heredity. 1996; 77:555-561.
46. Scott JG. Cytochrome P450 and insecticide resistance, Mol. Bio. 1999; 29:757-777.
47. Severini C, Romi R, Marinucci M, Guillemand T, Raymond M. Esterases A5-B5 in organophosphate-resistant *Culex pipiens* from Italy. Med. Vet. Entomol. 1997; 11:123-126.
48. Simpson DR, Bull DL, Linquist DA. A semimicrotechniqu for estimation of cholinesterase activity in boll weevils. Ann. Ent. Soc. Amer. 1964; 57:367-371.
49. Soluman A, Mohareb E, Sulman D, Saad M, Salama S, *et al*. Studies on West Nile Virus infection in Egypt J. Infec and Pup. Hel. 2010; 3(2):54-59.
50. Stone BF. Metabolism of fenthion by the southern house mosquito. J. Econ. Entomol. 1969; 62(5):977-981.
51. Stone BF, Brown AWA. Mechanisms of resistance to fenthion in *Culex pipiens* fatigans Wied. Bull. World. Health. Organ. 1969; 40:401-408.
52. Tabbabi A, Daaboub JL, aameri A, Ben Cheikh R, Ben Cheikh H. Pirimiphos –methyl resistance status of field populations of *Culex pipiens* (Diptera: Culicidae) from grand Tunis area, northeast Tunisia. Herditary Genet. 2017; 6(1):1-4.
53. Taylor RM, Work TH, Hurlbut HS, Rizk FA. Study of the ecology of West Nile Virus in Egypt. Am. J. Trop.Med. Hyg. 1956; 5:579-620.
54. Van Asperen K. A study of house fly esterase by means of sensitive colourimetric method. J. Insect. physiol. 1962; 8:401-416.
55. Wei SH, Clark AG, Syvanen M. Identification and cloning of a key insecticides-metabolizing glutathione S-transferase (Md GST-6A) from a hyper insecticide-resistant strain of the house fly *Musca domestca.* Insect. Bochem. Mol. Bol. 2001; 31:1145-1153.
56. Wheelock CE, Shan G, Ottea J. Overview of carboxylesterases and their role in the metabolism of insecticides. J. Pestic. Sci. 2005; 30(2):75-83.
57. Wirth MC, Georghiou GP. Organophosphate resistance in *Culex pipiens* from Cyprus. J. Amer. Mosq. Cont. Asso. 1996; 12(1):112-118.
58. Wirth MC, Marquine M, Georghiou GP, Pasteur N. Esterases A2 and B2 in *Culex quinquefasciatus* (Diptera: Culicidae): role in organophosphate resistance and linkage. J. Med. Entomol. 1995; 27:202-206.
59. Wirth MC. Isolation and characterization of two novel organophosphate resistance mechanisms in *Culex pipiens* from Cyprus. J. American. Control. Association. 1998; 14:397-405.
60. Yebakima A, Raymond M, Marquine M, Pasteur N. Resistance to organophosphorus insecticides in *Culex pipiens quinquefasciatus* (Diptera: Culicidae) from Martinique. J. Med. Entomol. 1995; 32(2):77-82.
61. Yu SJ, Terriere LC. Metabolism of juvenile hormone 1 by microsomal oxidase, esterases, and epoxide hydrase of *Musca domestica* and some comparisons with *Phormia regina* and *Sarcophaga bullata*. Pestic Biochem Physiol. 1978; 9:237-246.