Benha Journal of Applied Sciences (BJAS) **print: ISSN 2356–9751**
Vol. (8) Issue (5) (2023), (115-125) **online: ISSN 2356–976x**
http://bjas.journals.ekb.eg

Susceptibility of *Culex pipiens* L. in Sharkia Governorate, Egypt to Chitin Synthesis Inhibitors and their Biochemical Characterizations

Mona F.A. El-Sitiny[1], Mohammed E. Gad[2], Hanem F. Khater[3]* and Mohammed G. Mahmoud[1]
[1]Plant Protection Department, Agricultural Faculty, Zagazig University, Egypt
[2] Zoology Department, Faculty of Science (Boys) Al-Azhar University, Naser City, Cairo11884, Egypt
[3]Parasitology Department, Faculty of Veterinary Medicine, Banha University, Egypt
***E-mail**:- hanem.salem@fvtm.bu.edu.eg

Abstract

Culex pipiens is an important vector in Egypt. The tolerance levels of insect growth regulators (IGRs) on 4th instar larvae of *Cx. pipiens* collected from the Faqous region, Sharkia Governorate, Egypt, and their biochemical characterization were determined. The data indicated that LC_{50} values post-treatment (PT) of the field strain with diflubenzuron, novaluron, and lufenuron were 0.08, 0.60, and 0.16 μg/ml, respectively. Whereas the corresponding values for the laboratory strain were 0.07, 0.16, and 0.02 μg/ml, respectively. The relative tolerances of the field strain reached 1.14 (low), 3.75 (medium), and 8.00 (high) folds, respectively, when compared to the laboratory strain. The total protein levels in larvae treated with diflubenzuron, novaluron, and lufenuron and those of laboratory strain were 17.00, 20.00, 27.33, and 13.33 mg/g b.wt, respectively, whereas such values for the target enzyme of acetylcholine esterase, AChE, were 210.00, 283.33, 310.00, and 225.00 AChBr/min./g.b. wt, respectively. Their effect on the detoxifying enzymes reached 242.00, 386.67, 483.33, and 235.00 μ Meb min^{-1} mg^{-1} protein, respectively, for carboxylesterases; 28.00, 35.67, 62.33, and 22.00 μmol min^{-1} mg^{-1} protein in case of glutathione- S- transferase; 560.00, 723.33, 921.67, and 503.33 ηmol min^{-1} mg^{-1} protein, respectively, for α – esterase; and 128.33, 151.67, 175.00, and 131.67, ηmol min^{-1} mg^{-1} protein, respectively, for β – esterase. The tolerance levels for the applied IGRs revealed that the field strain from the Faqous region was highly tolerant to lufenuron, followed by novaluron, and diflubenzeron; therefore, diflubenzeron is the IGR of choice to be applied in such area.

Keywords: Lufenuron, diflubenzuron, Novaluron, Esterases, Acetylcholineesterase, Glutathione-S- transferase, Carboxylesterases, Total protein.

Introduction

Mosquitoes are one of the most important groups of insects since ancient civilizations (Khater, 2017) and widely spread life-threatening pathogens to humans and animals such as filariasis, malaria, dengue, and Rift Valley Fever (Onen *et al.*, 2023). *Culex pipiens* (Diptera: Culicidae) is a broadly distributed mosquito in Egypt (Ammar *et al*., 2012) acting as the primary vector of filariasis, *Wuchereria bancrofti*, besides the Rift Valley Fever virus (Gad et al., 1999; Abdel-Shafi et al., 2016; Onen et al., 2023). The prevalence and dispersal of diseases related to mosquitoes are mainly altered by some elements regulating mosquito biological parameters, such as growth, behavior, and survival (Sankar & Kumar, 2023). Chemical control is mainly used to control mosquito vectors. Over the past decades, the application of synthetic insecticides has grown in the field of agricultural and public health protecting millions of tons of agricultural food resources as well as human's and animal's lives (Khater, 2012a,b). However, such chemicals have resulted in pest resistance besides several side effects on health and the environment (Khater, 2012a,b; Ahmed et al., 2021; Iqbal et al. 2021; Mohammed et al. 2023; Nabil et al., 2023).

Searching for biorational pesticides for competing pests of medical and veterinary importance is one of the great importance during the last decade (Shalaby & Khater, 2005; Khater & Shalaby, 2008; Seddiek et al., 2013; Khater & Hendawy, 2014; Fouda et al., 2017; Hasaballah et al., 2018; Karthi et al., 2020; Khater et al., 2013, 2014, 2016, 2018, 2022; Baz et al., 2022a,b,c; 2023 Mohammed et al., 2023; Nabil et al., 2023). Shifting to eco-friendly pesticides has recently received great research and public interests (Roni et al., 2015; Murugan et al., 2015; Govindarajan et al., 2016a,b; Khater et al., 2009, 2013, 2018, 2019, 2022, 2023; Baz et al., 2021, 2022a,b,c; Hegazy et al., 2022; Radwan et al., 2022a,b; Abd Elgawad et al., 2023; Eltaly et al., 2023; Gad et al. 2023; Tolsá-García et al., 2023).

The insect growth regulators (IGRs) are regarded as promising chemical group of pesticides and considered as eco-friendly pesticides because of thier safety for most non-target creatures with minimal environmental toxicity. They do not directly kill insects, but alter their growth, interfering with their development and preventing them from reaching their(Khater, 2012a; Onen et al., 2023). According to their mode of action, IGRs could be classified into two main classes: Juvenile Hormone Analogs (JH) plus Chitin Synthesis Inhibitors (CSI) affecting the chitin synthesis via targeting specific enzymes and inhibiting the biosynthetic activity interfering with the cuticle formation. CSI includes the benzoyl phenyl urea (benzoylurea) class including lufenuron, diflubenzuron, and novaluron (Khater, 2012a; Sankar & Kumar, 2023). Diflubenzuron is widely utilized for mosquito management in public health programs (Gaaboub et al., 2017).

Some enzymes could be used as steadfast indicators for assessing the impression of toxic compounds on insects (Lushchak *et al.*, 2018). The insecticide resistance mechanisms mainly include Glutathione-S-transferase (GST), non-specific esterase, as well as P_{450}-mediated monooxygenase (MFOs) (Viswan et al., 2018). Therefore, this investigation aimed to evaluate the tolerance of Faqous' strain of *Cx. pipiens* collected from Sharkia Governorate in eastern Egypt to some IGRs such as CSI including diflubenzuron, novaluron, and lufenuron as well as their biochemical characterizations.

2. Materials and Methods

2.1. Study area and mosquitoes

Sharkia Governorate is the third most populous governorate in Egypt and Zagazig City is its capital, locating at the Eastern Nile Delta of Egypt (latitudes 29º 54` and 31º 12` N along with longitudes 31º20` and 32º 15` E). Topographically, it is approaching 13 m above the sea level (a.m.s.l.). Faqous district is located at 30.733333°N 31.8°E in Sharkia Governorate.

Cx. pipiens larvae were collected from Faqous district at Sharkia Governorate, Egypt. The laboratory strain of *Cx. pipiens* was mass reared according to a previous protocol (Mahmoud, 2013) for a minimum of six generations within a temperature range of 27±2°C and relative humidity (RH) range of 75-80%, whereas the photoperiod level was 14:10 h (light/dark). The laboratory strain was used as a reference strain for larval bioassays**.** Early 4^{th} instar larvae of both strains were used in this investigation.

Insect growth regulators

Difluorite® 25% WP (diflubenzuron, $C_{14}H_9ClF_2N_2O_2$, MOBEDECO company, import and distribution by Egypt Agricultural Development Company); Rocksy® 10% EC (novaluron, $C_{17}H_9ClF_8N_2O_4$, UPL company, India); and Match® 5% EC (lufenuron, $C_{17}H_8Cl_2F_8N_2O_3$, Syngenta Company, China).

Larval bioassays

The bioassys ere done according to Baz et al., (2021) with little modification. Against the early 4^{th} larval instars of *Cx. pipiens*, different concentrations were used for Diflubenzuron, Novaluron**,** and Lufenuron in case of the Faquos strain as follows: 0.01, 0.02, 0.04, 0.08, 0.1, and 0.2 μg/ml; 0.1, 0.2, 0.4, 0.8, 1.0, and 2.0 μg/ml; and 0.05, 0.1, 0.2, 0.4, 0.8, and 1.0 μg/ml, respectively. On the other hand, such concentrations for the laboratory strain were 0.01, 0.02, 0.04, 0.08, 0.1, and 0.2 μg/ml, (0.01, 0.05, 0.1, 0.2, 0.4, and 0.8 μg/ml; and 0.001, 0.005, 0.01, 0.05, 0.1, and 0.2 μg/ml, respectively.

The untreated control group was treated with de-chlorinated water. The bioassays were replicated five times and 25 larvae were used per replicate. Mortalities were checked (Khater & Shalaby, 2008) 72 h post-treatment (PT).

Biochemical analyses

Biochemical markers within *Cx. pipiens*, 4^{th} instar larvae, tolerant strain in Faqous region were compared with the susceptible laboratory strain after treating 4^{th} instar larvae of the field strain with LC_{50s} of diflubenzuron (the most sensitive), novaluron (medium sensitivity), and lufenuron (the least sensitive) compared to the same untreated larval instar.

Larval homogenates were established by homogenization of twenty larvae of each population using a plastic mini pestle in the 1.5 ml centrifuge tubes (ice-cold) using 250 μl of sodium phosphate buffer (0.1 M) and a pH 7.4 with 0.02% Triton X-100. The homogenate was centrifuged at 4°C (10,000 rpm for 15 min). Separation from the supernatant was done into a 0.5 ml eppendorf tube, and then kept at –20°C until used for biochemical analysis.

Some parameters were estimated, such as the total protein concentrations (Koller & Kaplan, 1984). Acetylcholinesterase, AChE, activity (Ellman et al., 1961). Moreover, the colorimetric esterase activity and carboxylesterase assays were determined for the general substrates, such as activity assays for α- or β- naphthyl acetate (Gomori & Chessick, 1953) and Glutathione-S-transferase, GST (Grant & Matsumura, 1988)**.**

Statistical analysis

Mortality data were subjected to BioStat program V. 2009 and the lethal concentrations providing 50% (LC_{50}) and 90% (LC_{90}) lethalities were measured. The SPSS, 10.0 for Windows software package, was used for running the One-way analysis of variance for biochemical analyses and variant groups were determined using the Duncan test**.** The relative tolerance (or resistance ratio, RR) values were calculated (Aziz et al., 2016) as follows; LC_{50} or LC_{90} value of the field strain/ LC_{50} or LC_{90} value of the laboratory strain where RR< 2 showed a susceptible strain, 10> RR> 2 indicated a tolerant strain, and RR> 10 referred to a resistant strain. Also, the enzyme activity ratio was evaluated as follows:

Activity ratio = enzyme activity in the strain/ enzyme activity in the laboratory strain

Results and Discussions

The applications of any CSI compound eventually result in mortality due to the disrupted molting process of the treated insects, which confirms the great specialization of such compounds for insects (Khater, 2012a). Respecting the field strain, this study indicated that diflubenzuron was the most toxic compound followed by lufenuron and novaluron (LC_{50}= 0.08, 0.16, and 0.60 μg/ ml, respectively). In contrast, the order of toxic potency according to LC_{90} values of the tested compounds against the field strain was lufenuron, followed by diflubenzuron, and novaluron (2.13, 2.40, and 4.32 µg/ ml, respectively). The slope values of the toxicity lines indicated heterogenic responses (Table 1).

Table (1) *In vitro* efficacy of the insect growth regulators against 4th instar larvae of *Culex pipiens* collected from Faquos district compared to the laboratory reference strain

Strain	Insecticide	LC_{50} (µg/ml)	Confidence Limits		LC_{90} (µg/ml)	Confidence Limits		Slope	Relative tolerance*	
			Lower	Upper		Lower	Upper		LC_{50}	LC_{90}
Faqous	Diflubenzuron	0.08	0.03	0.141	2.40	1.50	3.10	1.62	1.14	7.06
	Novaluron	0.60	0.40	0.81	4.32	2.63	5.60	1.48	3.75	3.60
	Lufenuron	0.16	0.06	0.47	2.13	1.30	3.82	1.15	8.00	19.36
Laboratory Reference	Diflubenzuron	0.07	0.05	0.09	0.34	0.14	0.72	1.80	-	-
	Novaluron	0.16	0.08	0.24	1.20	0.91	2.10	1.45	-	-
	Lufenuron	0.02	0.01	0.03	0.11	0.06	0.18	1.68	-	-

*Relative tolerance (or resistance ratio, RR) values were calculated as follows: LC_{50}, or LC_{90}, value of the field strain/ LC_{50}, or LC_{90} value of the laboratory strain; where RR< 2 showed a susceptible strain, 10> RR> 2 indicated to a tolerant strain, and RR> 10 referred to a resistant strain

According to the values of tolerance/ resistance of the field strain and according to the LC_{50} values, this study indicated different grades of tolerance/ resistance towards the tested compounds (Table 1). The highest levels of tolerance were recorded for lufenuron, followed by novaluron and diflubenzuron (8.00-, 3.75-, and 1.14- folds, respectively). On the other hand, high levels of tolerances at the LC_{90} level were also recorded towards lufenuron, diflubenzuron, and novaluron (19.36-, 7.06- and 3.60- folds, respectively) after comparing with the laboratory strain.

Similar findings about diflubenzuron (Dimilin®) revealed that it (0.04 - 40 ppm) effectively controlled the late 3rd and early 4th larvae of *Cx. pipiens* in Qalubia Governorate, Egypt, and its LC_{50} value was 1.26 ppm. Diflubenzuron also prolonged the larval durations for 11.9 days, when compared with only four days within the control group. It also elevated larval abnormalities, 46.7%, such as the formation of larvae with weak and transparent cuticles plus pharate pupae and pupal abnormalities. Diflubenzuron also retarded the development of *Musca domestica*. Moreover, similar hindrances in the development of *Cx. pipiens* and *M. domestica* were recorded PT with pyriproxyfen (Sumilarv®), a juvenile hormone (JH) analog (Khater, 2003).

Another similar finding was reported for IGRs belonging to JH such as pyriproxyfen and CSI like lufenuron, novaluron, and diflubenzuron against larvae (4th instars) of *Cx. pipiens*; pyriproxyfen was the highly potent one (LC_{50}= 44 ng/ml) followed by the other IGRs such as diflubenzuron, novaluron, and lufenuron (LC_{50}= 1127, 137, and 263 ng/ml, respectively) (Ahmed & Vogel, 2020).

A similar study regarding the resistance ratio revealed that *Cx. pipiens* was tolerant against diflubenzuron 4% (Dudim), but it did not reach the resistance level; on the other hand, it was susceptible to pirimiphos- methyl (Actikil), cyphenothrin 12%, d-tetramethrin (Pesguard), Bti ITU, *Bacillus thuringiensis var. israelensis* (Bacilod), and triflumuron (Baycidal) (Aziz et al., 2016). A like finding recorded that diflubenzuron (Hilmilin®), at a higher concentration (0.008 g/m2), effectively controlled several mosquito species, such as *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles culicifacies*, and *Anopheles stephensi* under laboratory and field conditions (Ansari *et al.*, 2005). Moreover, the intensive use of diflubenzuron against *Cx. pipiens* led to the development of tolerance/resistance (Belinato & Valle, 2015). Two mutations (I1043L and I1043M) in the chitin synthase (CHS) were documented and conferred low and high points of resistance (Fotakis *et al.*, 2020).

The present study confirmed that lufenuron was the least effective IGR with increased tolerance against *Cx. pipiens*. Such a finding might be due to the over or misuse of lufenuron in the Faqous region. In the same direction, laboratory selection with diflubenzuron against *Ae. aegypti* quickly led to the development of resistance (Belinato & Valle, 2015) and this highlights the relevance of careful use of insecticides.

Biochemical Characterization

Total proteins content

This study pointed out that the total protein contents in the body homogenates of treated *Cx. pipiens* larvae indicated high, median, and low tolerance in the Faqous strain when compared to the susceptible, laboratory strain. The data revealed that the activity ratio of the total protein content was highly significantly increased in the high tolerant Faquos to lufenuron (HTFL), followed by the medium tolerant, Faqous to novaluron (MTFN), and the least tolerant Faqous to diflubenzuron (LTFD) when compared to the laboratory reference strain (2.05, 1.50, and 1.28 , respectively) (Table 2).

The protein reduction in the current study could be due to a hormonal imbalance caused by the interference of tested IGRs with the endocrine system (Khater, 2012a) and affecting the protein synthesis or the metabolism in insects (Padmaja & Rao, 2000).

Table (2) Biochemical markers in 4th instar larvae of *Cx. pipiens*, tolerant in Faqous region strain compared with the susceptible laboratory strain.

Strain	Total proteins (mg/ g.b.wt) (Activity ratio)	AChE activity (µg AChBr min^{-1} mg^{-1} protein) (Activity ratio)	Carboxylesterases µ Meb min^{-1} mg^{-1} protein (Activity ratio)	GSH (µmoL min^{-1} mg^{-1} protein) (Activity ratio)	α – esterase (ηmoL min^{-1} mg^{-1} protein) (Activity ratio)	β – esterase (ηmoL min^{-1} mg^{-1} protein) (Activity ratio)
Less tolerant Faquos to diflubenzuron (LTFD)	**17.00± 0.58[bc]** (1.28)*	**210.00±20.82[c]** (0.93)*	**242.00±1.53[c]** (1.03)*	**28.00±1.53[bc]** (1.27)*	**560.00±30.55[c]** (1.11)*	**128.33±6.01[bc]** (0.97)*
Median tolerant Faquos to novaluron (MTFN)	**20.00±0.58[b]** (1.50)*	**283.33±8.82[ab]** (1.26)*	**386.67±8.82[b]** (1.65)*	**35.67±2.33[b]** (1.62)*	**723.33±14.53[b]** (1.44)*	**151.67±4.41[b]** (1.15)*
High tolerant Faquos to lufenuron (HTFL)	**27.33±1.20[a]** (2.05)*	**310.00±2.89[a]** (1.37)*	**483.33±8.82[a]** (2.06)*	**62.33±1.45[a]** (2.83)*	**921.67±10.93[a]** (1.83)*	**175.00±2.89[a]** (1.33)*
Laboratory Reference Strain (LRS)	**13.33±0.88[c]**	**225.00±10.41[bc]**	**235.00±2.89[c]**	**22.00±1.53[c]**	**503.33±8.82[c]**	**131.67±4.41[c]**
F- test	**	**	**	**	**	**

*Activity ratio = enzyme activity in the strain / enzyme activity in the laboratory strain
AChE: Acetylcholinesterase; GST: Glutathione-S-transferase

Acetylcholineesterase activity

Regarding the activity of AChE as a target enzyme, the findings of this study confirmed a high significant difference in the activity of this enzyme. The ratio of AChE activities in HTFL, MTFN, and LTFD field strains were 1.37-, 1.26-, and 0.93- times, respectively, compared with the laboratory reference strain (Table 2).

Acetylcholinesterase breaks down acetylcholine which is a neurotransmitter at the nerve synapsis. AChE is a target site for carbamates and organophosphates, which inhibit AChE's function. Surprisingly, the increased activity of AChE in the field population of Faquos in this study, which did not treat previously with any IGRs revealed tolerance to lufenuron insecticide.

Similar findings were recorded; a minimum of five point mutations at the acetylcholinesterase insecticide binding site (Ace) have been recognized, reducing the sensitivity of *Drosophila melanogaster* to carbamates and organophosphates (Mutero *et al.,* 1994).

Similar to our findings and in a field strain of *Aedes albopictus* populations collected from Malaysia, an insensitive AChE was recorded (Chen *et al.,* 2013). The insensitivity of the target-site could occur because of the structural modification or point mutation of the targeted proteins, decreasing the efficacy of insecticide inhibitions. Point mutations of the target protein reduce the response of the nervous system to insecticides or reduce the binding ability of such protein to insecticides (Narahashi, 1988) enhancing the development of resistance.

Carboxylesterases activity

Carboxylesterases, CarEs, EC 3.1.1.1, are related to a group of metabolic enzymes found in several microbes, animals, insects, and plants (Oakeshott *et al.,* 2005) for hydrolyzing carboxylic esters in order of procedure acids and alcohols. CarEs has a major role in performing resistance to insecticides like carbamates, organophosphates (OPs), and synthetic pyrethroids (SPs) via gene amplification (Liu, 2015). The data of the present study showed that the activity of carboxylesterases (CEs) differed significantly between the field strains compared with the laboratory strain. The ratio of CEs activities in HTFL, MTFN, and LTFD Field strains was 2.06-, 1.65-, and 1.03- times, respectively, when compared to the laboratory reference strain (Table 2).

The biochemical characterization of *Ae. albopictus* in Florida showed a significant resistance to IGR insecticides such as methoprene and pyriproxyfen and over-expressed CEs, ESTs, and GSTs compared with the susceptible strain. Such over-expression of the four detoxification enzyme families in the Florida strain of *Ae. albopictus* could lead to lowered sensitivity to IGRs **(**Marcombe *et al.,* 2014**)**. In addition, boosted carboxylesterase activities could impact insecticide tolerance, otherwise resistance within booklice (Wang *et al.,* 2004).

Glutathione-S-transferase activity

It has been documented that Glutathione-S-transferases (GSTs), enzymes have various activities and perform a major role in the cellular detoxification. They guard cells against toxins by binding them to glutathione to neutralize their electrophilic sites and turning the products to be more water soluble. The glutathione conjugates are then metabolized to the excreted mercapturic acid. Such classes comprise cytosolic as well as microsomal enzymes. GSTs detoxify a broad variety of xenobiotics. In insects, GSTs assist in biotransformation and detoxification of several insecticides (Hemingway *et al.,* 2004).

The biochemical analyses for the applied IGRs in this investigation showed the activation of GSTs in HTFL MTFN, LTFD when compared to that of the laboratory (reference) strain (62.33, 35.67, 28.00, and 22.00 µ Mol /mg protein/ min, respectively) (Table 2). The highest activity of GST enzymes was noticed in HTFL, followed by MTFN, and LTFD populations recording 2.83-, 1.62-, and 1.27- times, respectively. Similar studies showed that there is a significant increase in carboxylesterase, GST, and mixed function oxidase (MFO) in a field populations of *Cx. quinquefasciatus* after assessment with laboratory populations (Anju Viswan & Pushpalatha, 2021) and significant increase of AChE and GST activities in the water flea, *Daphnia magna* (Daphniidae: Anomopoda) treated with imidacloprid (Jemec *et al.,* 2007).

General esterases (α- and β- esterases)

Esterases, ESTs, are a main class of detoxification enzymes ever-present in organisms taking part in neurogenesis, developmental regulations, as well as many metabolic reactions (Panini *et al.,* 2016). This investigation revealed the general esterase (ESTs) activities of HTFL, MTFN, and LTFD populations were 921.67 and 175.00; 723.33 and 151.67; and 560.00 and 128.33, respectively, whereas those of the laboratory reference strain were 503.33 and 131.67µ Mol α and β- NA/ mg protein/ min. There was a high significant difference ($p \leq 0.01$) between the EST activities of different tolerant Faquos populations of different IGRs compared with the laboratory strain. The ratios of esterase activity in HTFL populations with α- and β- NA were 1.83- and 1.33- times, respectively, while the esterase ratios in MTFN with α- and β- NA were 1.44- and 1.15- times, respectively, and its ratios in LTFD were 1.11- and 0.97- times when compared with the esterases ratio in the laboratory reference strain (Table 2). A similar study pointed out that esterase genes could be correlated to malathion detoxification in the psocid, *Liposcelis bostrychophila*, a major stored product pest (Wei *et al.,* 2020).

General (*α* and *β*) esterases and carboxylesterase are major enzymes accountable for the metabolism or detoxification of toxins (Li *et al.*, 2007). The increased detoxification activities of the enzymes could weaken the defense responses of house flies to insecticides (Chen *et al.*, 2015). Treatment of *Cx. pipiens* (3rd larval instars) with the insecticides resulted in a notable increase in carboxylesterase and (*α* and *β*) esterases (Gharib *et al*., 2020).

Non-specific esterases could detoxify IGR insecticides such as methoprene and pyriproxyfen in the resistant Florida population of *Ae. albopictus* compared with the susceptible strain (Marcombe *et al.,* 2014). Through transcriptome studies after short-term exposure to malathion and deltamethrin, ESTs, GSTs, and P_{450}s could impact insecticide tolerance (Dou *et al.*, 2013). Some related studies have pointed out to the increased enzymatic activities of AChE, CE, GST, and GES (Rane *et al.*, 2019). The results of this study revealed elevated quantities of detoxification enzymes, AChE, CE, GST, and GEs (α- and β- esterases) activities in the Faquos population in contrast to those of the laboratory strain. This could be explained as the levels of the detoxification enzymes of *Cx. pipiens* were elevated wherever conventional insecticides were regularly sprayed. The activity of GST, MFO, and CarEs significantly increased within the field populations of *Cx. quinquefasciatus* when compared to that of the laboratory strain (Anju Viswan & Pushpalatha, 2021).

It was found through this study that there was a positive correlation between the levels of the total protein content and non-specific esterase activities and the level of insecticidal tolerance in larvae of *Cx. pipiens*. Diflubenzeron, in this study, provided the best results for controlling the field strain. On the contrary, it was found that *Cx. quinquefasciatus* populations showed a high resistance level against diflubenzuron (RR= 13.33–43.33) (Hafez & Abbas, 2021). This finding could be due to using different species, products, locality….etc.

Conclusion

This study revealed various degrees of tolerance to some non-traditional chitin synthesis inhibitors, mainly lufenuron, as an insecticide in the field strain of *Cx. pipiens* in Faqous. Therefore, diflubenzeron is the IGR of choice to be applied in such an area.

In fact, IGR pesticides are widely used to control agricultural pests in Faqous, but not applied for pests of medical and veterinary importance. Several detoxification enzyme families especially AChE, CEs, GST, and ESTs seemed to be involved in the tolerance process to the applied compounds. This could be explained as the presence of cross-resistance due to repeated conventional synthetic pesticide use and/ or repeated exposure due to the agriculture drainage.

To come to the point, the results of this work suggested that tolerance and the tendency toward resistance to commonly known CSIs are present in *Cx*. *pipiens* population in Faqous, Sharkia Governorate, Egypt, despite that such strain had never been exposed to any IGR as a field application.

Future studies could be directed toward studying cross-resistance and selection pressure on these CSIs under laboratory conditions and studying how various toxicants could affect protein synthesis in *Cx. pipiens* as well as the other insects. Alternative and safe insecticides should be evaluated and commercialized for effective and environmentally sound solutions for mosquito control.

Ethical approval

The protocol of this study has been reviewed and approved by the Ethical Committee of Zagazig University: Institutional animal care and Use committee, ZU-IACUC; the approval number is ZU-IACUC/2/F/169/2023.

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