

**GENETICAL AND HISTOCHEMICAL
STUDIES ON RESISTANCE TO BAYGON
IN CULEX PIPIENS**

A THESIS

Submitted in Partial Fulfilment
of the requirements for the Award
of the Degree of

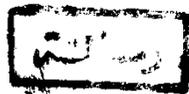
MASTER OF SCIENCE

BY

EHAD MAHMOUD SAID BARAKAT

B.Sc.

Department of Entomology
Faculty of Science
Ain Shams University



Cairo

1991

17302



Naima Abdel...

DEDICATION

*I have a great honour to dedicate this
dissertation and the including research to the
spirit of my Professor , Prof. Dr. Awni Mohamed
Guneidy. To whom I am really indebted for all
I have been learned in both moral and
scientific aspects.*

Emad Barakat



THESIS EXAMINATION COMMITTEE

NAME	TITLE	SIGNATURE
.....
.....
.....

BIOGRAPHY

Date and place of birth: 15 August, 1960, Cairo.

Date of graduation : May, 1984.

Degree awarded : B. Sc. Special Entomology.

Grade : Very good.

Occupation : Demonstrator in Entomology
Department, Faculty of
Science, Ain Shams University,
Cairo.

Date of appointment : October 1984.

Date of registration for
M. Sc. Degree : April, 1987.

Courses studied by the candidate in partial fulfilment
of the requirements for the M. Sc. degree

Language

English, M. Sc. courses.

Examination passed on: October, 1986.

Entomology courses

- 1- New approaches to insect control.
- 2- Environmental pollution.
- 3- Microbiology.
- 4- Radiobiology.
- 5- Insect Hormones and pheromones.
- 6- Chemistry of insecticides.
- 7- Advanced taxonomy.
- 8- How to write and publish a scientific paper.
- 9- Research subject.

Examination passed on: September, 1986.

Statistical courses

Biostatistics.

Examination passed on: September, 1986.

SUPERVISORS

Professor Dr. A. M. Guneidy

Professor Dr. Naima A. Abdel-Razik

Assistant Prof. Dr. Lyla S. El-Sherif

Dr. M. A. Hussein

Head of Department

Professor Dr. A. L. Merdan

Lyla S. El-Sherif

ACKNOWLEDEMENT

The author wishes to express his sincere thanks and appreciation to the late Dr. A. M. Guneidy, professor of Entomology and the former Dean, Faculty of Science, Ain Shams University, for suggesting the problem and for direct supervision of this work, his valuable advice and his kind encouragement.

Thanks are also due to Dr. Naima A. Abdel-Razik Professor of Entomology , Dr. Lyla S. El-Sherif, Assistant Professor of Entomology and Dr. M. A. Hussein, Lecturer of Entomology in Entomology Department Faculty of Science , Ain Shams University for Direct supervision , reading and correcting the manuscript.

The author is indebted with great thanks to professor Dr. M.S. Hamed and Dr. R.F. Bakr from the same Department for their useful advice and interest in the progress of the study .

Deep appreciation is also expressed to each of the staff members of Entomology Department, Faculty of Science, Ain Shams University, who helped in various ways throughout the period of this study.

C O N T E N T S

I- Introduction	1
II- Literature review :	4
1- Development and reversion of insecticide resistance.	4
2- Resistance spectra of the resistant strains to various insecticides.	10
3- Mode of inheritance of insecticide resistance.	16
4- Histochemical localization of cholinesterase.	24
5- Biochemical genetics of resistance.	29
III- Materials and methods :	40
1- Maintenance of culture ;	40
1) Strains employed.	40
2) Rearing methods.	40
2- Toxicological studies :	42
1) Insecticides used :	42
(1) Chemical formulae and structures.	42
(2) Preparation of insecticide concentrations.	44
2) Insecticide testing technique.	44
3) Selection procedure.	46
4) Statistical analysis of the data.	46
5) Resistance spectra of the selected strain to various insecticides.	48
3- Genetics of resistance to insecticides :	48
1) Factorial analysis.	48
4- Histochemical studies :	55
1) Histochemical study of cholinesterase :	55
(1) Reagents used.	56
(2) Preparation of larvae for enzymatic study	57
(3) Technique employed.	57
(4) The chemical reaction.	58
5- Biochemical genetics of resistance :	59
1) Biochemical assay of cholinesterase :	59
(1) Crossing experiments.	59
(2) Determination of cholinesterase activity :	60
a- Reagents used.	61
b- Enzyme extraction and assay of larval homogenate.	62
c- Preparation of the standard curve for substrate.	62
d- Technique employed.	65

IV- Results and discussion :.....	67
1- Toxicological studies :.....	67
1) The response of susceptible and resistant strains of <u>Culex pipiens</u> larvae to Baygon.....	67
2) Development and reversion of resistance to Baygon in <u>Culex pipiens</u> larvae :.....	69
(1) Selection for resistance.	69
(2) Relaxation.	74
3) Resistance spectra of the Baygon-resistant strain of <u>Culex pipiens</u> larvae to various insecticides.	77
2- Genetic of resistance to insecticides :.....	90
1) Mode of inheritance of Baygon-resistance in <u>Culex pipiens</u> larve :.....	90
(1) Test with parent colonies.	90
(2) Direct cross and progeny.	92
(3) Direct backcrosses :.....	97
a- Backcrosses to susceptible parents..	97
b- Backcrosses to resistant parents ...	98
(4) Backcrosses with selection :.....	100
a- To susceptible parents.	100
b- To resistant parents.	102
3- Histochemical studies of cholinesterase :.....	106
1) Histochemical localization of cholinesterase in susceptible and resistant mosquito larvae, <u>Culex pipiens</u>	106
4- Biochemical genetics of Baygon resistance in mosquito larvae, <u>Culex pipiens</u> :.....	111
V- Summary.	117
VI- Literature cited.	125
VII- Arabic summary.	--

INTRODUCTION

I. INTRODUCTION

The large scale use of insecticides against insects of either agricultural or medical importance has frequently led to the development of races or strains sufficiently resistant to the action of these chemicals . The development of resistance and particularly of multiple-resistance have led to great difficulties in the control of both disease vectors and agricultural pests.

Generally it has been accepted that the resistance arise by intensive selection with an insecticide, followed by inbreeding of survivors, i.e., selection of a mutant present in low frequency in an insect population. The present trend in biochemical genetics and the impetus provided by molecular biology emphasize the nature of genes and proteins as dynamic processes undergoing constant evolutionary changes. Many factors are involved in bringing about these changes including external pressures from foreign compounds and drugs . Certainly insecticides too, fall in the same category.

The mosquito, Culex pipiens was reported as among the most common and widely distributed species in

Egypt, plus being a vector of both parasitic and viral diseases dangerous to humans and animals . It is one of the most extensively reported species show as being able to develop resistance to insecticides of different chemical groups . Agricultural control operations, the sprays for malaria control and sporadic use of insecticides contributed to the fast development of resistance to insecticides in this insect.

The increasing threat of mosquito resistance to chlorinated hydrocarbons and organophosphorous insecticides has initiated research in alternative chemicals for mosquito control.

Carbamates are referred to as reversible acetylcholinesterase inhibitors . They inhibit true and pseudo-cholinesterase and ali-esterase enzymes. Poisoning from these compounds is clinically similar to that produced by the other group of anti-cholinesterase insecticides, the organophosphate (Strather, 1972).

Insect cholinesterase in particular, have been of interest for many years because of their importance in nerve transmission and for understanding their response to anti-enzyme action of certain chemical insecticides.

The determination of cholinesterase activity in various insects, was studied by many investigators. Most of these studies were based on biochemical techniques. These biochemical techniques gave only, quantitative estimates, which proved to have many drawbacks (Booth and Lee, 1971). The main drawback is the difficulty to trace the localization of the enzyme in the examined tissue; a matter, which is essential in understanding its sites of action .

The present work is, an attempt to study the mode of inheritance of resistance in Culex pipiens to the carbamate , Baygon, the stability of such resistance and cross-resistance to other insecticides of different chemical groups were studied in Baygon resistant strain. Also the activity and distribution of cholinesterase in resistant and susceptible larvae , in hybrids and different genotypes derived from different genetic crosses between them were assayed and investigated by biochemical and histochemical methods.

LITERATURE REVIEW