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Biochemical Changes in Both Adults and the 4th Instar Larvae of Sand Fly, *Phlebotomus papatasi* as Indicators for Tolerance to Insecticides at Sharkia Governorate

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ABSTRACT

Sandfly, *Phlebotomus papatasi* Scopoli was considered one of the dangerous medical insects that transmits a lot of diseases such as Leishmaniasis disease around the world. In Egypt, synthetic pyrethroids and organophosphorous insecticides were the main chemicals control methods for *P. papatasi*. So, the samples of adults and 4th larvae were collected from two different localities (10th of Ramadan city and Al- Quareen city), in addition, lab. strain. The common insecticides used synthetic pyrethroids (deltamethrin and lambda-cyhalothrin) and organophosphorous (chlorpyrifos and malathion) and diagnosed this tolerance biochemically. Respecting the biochemical diagnoses, electrophoresis protein separation utilizing sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE), some target and detoxifying enzymes acetylcholinesterase (AChE), general esterases (GES), cytochrome-P450 (C-450), glutathione-S-transferase (GST)} were characterized. Also, (amylase, invertase, alkaline phosphatase, acid phosphatase and total lipids) were determined. The obtained results showed that the 4th larvae of *P. papatasi* collected from the 10th of Ramadan locality was the most tolerant to cholorpyrifos (2.79- fold), deltamethrin (2.21- fold), malathion (1.76- fold) and lambda-cyhalothrin (1.16- fold) compared with the laboratory strain, while in adults the most tolerant was lambda-cyhalothrin (2.81-fold), cholorpyrifos (2.16-fold), deltamethrin (1.65-fold) and malathion (1.43). It was observed that there was a significant difference between the insect populations, the presence of different proteins bands in 10th of Ramadan population tolerant to these insecticides, followed by Al-Quareen city population compared with laboratory strain. Regarding the target and metabolic enzymes, data found that there was a highly significant increase in these metabolic enzymes activities (GES, P-450 and GST) in 10th of Ramadan locality, followed by Al- Quareen locality compared with laboratory strain in adults and larvae, while, other biochemical constituents, Total lipids, trehalase, alkaline phosphatase, acid phosphatase, amylase, invertase activities and their roles in energies for larvae and adults of *P. papatasi* showed a reduction. Generally, the results showed that there was a highly significant reduction in most of these energies enzymes. So, the current study recommended avoiding intensive insecticides used and randomly used, which led to increasing insecticides tolerance/ insecticides development in these the dangerous medical pests transmitting the epidemic diseases and human disaster.

INTRODUCTION

Leishmaniasis is transmitted by Phlebotominae sand flies, which are prevalent in over 98 countries throughout the world (WHO, 2018). Cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL), also known as "kala-azar," are the three primary clinical forms of the illness. An obligatory intracellular flagellated protozoan of the genus Leishmania is the causative agent. *Phlebotomus argentipes* (Diptera: Psycodidae) is the known vector of *Leishmania donovani*, the causative agent of leishmaniasis in Sri Lanka, (Desjeur, 1996; Arunaweera *et al.,* 2003). In Sri Lanka, leishmaniasis is a fast-expanding public health hazard (Weekly Epidemiological Reports, 2018), and there is currently no national control programme in place to address the problem. VL is the most virulent of the three types of parasitic disease and is responsible for the second-highest incidence of parasitic-related deaths (Picado *et al.,* 2012). Leishmaniasis can be controlled by interrupting the transmission cycle. Early detection and treatment of patients, as well as vector and reservoir host management, are all frequently utilised methods. Vector control is restricted due to the difficulty of discovering sand fly larval sites, despite its popularity as a leishmaniasis management technique (Alexander and Maroli, 2003).

As a result, vector management is primarily focused on adult flies control, which is typically achieved through chemical insecticides utilisation. Organochlorines, organophosphates, carbamates, and pyrethroids are the most commonly used chemical insecticides for vector control (Karunaratne *et al.,* 2018). Insecticide use, on the other hand, is only recommended after extensive research of the vector's biological features, such as resting behaviour and insecticide sensitivity patterns. Tolerance to such compounds is frequently developed as a result of haphazard or excessive usage of such chemicals.

Toxic insecticidal substances can be resisted by insects through a range of physiological methods, including interference with enzyme systems via the creation of detoxifying enzymes and/or effects on the insecticide's target site(s), rendering them insensitive. Acetylcholinesterase (AChE) destroys acetylcholine, a neurotransmitter that is a target for pesticides like organophosphates and carbamates. As a result, AChE gene alterations can lead to pesticide resistance (Karunaratne *et al.,* 2018; Hemingway *et al.,* 2004).

Insecticide resistance in natural vector populations must be monitored on a regular basis in order to detect resistance early and improve the effectiveness of operational control tactics (Aizoun *et al.,* 2014). Sandfly vectors in India, for example, have been found to be resistant to the pesticide dichlorodiphenyltrichloroethane (DDT) (Singh *et al.,* 2001 and Kishore *et al.,* 2006). Insecticide resistance research is currently focusing on the biochemical and molecular basis that can be utilised to identify resistant genotypes in insect populations (Surendran *et al.,* 2005; Lins *et al.,* 2008 and Liu *et al.,* 2011).

Only a few papers from throughout the world have suggested that enzymes may have a role in sand fly pesticide resistance (Lins et al., 2008 and Liu *et al.,* 2011). In Egypt, sand flies may have acquired pesticide resistance as a result of the widespread use of insecticides in malaria control programmes and agricultural practices, especially in the country's northern regions. Despite the fact that several studies on resistance in the malaria vector have been undertaken, no single study on pesticide resistance in sand flies has been conducted in the country to far (Abdallah *et al.,* 2008 and Himeidan *et al.,* 2011). As a result, the current study was conducted to determine the sensitivity and/or tolerance status of P. papatasi to several chemicals.

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enzymes may play a role in insecticide resistance in sand flies (Lins *et al.*, 2008 and Liu *et al.*, 2011). In Egypt, sand flies may have acquired pesticide resistance as a result of the widespread use of insecticides in malaria control programmes and agricultural practices, especially in the country's northern regions. Despite the fact that several studies on resistance in the malaria vector have been undertaken, no single study on pesticide resistance in sand flies has been conducted in the country to far (Abdallah *et al.*, 2008 and Himeidan *et al.*, 2011). As a result, the current research was conducted to determine the sensitivity and/or tolerance of P. papatasi to routinely used insecticides. Also, the biochemical diagnosis of *P. papatasi* tolerant to these insecticides as well as insecticides tolerance effects on some biochemical constituents.

MATERIALS AND METHODS

Sandflies, *P. papatasi*, Collection and Rearing:

P. papatasi flies were obtained from two different locations in Sharkia governorate (10th of Ramadan and Al-Quareen) using light traps set outdoors for ten consecutive nights and compared to the laboratory strain present in the Veterinary and Medical Insects Institute. For two generations, they were reared and maintained in a controlled laboratory environment at 26.3°C and relative humidity of 60%. Sandflies were placed in a freshly cleaned sand fly cage. In a sand fly cage, pigeons were exposed to sand flies for 30 minutes. Blood-starved sand flies were placed in oviposition vials lined with gypsum (calcium sulphate) and covered with mesh after being fed. The females in the vials were then kept at a temperature of 28–30° C. Using long syringes, the gypsum material lining the oviposition pots was wetted with distilled water. The newly emerged larvae were subsequently fed a larval diet made from ground rabbit faeces. Susceptibility and biochemical experiments were performed on adults and larvae of *P. papatasi*.

Insecticides:

Chlorpyrifos: diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate.

Malathion: S-(1,2-dicarboethoxyethyl) O, O-dimethyl phosphorodithioate

Deltamethrin: (S)- cyano- (3-phenoxyphenyl) methyl] (1R, 3R)- 3- (2,2-dibromoethenyl)- 2,2- dimethylcyclo propane-1-carboxylate

Lambda-cyhalothrin: reaction product comprising equal quantities of (R) -α-cyano- 3-phenoxybenzyl (1S, 3S)-3- [(Z)- 2- chloro-3,3,3- trifluoro propenyl]- 2, 2- dimethyl cyclopropane carboxylate and (S)- α- cyano- 3-phenoxybenzyl (1R, 3R) -3 -[(Z)- 2- chloro-3,3,3- trifluoro propenyl]- 2,2-dimethylcyclopropanecarboxylate.

Bioassay Tests:

The insecticides employed were chosen to reflect two different insecticide classes that have been utilised in Egypt, (chlorpyrifos, malathion, deltamethrin, and lambda-cyhalothrin). The insecticides were used to test the tolerance of *P. papatasi* adults and larvae to the tested insecticides. After 24 hours, the final mortality rate was recorded. Each insecticide was tested in three replicates at six different concentrations. Replicates were made by placing a Petri dish (90 mm x 20 mm) containing the weight of ground rabbit faeces on it and placing *P. papatasi* larvae on the treated ground rabbit faeces. Sandflies were exposed to the previous four tested insecticides, which were dissolved in acetone and placed in glass bottles. The solvent had volatilized, as had the residues in these bottles. The experiment was carried out in three replicates for each insecticide and one control. Twenty unfed females were used in each replicate. After 24 hours, the dead flies were counted. Female adults and larvae were kept in a controlled laboratory environment for 24 hours, and mortality was determined. Abbott's formula was used to correct the mortality percentages (Abbott, 1925). According to Finney (1971), the toxicity lines (Ld-P lines) were drowned and the LC50, LC90, and slope values were estimated. Bioassay of adults; in each test, 20 female sand flies from the F1 population were acclimatised for 0.5 hours at 26 26°C in a holding tube lined with insecticide-free paper. They were then transferred to the testing tube using insecticide-

impregnated filter paper. The insects were transferred to holding tubes for a further 24 hour recovery period with 30 percent sugar at 26°C after standing erect for 1 hour. For each insecticide concentration, five replicates were used. The percentages of flies that died after 24 hours of insecticide exposure were calculated. Each experiment's negative controls were oil and acetone-soaked papers. Only when the mortality rate in the control tubes was less than 20% was the data included, and the results were confirmed with the control samples using Abbott's formula.

Biochemical Analysis:

Adults from each population (10^{th} of Ramadan and Al-Quareen) were homogenized separately in 100 l of ice-cold distilled water. For each of the above standard assays; two duplicates of 10 l of crude homogenate produced following centrifugation at 13,000 rpm for 2 minutes were utilised. Each study site's 200 flies were homogenised individually in 70 l of ice-cold distilled water. Larval homogenate samples were prepared by homogenizing fifty 4^{th} instar larvae from each population (10^{th} of Ramadan and Al-Quareen) in a 2 ml centrifuge Eppendorf with 250 l of 0.1 M ice-cold sodium phosphate buffer, pH 7.4, containing 1.0% Triton X-100. At 4°C, the homogenate was centrifuged at 15,000 rpm for 15 minutes. The supernatant was then divided into clean 0.5 ml Eppendorf tubes and maintained at –20°C until colorimetric and electrophoretic measurements of total protein, acetylcholinesterase, general esterases, GST, and cytochrome P450-dependent monooxygenase activities were performed within 15 days.

SDS- PAGE Electrophoresis:

The total protein of sand flies was determined colorimetrically according to (Bradford, 1976). The protein pattern in two separate *P. papatasi* locales was studied using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and compared to the control. SDS-PAGE was performed at room temperature in vertical apparatus as described by (Laemmli, 1970).

Target and Metabolic Enzymes:

Acetylcholinesterase (AChE) Activity: The activity of AChE in entire larvae was measured using the procedure described by (Ellman *et al*.,1961).

Cytochrome p450 (Cyt p450) Monooxygenases: The activity of CP_{450} was measured using the method of p-nitroanisole oxidative demethylation (Hansen and Hodgson, 2001).

General Esterases (GES) Activity: Colorimetric esterase activity assay was measured utilizing the general substrates α- or β- naphthyl acetate as mentioned by (Gomori, 1953) with some modifications by (Grant *et al*., 1989).

Glutathione-S-Transferase (GST) Activity: The modified method of assaying GST activity was used (Grant and Matsumura, 1988).

Enzymes and Other Biochemical Constituents:

Determination of Trehalase: The amount of trehalose in the sample was determined using the method outlined by (Leyva, *et al.*, 2008).

Determination of Phosphatases: Acid and alkaline phosphatase activity were measured using the technique of **(**Powell and Smith, 1954).

Determination of Amylase: The dinitrosalicylic acid (DNS) technique (Bernfeld, 1955) was used to measure α--amylase activity, with 1% soluble starch (Merck) as a substrate (Bandani *et al.*, 2009).

Determination of Invertase: The activity of invertase was determined using the method described by (Ayre, 1967).

Statistical Analysis:

The median lethal concentrations (LC_{50}) and 90% (LC_{90}) mortalities were estimated using probit regression analysis utilising a Probit polo pc plus software v 3.1 (LeOra Software Inc., Cary, NC) that automatically compensated for control mortality according to the method of (Finney,1971). All results of biochemical characterizations were subjected to analysis of one-way ANOVA using the SPSS 14.00 software (SPSS Inc. Chicago, Il, USA).

RESULTS AND DISCUSSION

Insecticides Toxicity on The Relative Susceptibility in Adults and Larvae of *P. papatasi*:

The relative susceptibility in adults and larvae of *P. papatasi* of the two collected populations from 10th of Ramadan and Al-Quareen localities to the tested insecticides were assessed in (Figs.1-8). The relative tolerance levels values in the two different collected populations to the four different tested compounds, it seems clear that all populations exhibited different degrees of tolerance to these compounds compared with the laboratory strain. Data showed that in adults samples of 10th of Ramadan population the highest degree of tolerance at LC_{50} levels towards λ-cyhalothrin, chlorpyrifos, deltamethrin and

Malathion (2.818, 2.611, 1.65 and 1.434-fold), respectively, while in larvae data showed differences in tolerance to insecticides where the highest degree of tolerance at LC50 levels towards (chlorpyrifos, deltamethrin and Malathion and λ-cyhalothrin (2.79-, 2.21- and 1.76- and 1.16–fold), respectively.

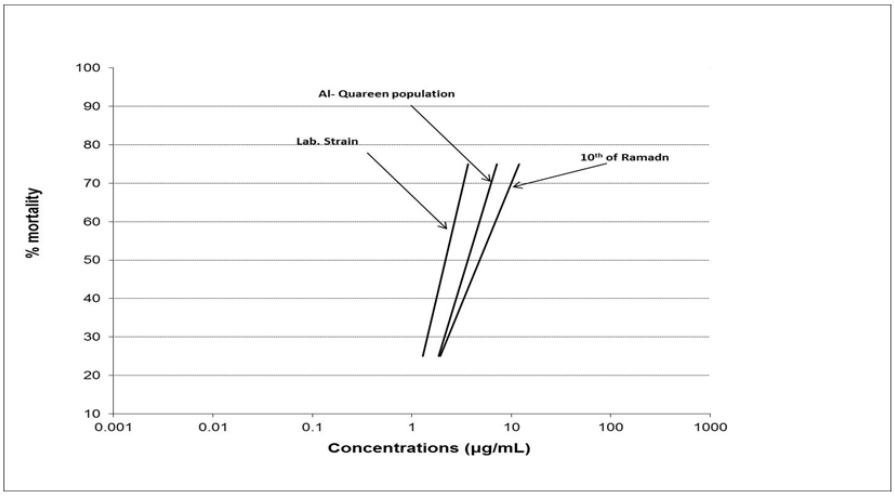

Fig. 1: Bioassay of the insecticide deltamethrin against *P. papatasi* larvae collected from two localities compared with a laboratory strain under controlled laboratory conditions.

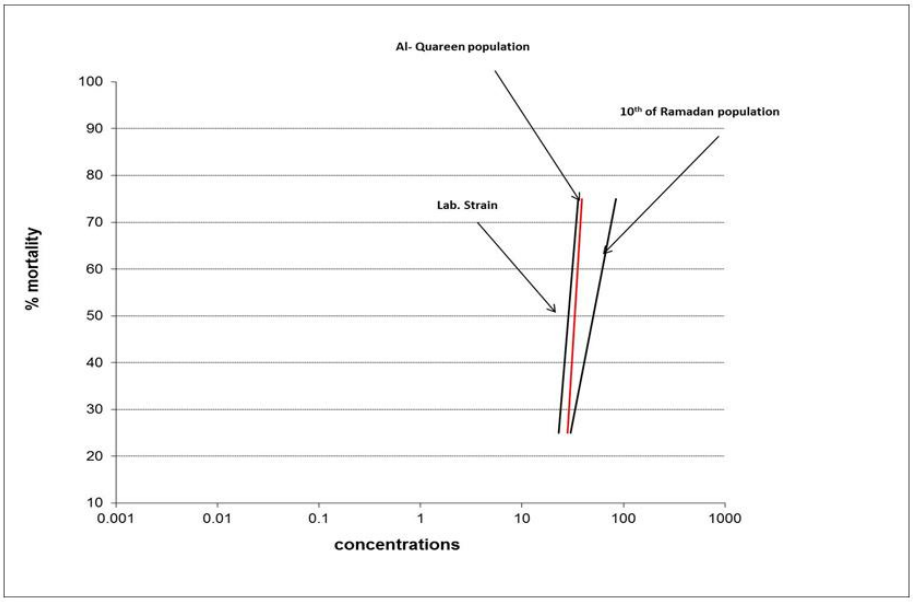

Fig. 2: Bioassay of the insecticide malathion against *P. papatasi* larvae collected from two localities compared with a laboratory strain under controlled laboratory conditions**.**

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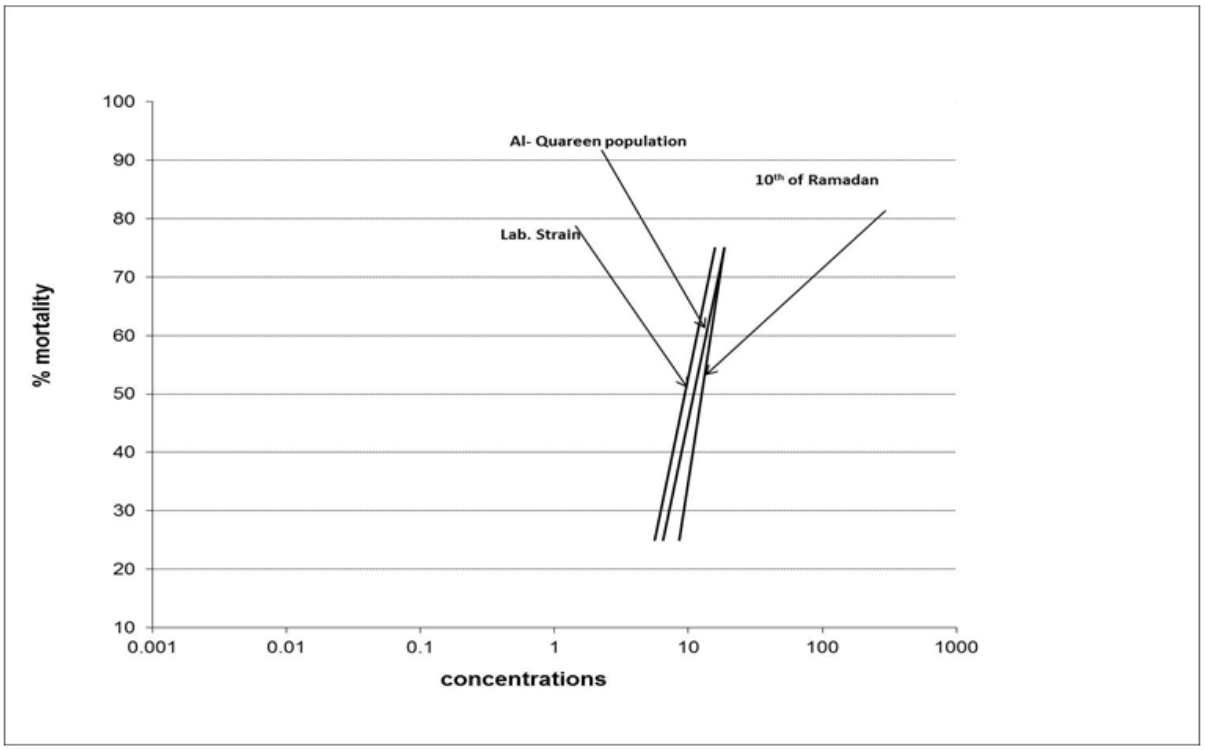

Fig. 3: Bioassay of the insecticide λ-cyhalothrin against *P. papatasi* larvae collected from two localities compared with a laboratory strain under controlled laboratory conditions.

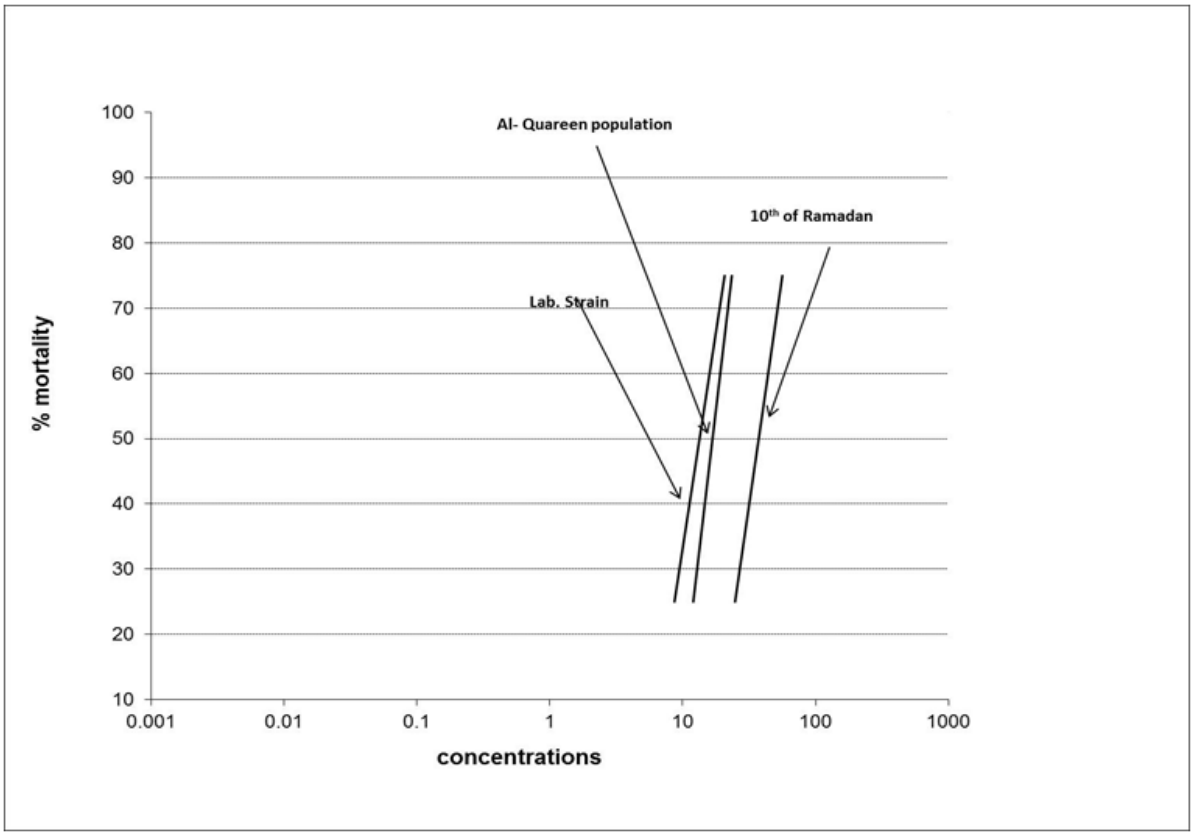

Fig. 4: Bioassay of the insecticide Chlorpyrifos against *P. papatasi* larvae collected from two localities compared with a laboratory strain under controlled laboratory conditions.

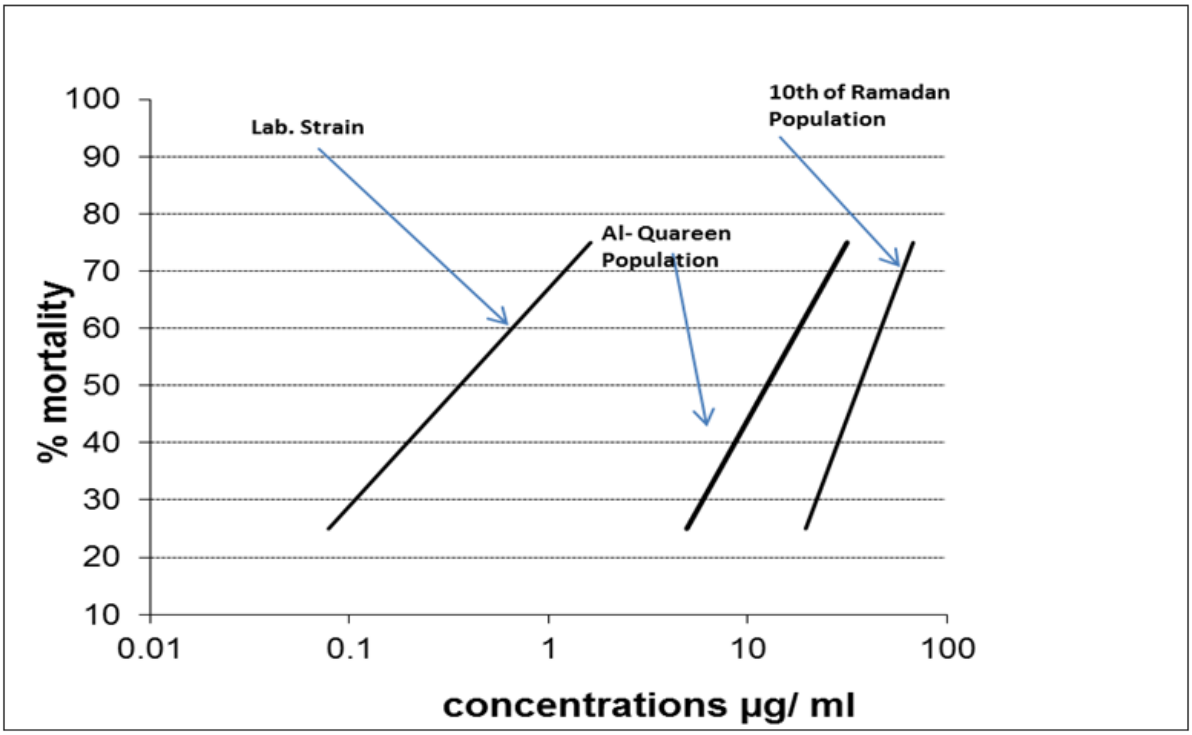

Fig. 5: Bioassay of insecticide deltamethrin against *P. papatasi* adults collected from two localities compared with a laboratory strain under controlled laboratory conditions.

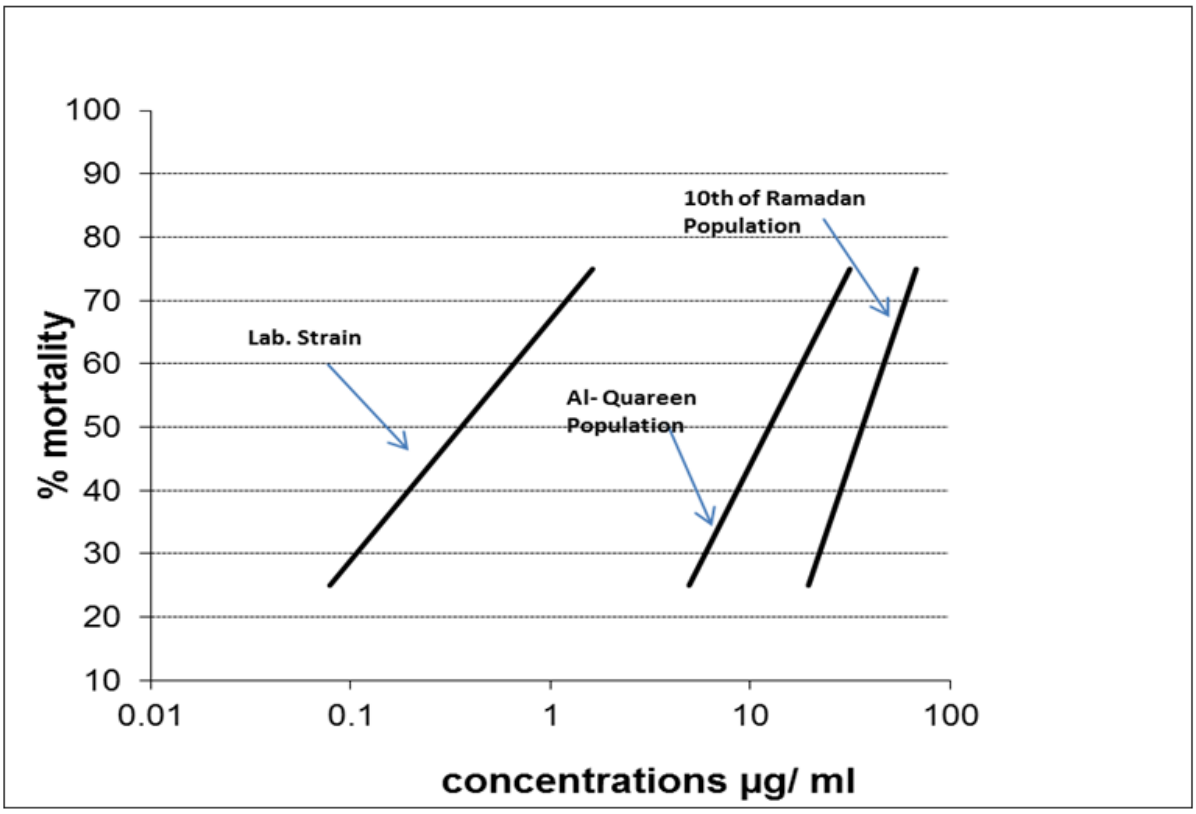

Fig. 6: Bioassay of the insecticide λ-cyhalothrin against P. papatasi adults collected from two localities compared with a laboratory strain under controlled laboratory conditions.

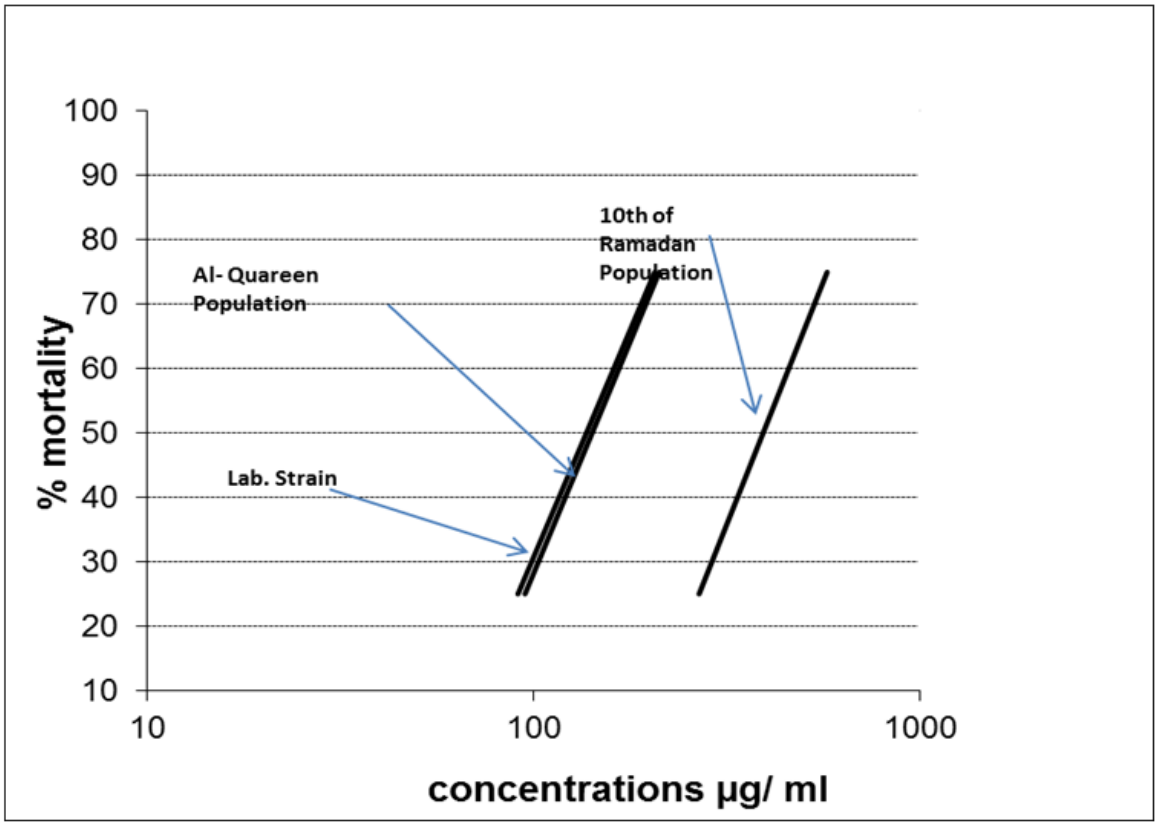

Fig. 7: Bioassay of the insecticide malathion against *P. papatasi* adults collected from two localities compared with a laboratory strain under controlled laboratory conditions.

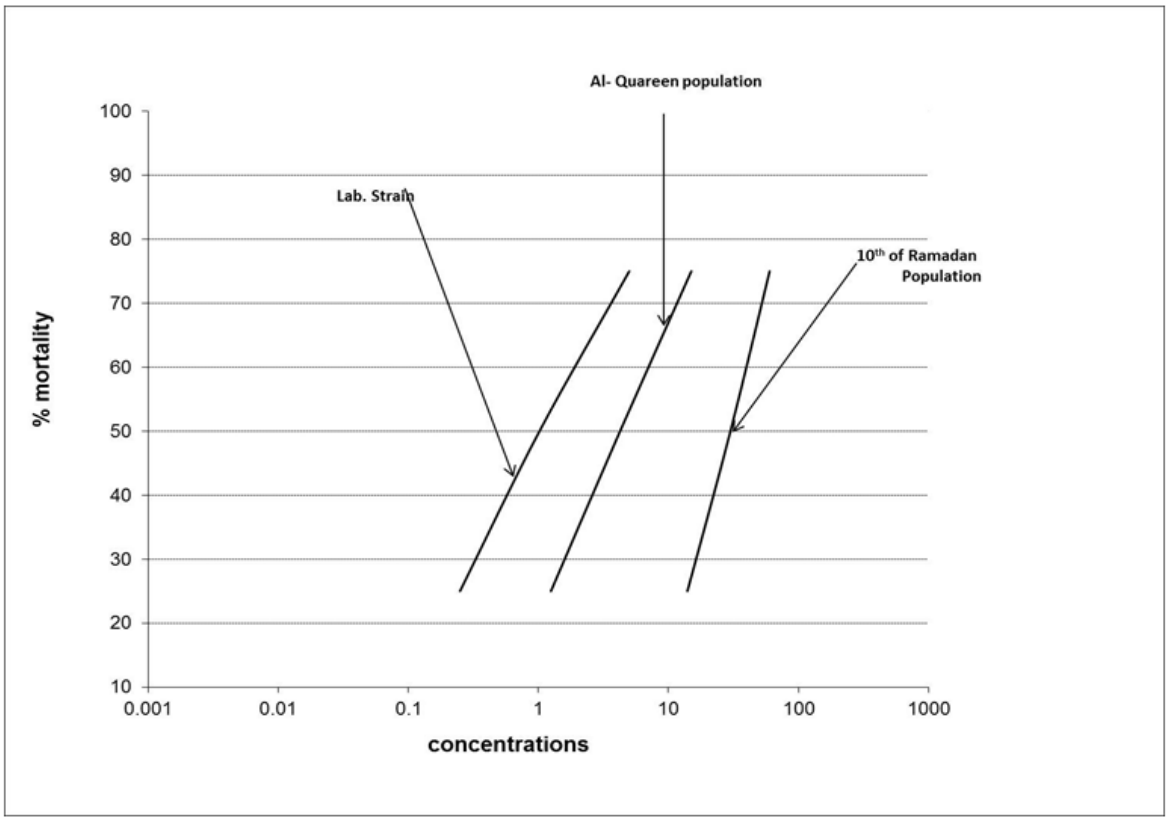

Fig. 8: Bioassay of the insecticide Chlorpyrifos against *P. papatasi* adults collected from two localities compared with a laboratory strain under controlled laboratory conditions.

The current findings matched those of (Dhiman and Mittal, 2000), who stated that the first record of sand-fly resistance to organophosphates and synthetic pyrethroids was discovered in India in 2000. They discovered resistance to the insecticides DDT, dieldrin, malathion, and deltamethrin when testing the susceptibility of *P. papatasi* sand flies to DDT, dieldrin, malathion, and deltamethrin. In terms of pyrethroid resistance, *P. papatasi* demonstrated resistance to permethrin and lambda-cyhalothrin, as well as tolerance to deltamethrin (Amalraj *et al.*, 1999), whereas *P. argentipes* showed resistance to permethrin and permethrin (Amalraj *et al.*, 1999). These were the first reports of pyrethroid pesticide resistance in sandflies. Fawaz *et al.* (2016) published the first evidence of sand-fly resistance outside of India in 2016. The species *P. papatasi* has been categorised as pyrethroid and permethrin resistant in Egypt. Karakus *et al.* (2017) studied a group of six sand fly species (*Ph. papatasi, Ph. tobbi, Ph. neglectus/syriacus, Ph. alexandri, Se. dentata, and Se. minuta*) in Turkey. Deltamethrin resistance and permethrin tolerance were demonstrated in laboratory bioassays. Using the WHO kits technique, Sardar *et al.* (2018) assessed the sensitivity of *Ph. argentipes* to the pesticides DDT, deltamethrin, and malathion.

Biochemical Assays:

Protein Profile:

Proteins Electrophoretic Separation (SDS-PAGE):

The obtained results from SDS- PAGE technique showed the presence of different protein bands in the tolerant population (10th of Ramadan), followed by Al- Quareen population may due to detoxifying proteins responsible for metabolizing the xenobiotic, such as synthetic insecticides (Fig.9).

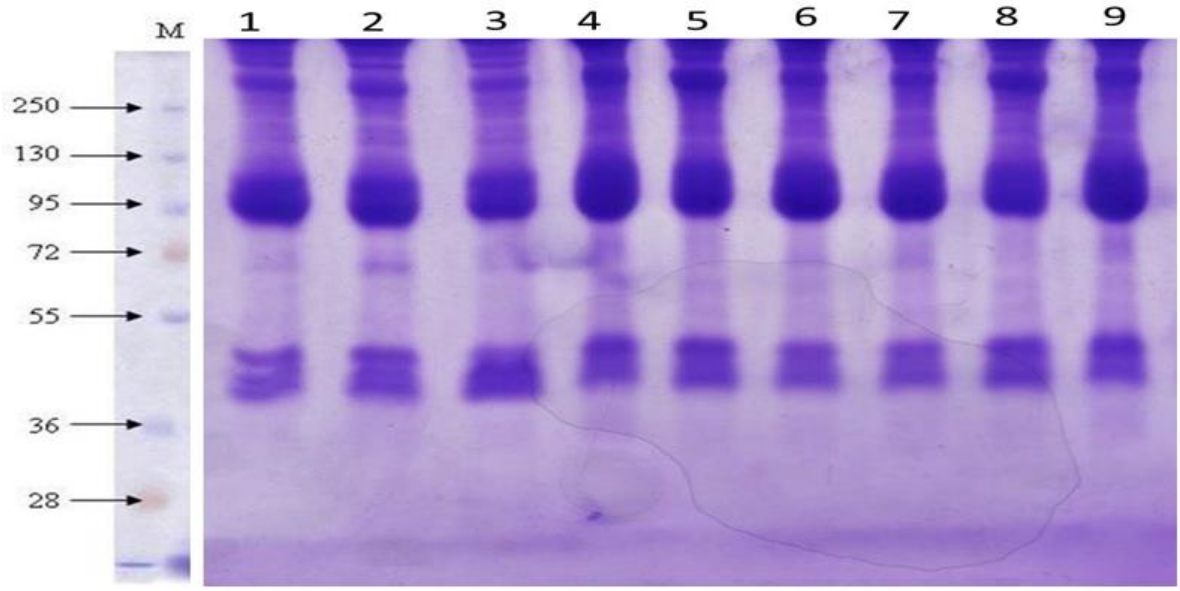

Fig. 9: SDS- PAGE of proteins in *P. papatasi* larvae, M: protein marker, L_1- L_3: Laboratory strain larvae, L_3- L_6: 10th of Ramadan locality larvae and L_7- L_9: Al- Quareen locality larvae, respectively.

Target and Metabolic Enzymes:

Acetylcholine Esterase Activity:

Data in Tables (1 & 2) showed the activity of acetylcholinesterase (AChE) in the two collected populations of *P. papatasi* adults and larvae compared with the laboratory strain. In adult insects the AChE activity was a highly significant inhibition in both the collected populations, 10th of Ramadan (2.48µgAchBr/min/mg protein and Al-

Quareen (4.14 µgAchBr/min/mg protein) compared with the laboratory strain (5.90 µgAchBr/min/mg protein), while AChE activity in larvae were in both the collected populations, 10th of Ramadan (47.33 µgAchBr/min/mg protein and Al- Quareen (93.00 µgAchBr/min/mg protein) compared with the laboratory strain (258.33 µgAchBr/min/mg protein).

Cytochrome P_{450} Monooxygenase (C450) Activity:

Cytochrome P_{450} monooxygenase (C_{450}) activity in adults of two different collected populations of *P. papatasi* at Sharkia governorate compared with the laboratory strain was determined. The results in (Tables 1 and 2) showed that C_{450} activity was increased intolerant regions, 10th of Ramadan (0.18 µmole substrate oxidized.min^{-1}.mg^{-1} protein and Al- Quareen 0.10 µmole substrate oxidized min^{-1} mg^{-1} protein) compared with the laboratory strain (0.07 µmole substrate oxidized.min^{-1}.mg^{-1} protein). The cross-pending picture in 4th larvae of two different collected populations of *P. papatasi* was C_{450} activity was increased intolerant regions, 10th of Ramadan (0.21 µmole substrate oxidized.min^{-1}.mg^{-1} protein and Al- Quareen (0.09 µmole substrate oxidized.min^{-1}.mg^{-1} protein) compared with the laboratory strain (0.08 µmole substrate oxidized.min^{-1}. mg^{-1} protein).

Table 1: Target and metabolic enzymes in the 4th larvae of *P. papatasi* collected from 10th of Ramadan and Al-Quareen localities at Sharkia governorate.

Strains	µgAchBr/min/mg protein	Cytochrome P450 activity (µmole substrate oxidized.min^{-1} mg^{-1} protein)	General esterases (GES)		GST* GSH (µmoL min^{-1} mg^{-1} protein)*
			µgα-naphthol/min/mg protein	µgβ-naphthol/min/mg protein	
Laboratory strain (LS)	258.33 ± 5.46[a]	0.08 ± 0.003[b]	5.07 ± 0.12[c]	2.90 ± 0.21[c]	0.14 ± 0.006[b]
10th of Ramadan	47.33 ± 4.33[c]	0.21 ± 0.02[a]	14.70 ± 0.43[a]	7.17 ± 0.09[a]	0.50 ± 0.09[a]
Al-Quareen	93.00 ± 2.52[b]	0.09 ± 0.003[b]	9.86 ± 0.13[b]	4.9jb 6 ± 0.08[b]	0.27 ± 0.03[ab]
LSD	22.4269	0.058	1.4281	0.73585	0.2838

* Means ± Standard error (N=3).

Table 2: Target and metabolic enzymes in adults of *P. papatasi* collected from 10th of Ramadan and Al-Quareen localities at Sharkia governorate.

Strains	µgAchBr/min/mg protein	Cytochrome P450 activity (n mole substrate oxidized.min^{-1}. mg^{-1} protein)	General esterases (GES)		GST* GSH (µmoL min^{-1} mg^{-1} protein)*
			µgα-naphthol/min/mg protein	µgβ-naphthol/min/mg protein	
Laboratory strain (LS)	5.90 ± 0.19[a]	0.07 ± 0.01[b]	15.51 ± 0.36[c]	1.98 ± 0.03[c]	0.11 ± 0.01[b]
10th of Ramadan	2.48 ± 0.22[c]	0.18 ± 0.006[a]	43.60 ± 0.78[a]	8.78 ± 0.26[a]	0.53 ± 0.07[a]
Al-Quareen	4.14 ± 0.12[b]	0.10 ± 0.006[b]	28.65 ± 1.26[b]	3.19 ± 0.23[b]	0.37 ± 0.09[ab]
LSD	0.9531	0.0391	4.6059	1.0454	0.3472

*Means ± Standard error (N=3).

General Esterases Colourmetrically:

General esterases (GES) activities of 10th of Ramadan and Al-Quareen populations in adults and 4th larvae were 43.60 ± 0.78,28.65±1.26,4.96±0.08 and 7.17±0.09 (µ Mol α and a- NA /mg protein/ min.) and 8.78 ±0.26,3.19±0.23, 7.17±0.09 and 4.96±0.08 (µ Mol α and β- NA /mg protein/ min.), respectively, compared with the laboratory strain (1.98±0.03,15.51±0.36,5.07±0.12 and 2.90±0.21 µ Mol αa and β- NA /mg protein/ min.) in Tables (1 & 2). There were highly significant differences (p> 0.01) between the EST activities of the 10th of Ramadan and Al-Quareen populations of *P. papatasi* compared with the laboratory strain.

Glutathione-S-Transferase Activity:

Glutathione-S-transferases (GSTs) activity of 10th of Ramadan population showed a highly significant more value of GST activity (p> 0.01) than Al- Quareen population Table (1 and 2). GST activities in 10th of Ramadan and Al-Quareen populations were 0.50±0.09, 0.27±0.03,0.53 ±0.07 and 0.37±0.09 µmoL.min^{-1}.mg^{-1} protein in the 4th larvae and adults of *P. papatasi* respectively.

The increase of inhibition AChE activity in the population collected from 10th of Ramadan region that appeared tolerance to chlorpyrifos insecticide, deltamethrin and malathion this may be an alternation or mutation in the target site (AChE) and consequently, the target enzyme does not inhibit by the tested insecticide, chlopyrifos. In *Drosophila melanogaster*, at least five-point mutations in the acetylcholinesterase pesticide binding site (Ace) have been found, resulting in reduced susceptibility to organophosphates and carbamates (Mutero *et al.*, 1994). The structural alteration or mutation (point mutation) of the target proteins causes target-site insensitivity, which reduces the effect of pesticide inhibition. Point mutations in the target protein reduce the nervous system's reaction to pesticides (or reduce the protein's binding affinity to insecticides) (Narahashi, 1988), increasing the insect's resistance to insecticides.

MFO was effectively reducing the efficacy of insecticides on insect pests (Wang *et al*., 2006). The cytochrome P450 enzymes are a group of heme proteins involved in the catabolism and anabolism of endogenous and exogenous substances such as steroids and pesticides. They are named after the absorption band at 450 nm of their carbon monoxide-bound form (Feyereisen, 2006). Clarifying the mechanism of resistance to spinosad, chlorpyrifos, and deltamethrin in P. papatasi could be a useful tool in pest management. (Wang *et al*., 2006 and Rodriguez *et al*. (2011).

Because of their propensity to hydrolyze insecticidal esters such as organophosphates and pyrethroids, non-specific esterases have been suggested as an insecticide resistance mechanism in a variety of insect pests (Reyes *et al*., 2007; Soleno *et al*., 2008 and Wu *et al*., 2011). This enzyme family, on the other hand, can sequester xenobiotics (Luna et al., 2007). In H. armigera, spinosad resistance is thought to be linked to esterase sequestration (Gunning and Balfe, 2002). Sequestration, a quick binding of insecticides that prevents them from reaching their target site, acetylcholinesterase, is the role of these esterases in pesticide resistance, followed by a slow turnover number (Karunaratne *et al*., 1993; Jayawardena *et al*., 1994 and Small *et al*., 1998). For decades, organophosphates (i.e., temphos, fenitrothion, malathion and chlorpyrifos), carbamates (i.e., propoxure and bendiocarb) had been heavily used for *P. papatasi* control before being replaced by synthetic pyrethroids in the 1980s (Salazar and Araya, 1997).

GSTs are of particular relevance in insects because of their function in pesticide resistance. They play a role in the o-dealkylation and o-dearylation of organophosphorus insecticides (Hayes and Wolf, 1988), as a secondary mechanism in the detoxification of organophosphate metabolites (Hemingway *et al.*, 1991), and in the dehydrochlorination of organochlorines (Clark and Shamaan, 1984). Insects resistant to a variety of insecticides, including organophosphates, have been found to have

GST activity (Wang *et al*., 1991; Wei *et al*., 2001 and Yang *et al*., 2009) and pyrethroids (Fournier *et al*., 1992; Grant and Hammock, 1992; Vulule *et al*., 1999; Vontas *et al*., 2001 and Rodrigouz *et al*., 2005).

Enzymes and Other Biochemical Constituents:

Total Lipids Content:

The results showed a highly significant reduction in the total lipids contents in both 10th of Ramadan and Al-Quareen populations compared with laboratory strain. Total lipids contents were 6.44±0.0.38, 4.18±0.38, 5.17±0.88 and 4.15±0.74 mg/ dL, in 4th larvae and adults, respectively, compared with the laboratory strain 12.28±0.32 and 9.82±0.26 mg/ dL (Tables 3 and 4).

Table 3: Impact of insecticides tolerance on some enzymes and other biochemical compounds in the 4th larvae of *P. papatasi* collected from 10th of Ramadan and Al-Quareen localities at Sharkia governorate.

Strains	Total lipids (mg/dL)	Trehalase (µg glucose/min/mg protein)	Alkaline phosphatase (Ux103 /mg protein)	Acid phosphatase (Ux103 /mg protein)	Amylase (µg glucosex10^3/min/mg protein)	Invertase (µg glucose/min/mg protein)
Laboratory strain (LS)	12.28 ±0.32^a	4.24 ± 0.15^a	16.24 ±0.21^c	3.82 ± 0.16^a	3.92 ± 0.19^a	6.18 ± 0.17^a
10th of Ramadan	6.44 ± 0.38^b	2.53 ± 0.04^b	34.11± 0.59^a	2.92 ± 0.02^b	1.31 ± 0.01^b	2.30 ± 0.03^c
Al-Quareen	4.18 ± 0.13^c	2.68 ± 0.09^b	26.17± 0.44^b	3.11 ± 0.10^b	1.54 ± 0.03^b	3.87 ± 0.04^b
LSD	1.56035	0.55215	2.30815	0.5594	0.59775	0.54365

*Means ± Standard error (N=3).

Table 4: Impact of insecticides tolerance on some enzymes and other biochemical compounds in adults of *P. papatasi* collected from 10th of Ramadan and Al-Quareen localities at Sharkia governorate.

Strains	Total lipids (mg/dL)	Trehalase (µg glucose/min/mg protein)	Alkaline phosphatase (Ux103 /mg protein)	Acid phosphatase (Ux103 /mg protein)	Amylase (µg glucosex10^3/min/mg protein)	Invertase (µg glucose/min/mg protein)
Laboratory strain (LS)	9.82 ± 0.26^a	14.75 ± 0.22^a	10.81 ± 0.65^b	5.75 ± 0.21^a	2100.00 ± 57.74^a	181.67 ± 4.41^a
10th of Ramadan	5.17 ± 0.88^b	8.90 ± 0.10^c	24.33± 0.21^a	5.50 ± 0.06^a	1750.00 ± 28.87^b	154.00 ± 3.06^b
Al-Quareen	4.15 ± 0.74^c	10.67 ± 0.44^b	12.73± 0.39^b	5.58 ± 0.04^a	2030.00 ± 25.17^b	164.33 ± 2.33^{ab}
LSD	0.85515	1.52495	4.298	0.6725	209.7238	17.70845

*Means ± Standard error (N=3).

Trehalase Activity:

Statistical analysis showed highly significant decreases in trehalase activities in both the 10th of Ramadan and Al-Quareen populations compared with laboratory strain (Tables 3 and 4). Trehalase activities were in both 10th of Ramadan and Al-Quareen populations compared with laboratory strain in both 4th larvae and adults were2.53, 4.24, 2.68,8.90,10.67 and 14.75 µg glucose/min/mg protein, respectively.

Phosphatases Activity:

Data in (Tables 3 and 4) showed high significantly increased alkaline phosphatase (ALP), while a significant reduction in acid phosphatase (ACP). ALP and ACP activities in both 4th larvae and adults of 10th of Ramadan and Al-Quareen populations compared with laboratory strain were 34.11, 26.17, 16.24;

2.92,24.33,12.73,5.50,5.58,10.81,5.75, 3.11 and 3.82 Ux10^3 /mg protein, respectively.

Amylase Invertase Activities:

The results shown in (Tables 3 and 4) indicated a highly significant reduction in amylase and invertase activities metabolizing sugars in both 4th larvae and adults of 10th of Ramadan and Al-Quareen populations compared with laboratory strain. Their activities were 1.31, 1.54, 3.92,1750,2030 glucosex10^3/min/mg protein; 2.30, 3.87, 6.18,154, 164.33 and 181.6 µg glucose/min/mg protein, respectively.

Total lipids, trehalase, alkaline phosphatase, acid phosphatase, amylase and invertase activities and their roles in energies for adults and 4th larvae of *P. papatasi*. The obtained results showed that there was a highly significant reduction in most of these energies enzymes.

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ARABIC SUMMARY

التغيرات البيوكميائيه في كلا من الطور البالغ والعمر اليرقي الرابع لحشره ذبابه الرمل *Phlebotomus papatasi* كمؤشرات لدرجه تحمل المبيدات الحشريه بمحافظة الشرقية

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تُعتبر حشرة ذبابة الرمل من الحشرات الطبية الناقلة لمرض الليشمانيا. في مصر، يعتبر استخدام المبيدات البيروثرويدية المُخلقة ومركبات الفوسفور العضوية من طرق المكافحة الكيماوية الأساسية لتلك الحشرة. لذلك الهدف من الدراسة تقصي تحمل تلك الحشرة في كلا من الطور اليرقي الرابع والحشرات الباغه التي تم تجميعها من منطقتين مختلفتين بمحافظة الشرقية (منطقتي العاشر من رمضان والقرين) لبعض المبيدات الحشرية شائعة الاستخدام في مكافحتها وتشخيص تحملها بيوكيميائياً. أوضحت النتائج أن يرقات حشرة **ذبابة الرمل** التي تم تجميعها من منطقة العاشر من رمضان هي الاكثر تحملاً لمبيدات الكلوربيرفوس، دلتامثرين، ملاثيون و اللمبدا- سيهالوثرين . فيما يخص التشخيص البيوكيماوي، التفريد الكهربي للبروتين، بعض الانزيمات المستهدفه والدفاعية (الجلوتاثيون-اس- ترانسفيريز، السيتوكروم بي- 450، الاستيريزس العامة)، بعض الانزيمات والمركبات الحيوية الأخرى (الاميليز، الانفيرتيز، الدهون الكلية، الالكالين فوسفاتيز، حامض الفوسفاتيز). لوحظ وجود اختلافات معنوية بين تعدادات الحشرة، حيث وُجد ظهور بندات بروتينات مختلفة في تعداد العاشر من رمضان المتحمل للمبيدات المستخدمة، تعداد منطقة القرين مقارنة بالسلالة الحساسة التي حدث فيها اختفاء لبعض بندات البروتين. ما يخص الانزيمات المستهدفة والدفاعية، لوحظ وجود زيادة عالية المعنوية في نشاط تلك الانزيمات (الجلوتاثيون-اس- ترانسفيريز، السيتوكروم بي- 450، الاستيريزس العامة) في تعداد العاشر من رمضان، متبوعاً بتعداد القرين مقارنة بالسلالة الحساسة. بينما، المكونات الحيوية الاخرى، محتوى الدهون الكلية، التريهاليز، الالكالين فوسفاتيز، الحمض فوسفاتيز، الأميليز والانفرتيز ودورها في اعطاء الطاقة ليرقات ذبابة الرمل. عموماً، أوضحت النتائج المتحصل عليها وجود انخفاضات عالية المعنوية في معظم تلك الانزيمات التي تعطي الطاقة.

لذلك نوصي بتجنب الاستخدام المُكثف للمبيدات وبطريقة عشوائية، مما ينتج عنه زيادة تطور المقاومة في تلك الآفات الطبية الخطيرة الناقلة للأمراض الوبائية وهلاك البشرية.