

**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

Nashwa Hussein Kamel Mohamed
Research and Training Center on Vectors of Diseases
M.Sc. in Biochemistry(2002)

Under supervision of

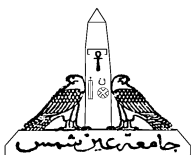
Prof. Dr. Ahmed M. Salem	Prof. Dr. Adel I. Merdan
Biochemistry Department	Entomology Department
Faculty of Science	Faculty of Science
Ain Shams University	Ain Shams University

Dr. Walid Elsayed Zahran
Lecturer of Biochemistry
Biochemistry Department
Faculty of Science
Ain Shams University

Ain Shams University
(2009)

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.

Nashwa Hussein Kamel



Biochemistry Department
Faculty of Science
Ain Shams University

Name : **Nashwa Hussein Kamel Mohamed**
Title : **STUDY ON THE EFFECT OF SOME
CHEMICAL AND BIOLOGICAL
INSECTICIDES ON THE DIGESTIVE AND
DETOXIFYING ENZYMES IN *CULEX
PIPIENS* MOSQUITO**
Scientific Degree : **Ph.D. in Biochemistry**

Supervision Committee

Prof. Dr. Ahmed M. Salem : Biochemistry Department - Faculty of Science -
Ain Shams University
Prof. Dr. Adel I. Merdan : Entomology Department - Faculty of Science -
Ain Shams University
Dr. Walid Elsayed Zahran : Biochemistry Department - Faculty of Science -
Ain Shams University

Discussion Committee

Prof. Dr. Hussein S. Abd-Elrahman : Entomology Department -National Center for
Research
Prof. Dr. Raouf Mohamed : Biochemistry Department – National
Organization for Drug Control and Research
Prof. Dr. Ahmed M. Salem : Biochemistry Department - Faculty of Science -
Ain Shams University
Prof. Dr. Adel I. Merdan : Entomology Department - Faculty of Science -
Ain Shams University

Postgraduates' Studies

Research Date

/ /2009

Approval Stamp

Date of Approval

/ /2009

Collage Aprooval

/ /2009

University Aprooval

/ /2009

BIOGRAPHY

Date and Place of Birth : 24/3/1973
Date of B.Sc.Graduation : May 1995
Degree Awarded : M.Sc. in Biochemistry (2004)
Grade : Ph.D. in Biochemistry
Date of Registration : 2004
Date of Appointment : 2009

Supervisors

- 1- Prof. Dr.Ahmed M. Salem (Professor of Biochemistry)
- 2- Prof. Dr.Adel Ibrahim Merdan (Professor of Entomology).
- 3- Dr.Walid Elsayed Zahran (Lecturer of Biochemistry).

Head of Department
Prof.Dr. Amr M. Karim

ACKNOWLEDGEMENT

My deepest thanks to Prof. Dr. Ahmed M. Salem, Professor of Biochemistry, Faculty of science, Ain Shams University, whose kind supervision, scientific advice, encouragement, and guidance is highly appreciated.

I am greatly indebted to Prof. Dr. Adel Ibrahim Merdan, Professor of Entomology, Faculty of Science, Ain Shams University, For his instructive guidance, sincere help and valuable advice throughout the course of this study. I am really very proud to be his student.

I wish to express my deepest appreciation and gratitude to Prof. Dr. Gamal El Kady, Professor of Plant Protection Department, Faculty of Agriculture and Dr. Iman Bahgat Assistant Professor of Entomology, Faculty of Science, Suez Canal University for their kind support and offering facilities during all phases of this work.

I wish also to thank Dr. Walid Elsayed Zahran, Lecturer of Biochemistry, Faculty of Science, Ain Shams University for his support during this work.

Deep appreciation to Dr. R. Ramzy, Professor of Immunology, Institute of Nutrition, and all staff members of the Research and Training Center on Vectors of Diseases, Ain Shams University.

CONTENTS

	Page
Abstract -----	i
List of abbreviations -----	ii
List of insects and parasites terminology -----	iv
List of figures -----	vi
List of Tables -----	x
I. Introduction	1
Aim of work -----	3
II. Review of literature	4
Mosquito and its associated problems -----	4
Historical development of insecticides -----	5
Classification of the insecticides used in the present study.-----	6
1- Chemical insecticides-----	6
<i>a</i> -Organophosphate insecticides (OP) -----	6
<i>b</i> -Carbamate insecticides-----	8
2- Biological insecticides-----	9
Mode of action of insecticides-----	13
Mode of action of chemical insecticides(organophosphate and carbamate) -----	13
Mode of action of <i>Bacillus thuringiensis</i> -----	14
Resistance -----	16
Methods of detecting resistance -----	17
<i>a</i> - WHO bioassay-----	17
<i>b</i> - Biochemical and Immunological assays-----	17
<i>c</i> - DNA and RNA probes-----	18
Mechanisms of resistance-----	18
1- Reduced penetration -----	21
2- Metabolic resistance (degradation of insecticide) -----	21
<i>I</i> -Enzyme involved in detoxification of chemical insecticides---	23
a-Glutathione S-transferases-----	23

b-Esterases -----	25
c-Acetylcholinesterases-----	30
II-Enzyme involved in degradation and resistance to -----	34
1. Endo-peptidases-----	34
Trypsin activities in insects: -----	36
2. Exo-peptidase-----	38
III. Materials and Methods -----	42
Mosquito colonies -----	42
Mosquito rearing-----	42
Insecticides used -----	43
Bioassay test for <i>Cx. pipiens</i> parental generation and field population-----	44
Resistance development in <i>Cx. pipiens</i> to chemical and biological insecticide-----	46
Biochemical assays-----	48
Total protein determination in larvae (Protein assay) -----	48
Enzymatic assays-----	49
1.Detoxifying enzymes-----	50
A-Assay on specific esterase -----	50
i- α -Esterase assay-----	50
Effect of substrate concentration on α -esterase activity	51
ii- β -Esterase-----	51
Effect of substrate concentration on β -esterase activity	52
B-Acetyl cholinesterase (AChE) -----	53
Enzyme Kinetics-----	53
C-Glutathione S-transferase (GST)-----	54
Enzyme Kinetics-----	54
2.Proteolytic enzymes -----	56
A-Trypsin assay-----	56
Effect of substrate concentration on trypsin activity-----	57
B-Aminopeptidase-----	57
Effect of substrate concentration on aminopeptidase activity -----	58
Stander curve-----	58
Statistical analysis-----	59

IV. Results	61
1-Susceptibility of mosquito <i>Cx. pipiens</i> to chemical and biological insecticides.-----	61
2-Resistance development in <i>Cx. pipiens</i> to chemical insecticides	62
A- <i>Methomyl</i> -----	62
B- <i>Malathion</i> -----	67
3-Resistance development in <i>Cx. pipiens</i> to biological insecticide- <i>Bacillus thuringiensis</i> H14 (<i>B.t.</i> H14) -----	70
Biochemical assays -----	74
Changes in protein content in <i>Cx. pipiens</i> resistant colonies	74
1-Effect of chemical and biological insecticides on detoxifying enzymes activities -----	79
a- α Esterase-----	79
Effect of substrate concentration on alpha esterase activity	79
Changes in alpha esterase activity in <i>Cx. pipiens</i> resistant colonies selected by methomyl, malathion and <i>B. t.</i> H14.	80
b- β Esterase -----	86
Effect of substrate concentration on β esterase activity	86
Changes in β esterase activity in <i>Cx. pipiens</i> resistant colonies selected by methomyl, malathion and <i>B. t.</i> H14.	87
2. Acetylcholinesterase-----	92
Effect of substrate concentration on acetylcholinesterase activity. -----	92
Changes in acetylcholinesterase activity in <i>Cx. pipiens</i> resistant colonies selected by methomyl, malathion and <i>B. t.</i> H14. -----	93
3-Glutathione S-transferase-----	98
Effects of substrate (CDNB) and cofactor (GSH) concentration on enzyme activity. -----	98
Changes in glutathione-S-transferase activity in <i>Cx. pipiens</i> resistant colonies selected by methomyl, malathion and <i>B. t.</i> H14. -----	99
Proteolytic enzyme-----	105
1-Trypsin-----	105
Effect of substrate concentration on trypsin activity-----	105

Changes in trypsin activity in <i>Cx. pipiens</i> resistant colonies selected by methomyl, malathion and <i>B. t.</i> H14.	106
2-Aminopeptidase-----	111
Effect of substrate concentration on aminopeptidase activity	111
Changes in aminopeptidase activity in <i>Cx. pipiens</i> resistant colonies selected by methomyl, malathion and <i>B. t.</i> H14.	112
V. Discussion -----	117
Bioassays: -----	118
Chemical insecticides-----	118
Biological insecticides (<i>Bacillus thuringiensis</i>)-----	120
Biochemical assay: -----	121
1-Detoxifying enzymes in mosquito larvae pressed by chemical insecticides -----	121
Esterases activities-----	121
Acetylcholinesterase-----	123
Glutathione S-transferases-----	124
2-Proteolytic enzymes in mosquito larvae pressed by chemical Insecticides.-----	125
3-Proteolytic enzymes in mosquito larvae pressed by biological insecticides-----	127
4-Detoxifying enzyme in mosquito larvae pressed by biological insecticides-----	129
Total protein content	130
VI. Summary -----	132
Conclusion-----	137
Recommendations-----	138
VII. References -----	140
Arabic Summary-----	
Arabic Abstract-----	

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

Faculty of Science, Biochemistry Department, Ain Shams University

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 LC₅₀ 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
ApNA	: Alanine <i>p</i> -nitroanilide.
ASChI	Acetylthiocholine iodide
BAEE	: N α - Benzoyl-L-arginine ethylester.
BApNA	: N α -Benzoyl DL-arginine- <i>p</i> -nitroanilide.
β -NA	β -Naphthyl acetate
BSA	: Bovine serum albumin
CDNB	1-chloro-2,4-Dinitrobenzene
C.I.	: Confidence limit
DMF	: N, N, Dimethyl formamide.
DMSO	: Dimethyl sulfoxide.
DTNB	Dithio- bis 2-nitrobenzoic acid
EU	: Enzyme unit of activity [is the amount of enzyme required to hydrolyse 1 μ 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 <i>p</i> -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

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

<i>R. americana</i>	:	<i>Rhychosciara americana.</i>
<i>R. prolixus</i>	:	<i>Rhodnius prolixus.</i>
<i>S. litura</i>	:	<i>Spodoptera litura.</i>
<i>T. molitor</i>	:	<i>Tenebrio molitor.</i>
<i>W. bancrofti</i>	:	<i>Wuchereria bancrofti.</i>

LIST OF FIGURES

	Page
Figure 1: Neurons communicate chemically at specialized structures called synapses.	32
Figure 2: The hydrolysis activity of acetylcholinesterase.	33
Figure 3: Standard calibration curve of bovine serum albumin.	49
Figure 4: Standard calibration curve of α - naphthol.	51
Figure 5: Standard calibration curve of β - naphthol.	52
Figure 6: Standard calibration curve <i>p</i> -nitroaniline.	58
Figure 7: Ld-p lines of the 3 rd instar larvae of <i>Cx. pipiens</i> treated with methomyl at different generation from F ₀ to F ₂₀ compared to the field strain.	66
Figure 8: Ld-p lines of the 3 rd instar larvae of <i>Cx. pipiens</i> treated with malathion at different generation from F ₀ to F ₂₀ compared to the field strain.	70
Figure 9: Ld-p lines of the 3 rd instar larvae of <i>Cx. pipiens</i> treated with <i>B.t.</i> H14 at different generation from F ₀ to F ₁₀ compared to the field strain.	73
Figure 10: Changes of protein content (mg) in control parental colony when compared with the field strain after pressed to methomyl, malathion and <i>B. t.</i> H14.	75