STUDY ON THE EFFECT OF SOME CHEMICAL AND BIOLOGICAL INSECTICIDES ON THE DIGESTIVE AND DETOXIFYING ENZYMES IN *CULEX PIPIENS* MOSQUITO

A Thesis
Submitted in Fulfillment of the Requirements of Ph.D. in Biochemistry

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I declare that myself have composed this thesis and the work therein has not been submitted for a degree at this or any other university.

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ABSTRACT

Nashwa Hussein Kamel Mohamed

STUDY ON THE EFFECT OF SOME CHEMICAL AND BIOLOGICAL INSECTICIDES ON THE DIGESTIVE AND DETOXIFYING ENZYMES IN *CULEX PIPIENS* MOSQUITO

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Culex pipiens is one of the main vectors of viral and parasitic diseases in Egypt. The proposed work aimed to study the enzymatic changes associated with the development of resistance in *Cx. pipiens* larvae treated with certain chemical (methomyl and Malathion) and biological (*Bacillus thuringiensis* H14) insecticides. Using chemical insecticides showed a decrease in susceptibility of the mosquito larvae with the progressive increase in selection pressure (increase in LC_{50} dose). In contract there was no significant difference in the susceptibility response to biological insecticides in the successive generations.

Larval bioassay results were supported by those of microplate assays in detection of changes in activities of proteolytic enzymes (trypsin & aminopeptidase) and detoxifying enzymes (non specific esterases, GST and acetylcholinesterase). The recorded changes in the enzymes activities could be used as indications of the multiple previous uses of these insecticides in the field.

Key Words: Insect, resistance, proteolytic enzymes, detoxifying enzymes, organophosphate, carbamate, *Bacillus thuringiensis*.

LIST OF ABBREVIATIONS

α-NA		α-Naphthyl acetate
AChE		Acetylcholinesterase
A*p*NA	:	Alanine *p*-nitroanilide.
ASChI		Acetylthiocholine iodide
BAEE	:	Nα - Benzoyl-L-arginine ethylester.
BA*p*NA	:	Nα-Benzoyl DL-arginine-*p*-nitroanilide.
β-NA		β-Naphthyl acetate
BSA	:	Bovine serum allbumin
CDNB		1-chloro-2,4-Dinitrobenzene
C.l.	:	Confidence limit
DMF	:	N, N, Dimethyl formamide.
DMSO	:	Dimethyl sulfoxide.
DTNB		Dithio- bis 2-nitrobenaic acid
EU	:	Enzyme unit of activity [is the amount of enzyme required to hydrolyse 1μ mol of substrate per minute].
$F_{(N)}$		Insect Generation
g		Centrifuge gravity
GSH	:	Glutathione reduced
GST	:	Glutathione S-transferase
K_m	:	The Michaelis constant
L: D		Light: Dark
LC_{50}	:	Lethal Concentration cause 50% mortality

LC_{90}	:	Lethal Concentration cause 90% mortality
L*p*NA	:	L-Leucine *p*-nitroanilide.
PBS	:	Phosphate buffered saline.
[S]	:	Substrate concentration
TAME	:	Tosyl-arginine methyl ester-HCl.
Tris- HCl	:	Trizma hydrochloride (Tris (hydroxymethyl) aminomethane hydrochloride).
v/v		Volume per volume
V_{max}		The maximal reaction velocity.
(w/v)	:	Weight per volume
X^2	:	Chi-2

LIST OF INSECTS AND PARSITIES TERMINOLOGY

Ae. aegypti	:	*Aedes aegypti.*
An. albimanus	:	*Anopheles albimanus.*
An. gambiae	:	*Anopheles gambiae.*
An. quadrimaculatus	:	*Anopheles quadrimaculatus.*
An. stephensi	:	*Anopheles stephensi.*
An. tessellatus	:	*Anopheles tessellatus.*
B. thuringiensis (B. t.)	:	*Bacillus thuringiensis*
Cx. fatigans	:	*Culex fatigans.*
Cx. nigripalpus	:	*Culex nigripalpus.*
Cx. pipiens	:	*Culex pipiens.*
Cx. tarsalis	:	*Culex tarsalis.*
H. virescens		*Heliothis virescens*
L. donovani	:	*Leishmania donovani.*
P. falciparum	:	*Plasmodium falciparum.*
B. germanica		*Blattella germanica*
O. nubilalis		*Ostrinia nubilalis*
P. interpunctella		*Plodia interpunctella*
P. gallinaceum	:	*Plasmodium gallinaceum.*
P. langeroni	:	*Phlebotomus langeroni.*
P. papatasi	:	*Phlebotomus papatasi.*
P. vivax grassi	:	*Plasmodium vivax* grassi.
P. yoeliinigeriensis	:	*Plasmodium yoeliinigeriensis.*

R. americana	:	*Rhychosciara americana.*
R. prolixus	:	*Rhodnius prolixus.*
S. litura	:	*Spodoptera litura.*
T. molitor	:	*Tenebrio molitor.*
W. bancrofti	:	*Wuchereria bancrofti.*

LIST OF FIGURES