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Monitoring of Development of Resistance to Pyrethroids in *Musca domestica* L. Population, Using Toxicological and Biochemical Features

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ABSTRACT

Pyrethroid insecticides have widely been used to control the house fly, *Musca domestica*. Toxicological and biochemical studies were conducted for monitoring housefly tolerance to three pyrethroid insecticides λ-cyhalothrin, deltamethrin and α-cypermethrin. Based on the LC_{50} values of third larval instar laboratory (LS) and field strains (FS) exposed for 72 h using a poisonous media technique, flies showed resistance ratios ranging from 4.06 to 7.59-fold. The highest house fly population homogeneity was observed with deltamethrin in LS (2.13) and α-cypermethrin in FS (1.66). The biochemical evaluation was conducted in the third larval instar after exposure to estimated LC_{50} values of the tested insecticides on both strains. The protein content of FS was significantly higher in the control and λ-cyhalothrin and decreased following treatment with deltamethrin and α-cypermethrin. In contrast, mixed function oxidase enzymes (MFOs) were significantly higher in FS under all treatments, while β-esterase was significantly highest in λ-cyhalothrin FS. The α-esterase activity declined significantly with different pyrethroid treatments of FS. Glutathione-S-transferase enzyme (GST) activity was highest in all treatments of FS, except α-cypermethrin was lower in LS. The activity of Acetylcholinesterase enzyme (AChE) following pyrethroid treatments decreased significantly in FS compared with LS and control treatments. Carboxylesterase was significantly higher in all pyrethroid treatments of FS. Significant interactions were observed between strains and pyrethroid treatments. The development of pyrethroid resistance in FS, and the role of mixed-function oxidases and β-esterase in the degradation of different pyrethroids, in addition to, β-esterase and GST with λ-cyhalothrin detoxification, may explain the highest tolerance ratio (7.59).

KEYWORDS: *Musca domestica*, resistance, pyrethroids, λ-cyhalothrin, deltamethrin and α-cypermethrin.

1. INTRODUCTION

The housefly *Musca domestica*, L. (Diptera, Muscidae), is one of the most important hygiene pests worldwide (Brock, 2020). The order Diptera is containing nearly 160,000 species worldwide (Courtney and Cranston 2015). The housefly is the main insect in the tropical and subtropical region especially in the summer season (Fusari *et al.*, 2018). It is widely distributed at waste sites such as livestock, poultry farms, hospitals and garbage dumps (Rozendaal, 1997). Due to feeding on animal and human excrement and wastes, it can spread microbiomes mechanically through contamination. The housefly is the main diseases' vector of bacteria, helminthic, viral, rickettsia and endemic and epidemic diseases such as dysentery, salmonellosis, cholera, typhoid and trachoma affecting the public health of humans and livestock (Fotedar, 2001; Graczyk *et al.*, 2001; Mullen and Durden, 2002; Ugbogu *et al.*, 2006; Malik *et al.*, 2007; Pavela, 2008, Palacios *et al.*, 2009 and Abbas *et al.*, 2014).

The high reproductive potential of houseflies as a pest requires precautions to control to protect humans and animal's health (Zimmer *et al.*, 2013). The chemical control methods of this insect pest are still the main component of integrated control using various insecticide formulations e.g., aerosols, spraying, poisonous baits and smoking (Ruiu *et al.*, 2011 and Sarwar *et al.*, 2014).

Since the middle of the last century, synthetic pyrethroids were widely used for the control of this insect pest (Zhang *et al.*, 2008), due to their effectiveness, especially with public health pests and due to their low toxicity to mammals upon exposure (Narahashi *et al.*, 2007). Pyrethroids metabolic resistance in insects is associated with an increase in cytochrome P450 activity, increase in general esterase, carboxylesterase special and elevated glutathione S-transferases due to high gene expression, enabling the resistant insect to produce detoxification enzyme vigorously (Aïzoun *et al.*, 2013).

The intensive and repeated use of pyrethroid insecticides leads to the selection of tolerant and resistant individuals in the housefly population and the exclusion of sensitive individuals (Scott and Georghiou, 1984). To solve the pyrethroids resistance problem researchers focus to understand

the resistance mechanism and revealed to using synergists (detoxification enzyme inhibitors) e.g. piperonyl butoxide (target MFOs) to enhance their potency (Gunning *et al*., 1991). But Gunning *et al*., (1999) proved that esterase inhibitors specific for enhancing organophosphates efficiency also can be used for solving pyrethroids resistance.

The present study was conducted for monitoring the sensitivity of field and laboratory strains of a housefly to three pyrethroid insecticides i.e., λ-cyhalothrin, deltamethrin and α-cypermethrin. The activity of some detoxifying enzymes were also determined to explain the pyrethroids tolerance/resistance in the housefly.

2. MATERIALS AND METHODS

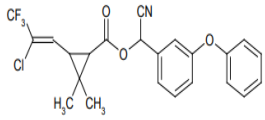
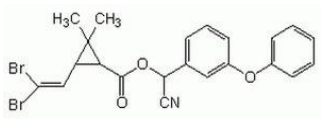
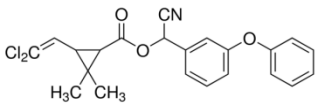
2.1. Housefly rearing:

The housefly *M. domestica*, L., cultures involved in this study were collected as adults from their natural breeding sites at Zagazig city, Sharkia Governorate, Egypt using baited traps. One part of the collected cultures was reared in the laboratory for 25 generations in an environment free of any insecticides at 28 ± 2 ˚C, 65 ± 5 % RH and 14 h illumination. This culture was considered a laboratory strain (LS). The second part of the collected adults was reared in the laboratory for one generation and used for testing as a field strain (FS). A diet (paste form consisted of wheat-bran, milk powder, brewer's yeast and tap water in a ratio of 15: 5: 0.3: 15 parts, respectively) was used as a standard rearing medium for larvae according to Busvine (1962); Selem (2011) and Khan *et al.* (2013). After pupation, pupae were placed in Petri dishes and kept in wooden cages (100 × 40 × 40 cm). Adult flies were provided with sugar and powdered milk (3:1) in Petri dishes as a diet with water-soaked cotton swabs in a 25 ml plastic jar filled with water inside the cages and it provided with a media for egg-laying (50 g of standard larval rearing medium in a separate plastic cup, 4.5 cm base diameter, 7.0 cm top diameter, and 8.5cm height). To avoid the entry of ants, the legs of cages are coated with grease.

2.2. Insecticides:

Three synthetic pyrethroid insecticides, namely λ-cyhalothrin, deltamethrin and α-cypermethrin were tested against third larval instar of *M. domestica* in the laboratory. The list of pyrethroid insecticides with the common name, chemical structure, trade name and source of availability is given in Table1.

Table 1. Common name, structural formula, trade name and source of the tested pyrethroid insecticides.

	Pyrethroids		
Common name	λ-cyhalothrin	Deltamethrin	α-Cypermethrin
structural formula			
Trade name & formulation	Cycon 10% EC	Decis 2.5 % EC	Super Alpha 10 % EC
Source	Plant Protection Research Institute, Giza, Egypt		

2.3. Bioassay Experiments:

Bioassay tests were performed according to Kristensen and Jespersen (2003). The larvicidal activity of the three selected pyrethroid insecticides was tested by mixing with a standard larval rearing medium. Based on the preliminary tests, serial dilutions of five different concentrations (mg/kg) were prepared in water as a dilutant for each insecticide. Each concentration was replicated three times and prepared in a container (100 ml volume) contained 20 g standard larval rearing medium and 2.5 ml of the insecticide concentration then mixed well with a glass spatula to ensure uniform distribution of the insecticide in the medium. The control treatment was treated with distilled water only and replicated three times. 50 larvae of the 3rd instar were put in the medium container (small plastic cup 7cm in diameter containing 50 g of the medium) and preserved at 28±2 ˚C, 65±5 % RH and 14 h illumination. The containers were covered with lids of muslin which were prepared to become permeable to air. The larval mortality was examined daily until three successive days post-exposure. Mortality percentages were calculated and corrected according to the Abbott formula (1925). Mortality dosage regression lines, LC_{50} value and slope were calculated according to (Finney, 1972) using Analyst soft Biostat Pro.V.5.8.4.3 Software. Resistance ratio values in fold were calculated for each insecticide, as follows:

Resistance ratio =

LC_{50} of the field strain ÷ LC_{50} of the laboratory strain

2.4. Biochemical Markers:

Samples of about 20 pyrethroid survival larvae each of both LS and FS were taken for determination of total protein and enzyme activities of the 3rd instar larvae which were collected from the media after 72 h post-treatment with median lethal concentration (LC_{50}) of the three pyrethroids. Both the control and the treated larvae were weighed and homogenized in sodium phosphate buffer (0.1 M, PH 7.4) using Universal Laboratory Aid homogenizer on the ice at 1000 rpm for 60 Sec. Larval homogenates were centrifuged at 4000 rpm for 10 minutes at 4 ºC. Filter paper of Whatmann1 was used for filtering supernatants, and then kept at -20 ºC until Spectrophotometric determination of total protein and some enzyme activities within one week Biochemical markers were prepared as described by Amin (1998) to determine the following:

1. Total protein was determined by the method of Bradford (I976).
2. Mixed function oxidase enzyme (MFOs) assay performed using P-nitroanisole O-demethylation to determine MFOs activity according to the method of Hansen and Hodgson (1971)
3. α- & ß - esterase enzyme activities were determined according to Asperen (I962)
4. Glutathione-S-transferase enzyme (GST) activity was determined by Habig *et al.* (I974) method.
5. Acetylcholinesterase enzyme (AChE) activity was measured using acetylcholine bromide as substrate according to the method described by Simpson *et. al*. (1964).
6. Carboxylesterase enzyme activity was measured according to the method described by Simpson *et. al.* (1964).

2.5. Statistical Analysis:

The different significance of biochemical parameters between different pyrethroid pesticides and housefly strains (laboratory and field) were analyzed by a two-way ANOVA using Costat Statistical Software (Cohort software, Berkeley). The means were compared by Duncan's multiple range test.

3. RESULTS

3.1. Bioassay Experiments:

Data concerning the responses of the two tested strains, LS and FS of housefly to the three tested pyrethroid insecticides i.e., λ-cyhalothrin, deltamethrin and α-cypermethrin are presented in Table (2) and Fig. (1). Based on the dose-response relationship of the tested insecticides against LS and FS, λ-cyhalothrin was the most toxic pyrethroid against the 3rd larval instar of the housefly. It recorded LC_{50} of 21.22 and 160.96 mg/kg for LS and FS, respectively, followed by deltamethrin (35.91 and 207.53 mg/kg for LS and FS, respectively). α-cypermethrin was the least effective insecticide, whereas LC_{50} values were 64.90 and 263.25 mg/kg for LS and FS, respectively. It was obvious that all tested pyrethroid insecticides were more toxic to the LS than to the FS, in other words, the LS become more susceptible to the tested pyrethroids as a result of colonies rearing in the laboratory comparing with its tolerance at the beginning continues rearing of FS in the laboratory to loss of some tolerant individuals each generation and the susceptibility was such increasing. The population of LS houseflies had the least homogeneity toward α-cypermethrin (1.46) followed by λ-cyhalothrin which recorded 1.75, while the most homogeneity was recorded for deltamethrin (2.13). On the contrary, the housefly field population was heterogeneous for its susceptibility to the tested pyrethroid than the LS with exception of α-cypermethrin: λ-cyhalothrin (1.06), deltamethrin (1.63) and α-cypermethrin (1.66). λ-cyhalothrin showed the highest resistance ratio (7.59 fold), then deltamethrin was 5.78 fold, and α-cypermethrin had the least resistance ratio of 4.06 fold.

3.2. Biochemical markers:

Results in Table (3) and Fig. (2) show the total proteins and activities of certain enzymes in both LS and FS 3rd larval instar homogenates of *M. domestica* treated with LC_{50} of the three tested pyrethroids insecticides. The total protein showed a highly significant reduction after pyrethroid treatments comparing to the control of both LS and FS. α-cypermethrin treatment showed the highest reduction (20.60±1.03 and 18.37±0.63 mg/g b.wt) with a reduction percent -34.19 and -46.29 % followed by λ-cyhalothrin (25.07±0.52 and 29.20±0.57 mg/g b.wt) with a reduction percent (-19.90 and -14.62%) and then deltamethrin (28.23±0.72 and 27.47±0.43 mg/g b.wt) with a reduction percent (-9.81 and -19.68 %) with highly significant in LS and FS, respectively. LS was the most influenced by pyrethroid treatment recorded a reduction % (-21.30 %) in total protein comparing with FS (-26.86 %), with a highly significant interaction between housefly strain and treatments (Table 3 and Fig. 2, A). As expected, this significant effect on the total protein content of pyrethroid-treated larvae on both tested strains will influence protein-related parameters (enzymatic activity), without affecting protein-independent parameters such as carboxylesterase.

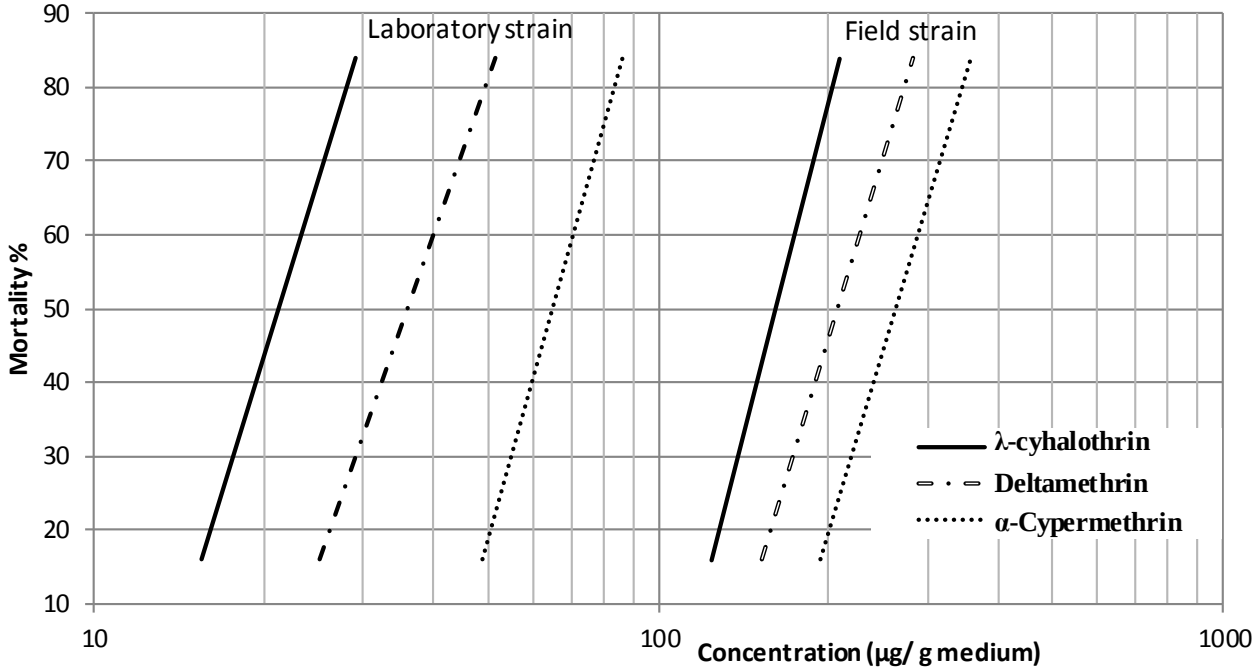


Fig. 1. Toxicity lines of the tested pyrethroids on 3rd instar larvae of laboratory and field strains of the housefly, *Musca domestica*.

Table 2. Toxicity of the tested pyrethroids to 3rd instar larvae of laboratory and field strains of the housefly, *Musca domestica*.

Strain	Parameters	Pyrethroid insecticides		
		λ-cyhalothrin	Deltamethrin	α-cypermethrin
Laboratory	LC_{50}	21.22	35.91	64.90
	Slope	1.75	2.13	1.46
Field	LC_{50}	160.96	207.53	263.25
	Slope	1.06	1.63	1.66
Resistance ratio		7.59	5.78	4.06

Mixed function oxidase activity (MFOs) showed a highly significant increase with α-cypermethrin treatment recorded (47.17±1.09 m mol sub./min./g b.wt with a change of -20.33% and 58.00±1.53 m mol sub./min./g b.wt with -23.22%) for LS and FS, respectively. Meanwhile, decrease 38.37±1.17 and 46.83±0.80 m mol sub./min./g b.wt in MFOs activity measured following treatment with deltamethrin and scored change percent of 2.12 and 0.51% for LS and FS, respectively. λ-cyhalothrin treatment showed different effects on MFOs activity, an increase by 52.20±1.01 m mol sub./min./g b.wt with -10.90 % reduction for FS, while a decrease for LS was reported (37.97±1.02 m mol sub./min./g b.wt) with a change percent equal 3.14 % compared with the control (Table 3 and Fig. 2,B). This evidence enhances the assumption of the presence of MFOs in both strains with a high activity due to MFOs have the main role in pyrethroid detoxification especially α-cypermethrin treatment.

Esterase activity included alpha and beta esterase showed the same trend. alpha esterase activity showed a decrease with highly significant after application with the three pyrethroids compared with control treatments in both strains. As for alpha esterase, α-cypermethrin elicited the most potent effect of 29.90 and 49.37 % change percent from control in LS and FS, respectively. While λ-cyhalothrin showed the least effect in this respect in both LS and FS (1204.00±9.45 and 1258.00±22.30 µg α naphthol/ min./g b.wt) with reducing percent 8.93 and 6.91 %, respectively (Table 3 and Fig. 2, C). Also, beta-esterase activity was significantly decreased after treatment of the two strains with the three tested insecticides except for λ-cyhalothrin treatment in FS, a highly significant increase recorded (437.00±9.07 µg β naphthol/ min./g b.wt) compared with 406.33±8.51 µg β naphthol/ min./g. b.wt in control. α-cypermethrin and deltamethrin showed high significant inhibition in β esterase activity in LS and FS (Table 3 and Fig. 2, D).

Table 3. Changes in total protein and some enzyme activity of the 3rd larval instar homogenates of the housefly, *Musca domestica* strains treated with LC_{50} of the tested pyrethroids insecticides.

Parameter	Strain	Control	λ-Cyhalothrin	Deltamethrin	α-Cypermethrin	Mean
Total protein concentration (mg/g b.wt)	Lab.	31.30±0.60	25.07±0.52 (-19.90)	28.23±0.72 (-9.81)	20.60±1.03 (-34.19)	**26.30[b] (-21.30)**
	Field	34.20±1.15	29.20±0.57 (-14.62)	27.47±0.43 (-19.68)	18.37±0.63 (-46.29)	**27.31[a] (-26.86)**
	Mean	**32.75[a]**	**27.14[b] (-17.26)**	**27.85[b] (-14.75)**	**19.49[c] (-40.24)**	**
MFO activity (m mole sub./ min/g b.wt)	Lab.	39.20±1.46	37.97±1.02 (3.14)	38.37±1.17 (2.12)	47.17±1.09 (-20.33)	**40.68[b] (-5.02)**
	Field	47.07±1.51	52.20±1.01 (−10.90)	46.83±0.80 (0.51)	58.00±1.53 (−23.22)	**51.03 [a] (-11.20)**
	Mean	**43.14[b]**	**45.09[b] (-3.88)**	**42.60[b] (1.32)**	**52.59[a] (-21.78)**	**
α-Esterase activity (µg α-naphthol/ min/g b.wt)	Lab.	1322.00±11.72	1204.00±9.45 (8.93)	1140.67±27.09 (13.72)	926.67±18.41 (29.90)	**1148.34[a] (17.52)**
	Field	1351.33±17.33	1258.00±22.30 (6.91)	984.67±8.67 (27.13)	679.33±10.35 (49.37)	**1068.33[b] (27.80)**
	Mean	**1336.67[a]**	**1231.00[b] (7.92)**	**1062.67[c] (20.43)**	**803.00[d] (39.64)**	**
ß -Esterase activity (µg α-naphthol/ min/g b.wt)	Lab.	393.33±8.11	390.67±5.21 (0.68)	333.67±10.20 (15.17)	309.00±5.51 (21.44)	**356.67[a] (12.43)**
	Field	406.33±8.51	437.00±9.07 (−7.55)	289.33±6.36 (28.79)	262.67±3.71 (35.36)	**348.83[a] (18.87)**
	Mean	**399.83[a]**	**413.84[a] (-3.44)**	**311.50[b] (21.98)**	**285.84[c] (28.40)**	**
GST activity (m mole sub conjugated/min/g b.wt)	Lab.	28.67±1.45	28.67±1.76 (0.00)	20.00±4.04 (30.24)	27.33±1.20 (4.67)	**26.17[b] (11.64)**
	Field	33.33±2.03	53.67±1.86 (−61.03)	37.00±1.53 (−11.01)	20.67±1.76 (37.98)	**36.16[a] (-11.35)**
	Mean	**31.00[b]**	**41.17[a] (-30.52)**	**28.50[b] (9.62)**	**24.00[c] (21.33)**	**
AChE activity (µg AchBr/min/g b.wt)	Lab.	283.33±8.82	261.33±5.70 (7.76)	273.33±4.40 (3.53)	158.00±4.04 (44.23)	**244.00[a] (18.51)**
	Field	335.00±12.58	267.33±5.36 (20.20)	192.33±3.93 (42.59)	54.00±2.08 (83.88)	**212.17[b] (48.89)**
	Mean	**309.17[a]**	**264.33[b] (13.98)**	**232.83[c] (23.06)**	**106.00[d] (64.06)**	**
Carboxylesterase activity (µg product /min/g b.wt)	Lab.	35.53±1.53	39.86±1.47 (-12.19)	38.23±1.29 (-7.60)	46.43±2.57 (-30.68)	**40.01[b] (-16.82)**
	Field	46.00±2.08	51.07±1.61 (−11.02)	48.03±0.99 (−4.41)	52.97±3.06 (−15.15)	**49.52[a] (-10.19)**
	Mean	**40.77[c]**	**45.47[b] (-11.61)**	**43.13[bc] (-6.01)**	**49.70[a] (-22.92)**	**

Means in the same column or row followed by different letters are significantly different (at $p \leq 0.01$) when analyzed using ANOVA and separated by Duncan test; numbers between parentheses refer to "Change percentage (%)"; ** refer to the interaction between house fly strain (LS and FS) with pyrethroids.

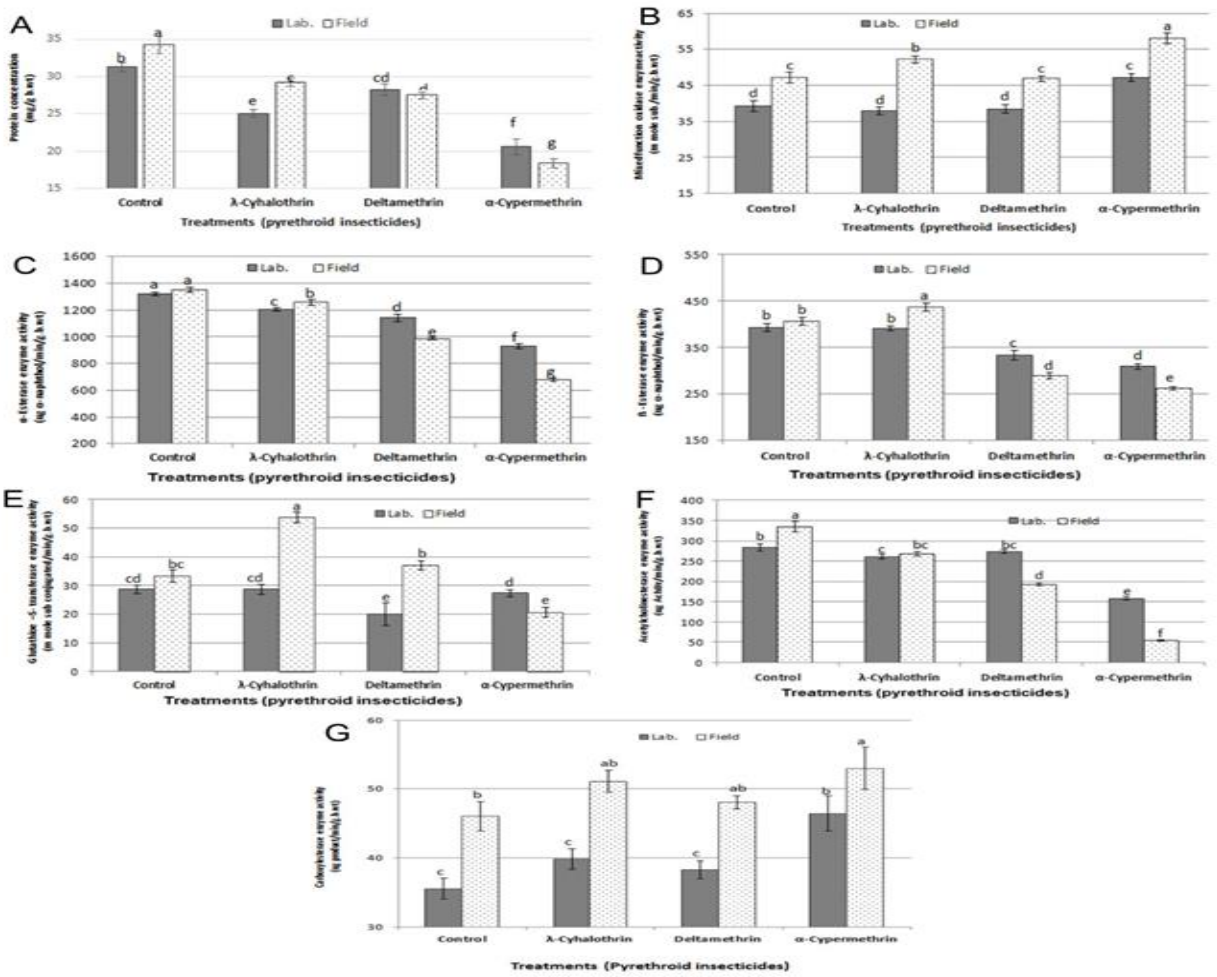


Fig. 2. Total protein and some enzyme activities of laboratory and field strains of 3rd instar larvae *Musca domestica* exposed to LC_{50} of the tested pyrethroid insecticides.

GST enzyme activity showed a unique trend represented in a highly significant increase in λ-cyhalothrin treated FS (53.67±1.86 mmol. Sub. conjugated/min/g b.wt) while, the lowest decrease was measured in α- α-cypermethrin treated FS (20.67±1.76 mmol. Sub. conjugated/min/g b.wt) and scored 37.98% change percent. GST enzyme activity was higher in FS than LS at all treatments and control but on the contrary in α-cypermethrin treatment Table (3) and Fig. (2, E).

AChE activity showed increased in the control treatment but reduced highly significant in all pyrethroid treatments in both LS and FS. Data in table (3) Fig. (2, F) showed that treatment with α-cypermethrin proved the most inhibitory effect by 54.00±2.08 µg AChBr/min/g b.wt compared with 335.00±12.58 µg AChBr/min/g b.wt in the control experiment, deltamethrin followed α-cypermethrin by 192.33±3.93 µg AChBr/min/g b.wt and finally by 267.33±5.36 µg AChBr/min/g b.wt after λ-cyhalothrin treatment. Highly significant reduction in AChE activity after treatment of LS with α-cypermethrin 158.00±4.04 µg AChBr/min/g b.wt with 44.23 % change percent compared to LS control 283.33±8.82 µg AChBr/min/g b.wt Table (3) and Fig.(2,F).

Data illustrated in Table (3) and Fig. (2, G) show that carboxylesterase activity was highly significant decreased in all LS treatments compared with all FS treatments. Carboxylesterase activity was the highest increase in α-cypermethrin treatment (46.43±2.57 µg product/min/g. b.wt) with -30.68 % reduction percent at LS, followed by λ-cyhalothrin (39.86±1.47 µg product/min/g b.wt) then deltamethrin (38.23±1.29 µg product/min/g b.wt.) with -12.19 and -7.60 % change percent in LS.

4. DISCUSSION

Among the most common hygienic insects, the housefly (*M. domestica* L.) is an annoying insect and a vector for many diseases in humans and livestock (World Health Organization, 2000). Pyrethroids are mainly recommended for use indoors and outdoors especially with public health-related insects because of frequent application, insect susceptibility changed, which is summoned conducting the current study to assess insect susceptibility houseflies for synthetic pyrethroids. This offered study focusing on the response of 3rd

larval instar of houseflies toward pyrethroids by toxicity and biochemical markers. Synthetic pyrethroids were developed to enhance the natural insecticide pyrethrum's specificity, persistence, and activity. The pyrethroids are a toxin, secondarily causing adverse effects, because of neuronal hyperexcitability (Rasheed *et al*., 2016). The nerve excitation occurs because of changes in nerve membrane permeabilities to sodium and potassium ions (Narahashi, 1971). For most adult insects, pyrethroid treatment is always with insect knockdown. The knockdown resulted in houseflies from exposure to pyrethroids occurred more rapidly at higher temperatures. pyrethroids caused higher mortality at a lower temperature, at 18 than at 32°C, even though the toxicity differed by compound and, to a lesser extent by strain (Scott and Georghiou, 1984). The presence of the α-cyano group, as seen in type II pyrethroids, reduces the rate of ester bond hydrolysis causing a substantially longer sodium action potential than type I pyrethroids (Ensley, 2018). Because of the high selective toxicity, the safety margin of pyrethroids, household pyrethroids and their preparations use in living environments around humans and pets (Katsuda, 2011).

The bioassay results showed high variability, which may be due to the environmental conditions, the tested stage of house fly (larva stage), pyrethroid introducing technique, the tested concentration, and exposure period…etc. The toxicity of pyrethroid compounds against houseflies was variable (Hong *et al*., 2000 and Ahmed *et al*., 2004).

Pyrethroids are insecticides that have neurotoxic actions affecting GABA receptor function as a primary target. The treatment of third larval instars of house flies with micromolar concentrations of pyrethroids blocks synaptic transmission from excitatory axons to intersegmental muscles in one hour. The synaptic vesicle depletion reflects a presynaptic action of deltamethrin on house fly larvae motor nerve terminals, as previously indicated by intracellular recordings (Schouest *et al*., 1986). However, many secondary targets respond to pyrethroid treatment, especially at sublethal concentrations.

In the current investigation, the total protein content decreased significantly in both LS and FS after treatment with LC_{50} of the three tested pyrethroids. Similar kinds of literature are in harmony with these results. The total protein content mainly decreased in the resistant strain of house fly when exposed to λ-cyhalothrin. In *Spodoptera littoralis* exposure to pyrethroids was accompanied by a significant change in biological (larval duration, pupal duration, adult longevity, fecundity and fertility) and biochemical (total carbohydrates, and total lipid contents) aspects of treated larvae. It also caused significant changes in the enzyme activity of the treated larvae (Rasheed *et al*., 2016).

Females of *Pimpla turionellae*, an endoparasitoid fed on *Galleria mellonella* treated to various sublethal doses of cypermethrin presented to the host larvae's meal. Females showed the most striking decrease in glycogen content and larvae were more susceptible to cypermethrin than pupae and adults in terms of decreases in protein and lipid contents (Sak *et al*., 2006). Also, Total protein levels in *Culex fatigans* were decreased by λ-cyhalothrin treatment (Yousuf *et al*., 2014). Total protein content in *Culex pipiens* declined, while total lipids increased gradually over generations (Gharib *et al*., 2020).

On the other hand, many enzymes which responsed to pyrethroid treatment can be classified as detoxifying enzymes and target enzymes. Three major mechanisms of metabolic transformation of insecticides underly biotransformation-based resistance: (i) oxidation; (ii) ester hydrolysis; and (iii) glutathione conjugation. Pyrethrins and pyrethroids are degraded by hydrolysis. The detoxification of insecticides occurs predominantly by molecular hydrolysis at several locations, which splits ester, carboxyl-ester, amide, and other chemical bonds (Stankovic and Kostic (2017).

The main detoxification enzyme of pyrethroid is cytochrome P450, which known to metabolize neurotoxic pyrethroid insecticides. The nervous system capability of metabolizing pyrethroids in the thoracic ganglia in pyrethroid-resistant and susceptible strains of house fly is due to the presence of cytochrome P450.

Tissue-specific analysis indicated that these constitutively expressed, and permethrin-induced P450 genes were overexpressed in the abdominal tissue, in which the primary detoxification organs of houseflies are located (Fang, 2007 and Wheelock and Scott, 1992).

The most important hydrolytic enzymes are carboxylesterases. Structural mutations in mutant carboxylesterases have now been widely described, and show to produce metabolic resistance to pyrethroid insecticides (Stankovic and Kostic, 2017 and Xuechun, 2018). Resistance to organophosphorus pesticides is caused by carboxylesterase (Devonshire and Moores 1982). Pyrethroid resistance in insects was also conferred by house fly carboxylesterases. The resistant strain carboxylesterases degrade cypermethrin / deltamethrin substrates, causing a raise resistance ratio 9.05- and 13.53-fold, respectively. The carboxylesterase alterations included quantitative and qualitative changes, which may lead to pyrethroid resistance in the resistant strain (Zhang *et*

al., 2010). Also, various microorganisms use carboxylesterase in the degradation of pyrethroids (Gajendiran and Abraham 2018). Permethrin degraded by carboxylesterases to 3-phenoxybenzyl alcohol and 3-phenoxybenzaldehyde in resistant houseflies (XueChun and Nannan (2020), which is the reason for metabolizing λ-cyhalothrin (Hamed *et al*., 2019) and beta-cypermethrin (Zhang *et al.*, 2007) in the resistant strain. The high levels of degradation of deltamethrin in the resistant strain compared to the sensitive one were inhibited by DEF (S,S,S-tributyl phosphorotrithioate) and paraoxon, indicating that esterases are likely to be involved in the metabolism of this insecticide (Delorme *et al.*, 1988 and Riskallah, 1983).

Esterases also contribute to the resistance against synthetic pyrethroids in *S. littoralis* larvae (Riskallah, 1983). However, purified esterases hydrolyzed permethrin at a slow rate (Vontas *et al*., 2001).

Although GSTs are susceptible to pyrethroid inhibition, they are not pyrethroid targets. In insects, increased GSTs with a high peroxidase activity cause oxidative stress and lipid peroxidation. Pyrethroid exposure resulted in the formation of lipid peroxides, protein oxidation, and a reduction in reduced glutathione levels. Elevated GSTs in the resistant strains attenuated the pyrethroid-induced lipid peroxidation and reduced mortality, whereas their *in vivo* inhibition eliminated their protective role (Vontas *et al*., 2001). There was independent regulation and differential response of the GST isoenzymes to the administration of the specific insecticides (Papadopoulos *et al.*, 1999). Furthermore, the limited increase in GST activity compared to resistance levels, idicates that GSTs may not play a significant role in λ-cyhalothrin resistance in *H. armigera* (Ugurlu *et al*., 2007).

All these earlier evidence does not idicate a limited role of these metabolic enzymes, as they do not assert their existence at the same time in resistant strains (Zhang *et al.*, 2007). Nonetheless, as the generation numbers increased, the activities of detoxifying enzymes increased progressively, indicating that increased resistance is likely to be linked to increased activity of target and metabolic enzyme systems (Gharib *et al*., 2020). These enzymes with significant elevation in the activity of α- and β-esterases, glucose-6-phosphate dehydrogenase, cytochrome P450, and GST play an important role in deltamethrin resistance in the resistant strain of *Aedes aegypti* (Jagadeshwaran and Vijayan 2009 and Pimsamarna *et al*., 2009).

Activity levels of another pyrethroid secondary target, Acetylcholinesterase (AChE) were elevated in *Culex fatigans* treated with λ-cyhalothrin (Yousuf *et al*., 2014). The maximum AChE activity in treated larvae of *H. armigera* was recorded for λ-cyhalothrin and bifenthrin (Bilal *et al*., 2018).

5. CONCLUSION

The effect of three tested pyrethroids treatment on 3rd larval instar of *M. domestica* showed toxic effects. λ-cyhalothrin exhibited high toxicity to both strains comparing with deltamethrin and α-cypermethrin. All the tested pyrethroids caused changes in the tested biochemical markers in this study. The continued use of pyrethroids will eventually lead to the development of resistant generations of houseflies, so constant monitoring of insect resistance status is necessary to ensure the efficiency of pyrethroids and discovery resistance early, whether by reducing their use or by directing pyrethroid manufacturers to use enzyme inhibitors, either MFOs or esterases, especially carboxylesterase.

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الملخص العربي

رصد تطور صفة المقاومة لمبيدات البيروثريد على تعداد الذبابة المنزلية، باستعمال خصائص سمية وبيوكيميائية

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تستخدم مبيدات البيرثرويد على نطاق واسع لمكافحة حشرة الذباب المنزلي. ولرصد تحمل الذبابة المنزلية للبيروثرويد أجريت دراسات سمية وبيوكيميائية لثلاثة مبيدات حشرية بيرثرويدية شملت اللمبادا–سيهالوثرين والدلتاميثرين والفا–سيبرمثرين على سلالة معملية (حساسة) وأخري حقلية. بناءً على قيم LC_{50} للمبيدات البيروثريدية المختبرة على يرقات العمر الثالث للسلالتين المعرضة للمبيدات لمدة ٧٢ ساعة بالبيئة المسممة. أظهرت النتائج معدلات مقاومة تراوحت بين ٤.٠٦ و٧.٥٩ ضعفًا، بالإضافة لتسجيل أعلى تجانس لاستجابة السلالة المعملية مع الدلتاميثرين (٢.١٣) والسلالة الحقلية مع السايبرمثرين (١.٦٦). وعند رصد التغيرات البيوكيميائية ليرقات كلا السلالتين بعد التعرض للمبيدات المختبرة بالتركيزات النصفية القاتلة. لوحظ ارتفاع المحتوى البروتيني في السلالة الحقلية بشكل ملحوظ في اليرقات غير المعاملة (الكنترول) واليرقات المعاملة بالمبادا–سيهالوثرين وانخفض مع الدلتامثرين وألفا–سيبرمثرين. وفي المقابل ارتفع نشاط انزيم اكسيداز متعدد الوظائف بشكل ملحوظ في السلالة الحقلية في جميع المعاملات بينما كانت زيادة نشاط انزيم البيتا استيريز ملحوظ في السلالة الحقلية المعاملة بلمبادا سيهالوثرين. بينما انخفض نشاط إنزيم الفا استيريز بشكل ملحوظ مع معاملة الألفا–سيبرمثرين للسلالة الحقلية. أما نشاط إنزيم جلوتاثيون–أس–ترانسفيراز فسجل ارتفاعا في جميع معاملات السلالة الحقلية باستثناء اليرقات المعملية المعاملة بالفا–سيبرمثرين. في حين انخفض إنزيم الأستيل كولين استيريز بشكل ملحوظ في معاملات البيرثرويدات للسلالة الحقلية مقارنة بالسلالة المعملية والكنترول. من ناحية أخرى ارتفع نشاط كربوكسيل إستيراز بشكل ملحوظ في جميع معاملات البيرثرويدات على السلالة الحقلية. كما أظهر التحليل الإحصائي وجود تفاعل معنوي عالي بين السلالات ومعاملات البيرثرويدات. وقد خلصت الدراسة إلى أن تطور مقاومة السلالة الحقلية للبيرثرويدات، يكون مصحوبا بزيادة نشاط انزيمات الاكسيداز متعدد الوظائف والبيتا استيريز لدورهم في تحلل البيرثرويدات المختلفة، بالإضافة إلى دور انزيمات البيتا استيريز والجلوتاثيون–أس–ترانسفيراز في إزالة سمية مبيد اللمبادا سيهالوثرين ولعل هذا ما يفسر ذلك سبب حدوث أعلى معدل مقاومة لمبيد اللمبادا–سيهالوثرين (٧.٥٩ ضعف).

الكلمات المفتاحية: الذبابة المنزلية، مقاومة، بيروثرويدات، لمبادا– سيهالوثرين، دلتاميثرين والفا– سيبرمثرين.