



**Faculty of Science
Dept. of Entomology**

**Efficacy of some insecticides and botanical extracts on
two dipterous insects and testing possibility of using
these insects as bioindicators for detecting toxic
residues.**

A Thesis

Submitted to the Faculty of Science
Ain Shams University

For the award of Ph.D. Degree
(Entomology)

By

Reham Ibrahim Mohamed Abdel- Aziz

B.Sc. & M. Sc. (Entomology)

Supervised by:

Prof.Dr.	Reda Fadeel Ali Bakr
Prof.Dr.	Sameeh Abdel-Kader Mansour
Prof.Dr.	Laila Sayed Ali Hamouda
Dr.	Nasra Mohammed Hasaneen Zohry

2012

Board of supervision

Prof.Dr. Reda Fadeel Ali Bakr

Professor of Entomology, Faculty of Science , Ain Shams University

Prof.Dr. Sameeh Abdel-Kader Mansour

Professor of Environmental, Toxicology of pesticides, National Research Centre

Prof.Dr. Laila Sayed Ali Hamouda

Professor of Entomology, Faculty of Science , Ain Shams University

Dr. Nasra Mohammed Hasaneen Zohry

Dr. of Zoology, Faculty of Science , Sohag University, Egypt

Biography

Date and place of birth: 6, October,1977, Egypt

Date of graduation: June, 1999

Degree awarded : B.Sc. (Special Entomology), 1999

M. Sc. 2005

Grade: Excellent

Occupation: Assistant Researcher

Pesticide Chemistry Dept.,

National Research Centre

Date of Registration

For the Ph.D.Degree: 17, February, 2007

ACKNOWLEDGEMENT

Firstly, all thanks to **Allah**, by the grace of whom, the present work was realized.

I would like to thank **Prof. Dr. Reda F.A. Bakr** , Professor of Entomology, Faculty of Science, Ain Shams University, for his kind help and guidance during supervision of the present work.

I wish to express my sincere gratitude and my appreciation to **Prof. Dr. Sameeh A.Mansour**, Professor of Environmental Toxicology of pesticides, National Research Centre , For suggesting the subject, supervising this work, for his continuous encouragement, kind help, indispensable advice and valuable comments he displayed during the course of this study and for reading and correcting the manuscript. It was a great honor to learn on his hands the basis of the scientific research.

Deep thanks also extened to **Prof.Dr. Laila Sayed Hamouda** , Professor of Entomology, Faculty of Science , Ain Shams University, for her kind help during supervision of the present work ,reading and correcting the manuscript .

My deep thanks to **Dr. Nasra Mohammed Hasaneen Zohry,Dr.** of Zoology, Faculty of Science, Sohag University, Egypt.

I am so grateful to **Prof.Dr. Souad E. EL-Gengaihi**, Phytochemical Sciene Department, National Research Centre, for her unlimited kind help for identifying the plant constituents used in this study.

Deep gratitude to the **President of National Research Centre**, for the facilities provided for such an accomplishment.

Finally, I wish to thank all colleagues in the department of Pesticide Chemistry, National Research Centre , Dokki, Egypt, especially my dear colleague, **Amina Rashad Ali** for her greatly support.

Abstract

The present study was carried out to evaluate the toxic effect of 12 ethanolic plant extracts (e.g., *Piper nigrum*, *Zea mays*, *Opuntia vulgaris*, *Punica granatum*, *Salix safsaf*, *Cichorium intybus*, *Sonchus oleraceus*, *Conyza aegyptiaca*, *Eucalyptus globulus* (fruits and leaves), *Saccharum sp.*, *Citrus aurantifolia* and *Azadirachta indica*) belonging to 10 families and four insecticides (e.g., chlorpyrifos, methomyl, deltamethrin and flufenoxuron) for their efficacy against larvae and adults of the mosquito, *Anopheles pharoensis* and *Musca domestica*.

All the tested plant extracts, except *Saccharum sp.* and *Opuntia vulgaris* were toxic to variant extend to the tested insects. *Cichorium intybus* was the most effective extract against *Anopheles pharoensis* larvae, while *C. aegyptiaca* was highly toxic against adult females of *Anopheles pharoensis*. For *Musca domestica* larvae and adults, *Piper nigrum* was the most active plant extract. The tested insecticides showed that chlorpyrifos was the most toxic one against *Anopheles pharoensis* larvae, while deltamethrin was the highest toxic against the adult stage of both insects as well as against larval stage of *Musca domestica*. Many morphological changes and abnormalities were observed due to exposure of *Musca domestica* larvae to the tested botanicals and insecticides. Also, the results showed highly significant differences in the larval, pupal and adult durations, compared to control tests.

Some biological aspects, such as the repellent efficacy and attractant activity of the tested plant extracts against the adults were studied. The results revealed that the most effective one as repellent agent against *Anopheles pharoensis* was *C. intybus* while the lowest one was *A. indica*. For *Musca domestica*, *S. safsaf* was the most effective plant extract as repellent agent but *C. intybus*, *C. aurantifolia*, *P. nigrum*, *S. oleracues* and *Z. mays* were the lowest repellent agents. The residual toxicity of the used plant extracts and commercial insecticides against the house fly adults was investigated; *C. aegyptiaca* possessed the highest t_{50} value (10.6 days), while the lowest t_{50} was entitled to *S. safsaf* (3.2 days).

The influence of temperature (10 to 30°C) on the toxicity of chemical insecticides showed positive effect after treatment with chlorpyrifos and methomyl. The opposite trend was observed for the insecticide deltamethrin.

Some biochemical parameters were studied following exposure to the tested botanical extracts and insecticides. The results indicated an increase in the protein content, inhibition in AChE level and increase in GST activity.

In addition to the laboratory house fly strain, the field collected strains from different locations in Cairo were subjected to some biochemical measurements where total protein content, AChE and GST activities were determined. The results revealed the possibility of using house fly adult as a bioindicator/ biomarker for assessing potential exposure of the insect to household pesticides in open areas.

Finally, some pure substances were isolated from *Cichorium intybus*, *Sonchus oleraceus* and *Conyza aegyptiaca* oil and screened against the tested insects and were found more toxic than the parent crude extracts.

The overall results of the present investigation revealed the broad-spectrum toxic properties of the tested plant extracts against the tested insects; which may encourage further research on mosquito and house fly control in tropics.

Key words

Insecticides - plant extracts- *Musca domestica* – *Anopheles pharoensis* - potency – joint action – larvicidal action - adulticidal action - repellent action- insect development-bioindicator.

CONTENTS

Content	Page
Acknowledgment	
Abstract	
1-Introduction	1
2-Literature review	5
2.1. Phytochemical screening	5
2.2. Toxicological studies against <i>Anopheles</i> mosquitoes	9
2.3. Toxicological studies against <i>Musca domestica</i>	26
2.4. Synergistic action studies	38
2.5. The effect of temperature change on the insecticides' toxicity	43
2.6. Isolation and identification of insecticidal constituents of certain plants	47
2.7. Biochemical studies	55
2.8. Insects as bioindicators/ biomarkers	60
3-Materials and Methods	71
3.1. Tested insects	71
A) <i>Anopheles pharoensis</i>	71
B) <i>Musca domestica</i>	71
3.2. Tested Materials	73
3.2.1. Tested Plants	73
3.2.2. Insecticides.	73
3.3. Tested procedures	77
3.3.1. Tested procedures against <i>Anopheles pharoensis</i>	77
3.3.1.1. Larvicidal activity against <i>Anopheles pharoensis</i>	77
3.3.1.2. Efficacy of tested plant extracts	77
3.3.1.3. Efficacy of tested Insecticides.	78
3.3.1.4. Joint action Studies.	78
3.3.1.5. Synergistic action.	79

3.3.1.6. Developmental studies.	80
3.3.2. Adulticidal activity against <i>Anopheles pharoensis</i>.	81
3.3.2.1. Toxicity screening tests.	82
3.3.2.2. Repellency action.	83
3.3.2.3. Joint action.	83
3.3.2.4. Synergistic action.	84
3.3.2.5. Developmental studies.	
3.4. Tested procedures for <i>Musca domestica</i>.	
3.4.1. Larvicidal activity against <i>Musca domestica</i>.	
3.4.1.1. Toxicity screening by Bait Technique.	84
3.4.1.2. Joint action.	85
3.4.1.3. Synergistic action.	86
4.4.1.4. Developmental studies.	86
3.4.2. Adulticidal activity against <i>Musca domestica</i>.	
3.4.2.1. Toxicity screening by Topical Application.	87
3.4.2.2. Toxicity screening by (sugar bait) method.	88
3.4.2.3. Residual toxicity.	89
3.4.2.4. Repellency action.	89
3.4.2.5. Joint action studies.	90
3.4.2.6. Synergistic action.	91
3.4.2.7. Attractant action.	91
3.4.2.8. Developmental studies.	92
3.4.2.9. Effect of temperature on the insecticides' toxicity.	92
3.5. Effect of insecticides and plant extracts on some biochemical parameters in <i>Musca domestica</i>	93
3.6. Using of house fly, <i>Musca domestica</i> as a bioindicator / biomarker.	97
3.6.1. Field collection of house fly	97
3.6.2. Potency of Insecticides	98
3.6.3. Estimation of biochemical parameters in	99

house fly	
3.7. Identification of tested plant constituents	
3.7.1. Primary Phytochemical screening.	99
3.7.2. Isolation and identification of insecticidal constituents of certain plants.	102
4- Results	111
4.1. Phytochemical screening	111
4.2. Toxicological and biological studies	114
4.2.1. Efficacy of tested toxicants against <i>Anopheles pharoensis</i>	
4.2.1.1. Larvicidal activity of tested toxicants against 4 th instar of <i>Anopheles pharoensis</i>	114
4.2.1.2. Adulticidal activity of tested toxicants against adult females of <i>Anopheles pharoensis</i>	126
4.2.1.3. Developmental studies	138
4.2.1.4. Joint action studies	141
4.2.1.4.1 Joint action studies on <i>An. pharoensis</i> larvae	141
4.2.1.4.2 Joint action studies on adult females of <i>An. Pharoensis</i>	154
4.2.1.5. Synergistic/antagonistic studies	
4.2.1.5.1 Synergistic / antagonistic studies on <i>An. Pharoensis</i> larvae	147
4.2.1.5.2. Synergistic / antagonistic studies on adult females of <i>An. Pharoensis</i>	160
4.2.1.6. Repellent activity of the tested plant extracts	167
4.3. 4.3. Toxicological and Biological studies against <i>Musca domestica</i>	174
4.3.1. Toxicological and biological studies against 4th instar larvae of <i>Musca domestica</i>	174
4.3.1.1. Larvicidal activity of the tested toxicants against 3 rd instar larvae of <i>Musca domestica</i> by bait method	174
4. 4.3.1.2. Joint action studies on 4 th instar larvae of <i>Musca domestica</i>	187

4. 4.3.1.3. Synergistic /antagonistic studies on 4 th instar larvae of <i>Musca domestica</i>	193
4.3.1.4. Developmental studies on 3 rd instar larvae of <i>Musca domestica</i>	203
4.3.2. Toxicological and Biological studies against adult stage of <i>Musca domestica</i>	212
4.3.2.1. Adulticidal activity of the tested toxicants against adult stage of <i>Musca domestica</i> by topical application	212
4.3.2.2. Developmental studies on adult stage of <i>Musca domestica</i>	224
4.3.2.3. Adulticidal activity of the tested toxicants against adult stage of <i>Musca domestica</i> by sugar bait	227
4.3.2.4. Residual toxicity studies	241
4.3.2.5. Repellent activity studies	247
4.3.2.6. Attractant activity studies	254
4.3.2.7. Joint action studies	257
4.3.2.8. Synergistic/antagonistic studies	262
4.3.2.9. The effect of temperature on the insecticides toxicity	271
4.4. Toxicity of the tested insecticides against adults of <i>Musca domestica</i> collected from different locations.	277
4.5. Effect of insecticides and plant extracts on some biochemical parameters in <i>Musca domestica</i> adults .	290
4.6. Using of house fly, <i>Musca domestica</i> adults as a bioindicator/ biomarker.	299
4.7. Isolation and indentification of insecticidal constituents for certain plants.	301
5- Discussion	328

5.1. Identification of plant extracts constituent	328
5.2. Toxicicological studies	328
5.2.1. Mosquitocidal studies	330
5.2.2. Muscacidal studies	338
5.2.3. Joint action studies	345
5.2.4. Developmental studies	346
5.2.5. Repellent studies	350
5.2.6. Residual studies	350
5.3. Effect of temperature on the insecticides' toxicity	351
5.4. Biochemical studies	354
5.5. Using of <i>Musca domestica</i> as bioindicator / biomarker.	359
5.6. Isolation and identification of insecticidal constituents from certain plants.	366
6- Summary	369
7- Conclusion	384
8- Literature cited	385
9- Arabic summary	١

LIST OF TABLES

Table number		Page
1	Plants investigated for their insecticidal activities against <i>Anopheles pharoensis</i> and <i>Musca domestica</i> .	112
2	Screenin Screening for yield of crude ethanolic plant extracts, potency against 4 th instar larvae of <i>Anopheles pharoensis</i> at a definite concentration , and major phytochemical constituents.	113
2	Mortalities resulted from exposing 4 th instar larvae of <i>Anopheles pharoensis</i> to different concentrations of ethanolic extract of <i>Azadirachta indica</i> after 24 & 48 hours.	115
4	Mortalities resulted from exposing 4 th instar larvae of <i>Anopheles pharoensis</i> to different concentrations of ethanolic extract of <i>Cichorium intybus</i> after 24 & 48 hours.	115
5	Mortalities resulted from exposing 4 th instar larvae of <i>Anopheles pharoensis</i> to different concentrations of ethanolic extract of <i>Citrus aurantifolia</i> after 24 & 48 hours.	116
6	Mortalities resulted from exposing 4 th instar larvae <i>Anopheles pharoensis</i> to different concentrations of ethanolic extract of <i>Conyza aegyptiaca</i> after 24 & 48 hours.	116
7	Mortalities resulted from exposing 4 th instar larvae <i>Anopheles pharoensis</i> to different concentrations of ethanolic extract of <i>Eucalyptus globulus</i> (fruits) after 24 & 48 hours.	117
8	Mortalities resulted from exposing 4 th instar larvae <i>Anopheles pharoensis</i> to different concentrations of ethanolic extract of <i>Eucalyptus globulus</i> (leaves) after 24 & 48 hours.	117
9	Mortalities resulted from exposing 4 th instar larvae <i>Anopheles pharoensis</i> to different concentrations of ethanolic extract of <i>Piper nigrum</i> after 24 & 48 hours.	118
10	Mortalities resulted from exposing 4 th instar larvae <i>Anopheles pharoensis</i> to different concentrations of ethanolic extract of <i>Punica granatum</i> after 24 & 48 hours.	118

11	Mortalities resulted from exposing 4 th instar larvae of <i>Anopheles pharoensis</i> to different concentrations of ethanolic extract of <i>Salix safsaf</i> after 24 & 48 hours.	119
12	Mortalities resulted from exposing 4 th instar larvae of <i>Anopheles pharoensis</i> to different concentrations of ethanolic extract of <i>Sonchus oleraceus</i> after 24 & 48 hours.	119
12	Mortalities resulted from exposing 4 th instar larvae of <i>Anopheles pharoensis</i> to different concentrations of ethanolic extract of <i>Zea mays</i> after 24 & 48 hours.	120
14	Mortalities resulted from exposing 4 th larval of <i>Anopheles pharoensis</i> to different concentrations of Chlorpyrifos after 24 & 48 hours.	122
15	Mortalities resulted from exposing 4 th instar larvae of <i>Anopheles pharoensis</i> to different concentrations of Deltamethrin after 24 & 48 hours.	122
16	Mortalities resulted from exposing 4 th instar larvae of <i>Anopheles pharoensis</i> to different concentrations of Flufenoxuron after 24 & 48 hours.	123
17	Mortalities resulted from exposing 4 th instar larvae of <i>Anopheles pharoensis</i> to different concentrations of Methomyl after 24 & 48 hours.	123
18	LC ₂₅ , LC ₅₀ , LC ₉₅ , 95% fiducial limits and slope values for the tested ethanolic plant extracts and insecticides against 4 th instar larvae of <i>Anopheles pharoensis</i> , as estimated after 24 and 48 hrs exposure times.	124
19	Mortality % resulted from exposing adult females of <i>Anopheles pharoensis</i> to 0.16 mg/cm ² concentration of plant extracts.	127
20	Mortalities resulted from exposing adult females of <i>Anopheles pharoensis</i> to different concentrations of ethanolic extract of <i>Azadirachta indica</i> after 24 & 48 hours.	127
21	Mortalities resulted from exposing adult females of <i>Anopheles pharoensis</i> to different concentrations of ethanolic extract of <i>Cichorium intybus</i> after 24 & 48 hours.	128
22	Mortalities resulted from exposing adult females of <i>Anopheles pharoensis</i> to different concentrations of ethanolic extract of <i>Citrus aurantifolia</i> after 24 & 48 hours.	128

23	Mortalities resulted from exposing adult females of <i>Anopheles pharoensis</i> to different concentrations of ethanolic extract of <i>Conyza aegyptiaca</i> after 24 & 48 hours.	129
24	Mortalities resulted from exposing adult females of <i>Anopheles pharoensis</i> to different concentrations of ethanolic extract of <i>Eucalyptus globulus</i> (fruits) after 24 & 48 hours.	129
25	Mortalities resulted from exposing adult females of <i>Anopheles pharoensis</i> to different concentrations of ethanolic extract of <i>Eucalyptus globulus</i> (leaves) after 24 & 48 hours.	130
26	Mortalities resulted from exposing adult females of <i>Anopheles pharoensis</i> to different concentrations of ethanolic extract of <i>Piper nigrum</i> after 24 & 48 hours.	130
27	Mortalities resulted from exposing adult females of <i>Anopheles pharoensis</i> to different concentrations of ethanolic extract of <i>Punica granatum</i> after 24 & 48 hours.	131
28	Mortalities resulted from exposing adult females of <i>Anopheles pharoensis</i> to different concentrations of ethanolic extract of <i>Salix safsaf</i> after 24 & 48 hours.	131
29	Mortalities resulted from exposing adult females of <i>Anopheles pharoensis</i> to different concentrations of ethanolic extract of <i>Sonchus oleraceus</i> after 24 & 48 hours.	132
30	Mortalities resulted from exposing adult females of <i>Anopheles pharoensis</i> to different concentrations of ethanolic extract of <i>Zea mays</i> after 24 & 48 hours.	132
31	Mortalities resulted from exposing adult females of <i>Anopheles pharoensis</i> to different concentrations of Chlorpyrifos after 24 & 48 hours.	134
32	Mortalities resulted from exposing adult females of <i>Anopheles pharoensis</i> to different concentrations of Deltamethrin after 24 & 48 hours.	134
33	Mortalities resulted from exposing adult females of <i>Anopheles pharoensis</i> to different concentrations of Flufenoxuron after 24 & 48 hours	135
34	Mortalities resulted from exposing adult females of <i>Anopheles pharoensis</i> to different concentrations of Methomyl after 24 & 48 hours.	135