Evaluation of Insecticidal and Genotoxic Activities of some Essential Oils Against *Culex pipiens*

Thesis

submitted to Faculty of medicine, Ain-Shams University for partial fulfillment of Master Degree in Medical Sciences (Medical Parasitology)

By

ASMAA MUSTAFA ABD EL MOATY

Demonstrator of Medical Parasitology

Faculty of Medicine, Ain-Shams University

Supervised by

Prof. Dr. ELHAM ABBAS El ZAYYAT

Professor of Medical Parasitology

Faculty of Medicine, Ain-Shams University

Prof. Dr. AZZA ABDEL FATTAH MOSTAFA

Professor of Insecticides, Research Institute

Of Medical Entomology

Assistant Prof. Dr. NOHA ABDEL FATTAH ELLEBOUDY

Assistant prof of Medical Parasitology

Faculty of Medicine, Ain-Shams University

2016

Aknowledgement

First and foremost, thanks are due to **Allah**, the creator of all, the most beneficent and merciful of all, to whom I related any success that can be achieved at work in my life.

I cannot find words to express my feelings towards my supervisors for their great help and guidance in production this work.

I would like to express my high appreciation and gratitude to **Prof. Dr/ Elham Abbass El Zayyat**, Professor of Medical Parasitology, Faculty of Medicine, Ain-Shams University, for her gracious supervision, precious advice, and continuous encouragement through the whole research.

My special thanks and appreciation to **Prof. Dr/ Azza Abdel Fattah Mostafa** Professor of Insecticides, Research Institute Of Medical Entomology, for her kind help, meticulous supervision and continuous support and guidance throughout this work.

Also I would like to express my gratitude and appreciation to **Assistant Prof. Dr. Noha Abdel Fattah Elleboudy**, Assistant prof of Medical Parasitology, Faculty of Medicine, Ain-Shams University, for her fruitful encouragement, vast knowledge and valuable advice.

Finally, I wish to thank all **my family** especially **my mother**, **my husband**, **my sisters**, all the member of parasitology department and every one contributed with an effort in this work.

لجنه المناقشه

أ . د / عائشه توفيق حسن أستاذ علم الطفيليات - كلية الطب - جامعة الازهر
(ممتحن خارجي)

أ . د / فايزه سيد محمد حبيب أستاذ علم الطفيليات - كلية الطب - جامعة عين شمس
(ممتحن داخلي)

أ . د . /إلهام عباس الزيات أستاذ علم الطفيليات - كلية الطب - جامعة عين شمس
(عن المشرفين)

Contents

Subject	page
Abstract	I
List of Tables	II
List of Figures	III
List of Abbreviations	IV
Introduction	1
Review of literature Culex pipiens Taxonomy Morphology Life cycle Ecology Medical importance Control Essential oils Mosquitoes detoxifying enzymes The Single Cell Gel Electrophoresis	3 4 5 9 10 14 20 34 41
Materials and methods	46
Results	71

Discussions	91
Summary	107
Conclusion	112
Recommendations	113
Reference	115
Arabic summary	124

Abstract

Culex pipiens is a worldwide mosquitoes causing nuisance and transmitting many dangerous diseases. In Egypt, it is the main vector of filarial worm Wuchereria bancrofti as well as Rift valley fever virus.

Insecticides overuse led to several ecological drawbacks over the past years. the residual effects of The toxicity, development of resistance phenomenon and these insecticides are the main concern of scientists. The urging need for developing environmental friendly insecticides is rising.

Botanical insecticides are one of the successful alternatives as recorded by studies on plant-insect interaction. They are safe, specific, biodegradable, eco-compatible components. Therefore, this study aimed at evaluation of the Insecticidal and genotoxic Activities of three aromatic substances; eucalyptus oil , clove oil and rose water on Culex pipiens adults and larvae.

The chemical composition of essential oils were determined by gas chromatography/mass spectrometry (GC/MS) analysis .The main components of clove essential oil were Eugenol(88.08%) , Eugenolacetate(3.40%) and b-Caryophyllene(3.24%).The main components of eucalyptus essential oil were 1, 8-eucalyptol(46.76%) and D-Limonene (9.61%) . The main components of rose essential oil were nerol(20.88%), Citronellol(18.05%) and Geraniol (10.42%).

Fumigation bioassay was performed on *Culex pipiens* adults, eucalyptus oil has the highest toxicity index (LC50 :0.108%) and rose water has the lowest toxicity (LC50: 0.732%) after 24h.

In larval bioassay of *Culex pipiens* eucalyptus oil has the highest toxicity index (LC50 : 0.014%) and rose water has the lowest toxicity (LC50: 0.119%) after 48h.

The effects of three aromatic substances on the larval duration, pupation percent, pupal duration, adult emergence percent were determined, The induced malformed larvae, pupae were recorded and photographed.

The present study showed effect of tested aromatic substances on activities of Glutathione peroxidase (GPx) ,catalase and superoxide dismutase (SOD). The activities of GPx are significantly inhibited ($P<0.05$) compere to control and gradually decreased with the increasing concentrations of tested aromatic substances on *Culex pipiens* larvae. In *Culex pipiens* adult the activities of GPx are increased than that of the control and gradually decreased with the increasing concentrations, except in rose water the GPx activity is gradually increased with the increasing concentrations. In high concentration of eucalyptus oil there is no significant differences are found as compared to control ($p>0.05$).

The activities of catalase are increased about two fold as compared to control in low concentrations ($P<0.05$) but there is no significant differences were found as compared to control in high concentrations ($P>0.05$) in both *Culex pipiens* adult and larvae.

In *Culex pipiens* adult the activities of SOD enzyme are increased than that of the control and gradually decreased with the increasing concentrations after exposure to clove oil and rose water.In case of eucalyptus oil at low concentration

there is no significant difference (P>0.05) but, in high concentration there is significant decrease in enzyme activities (P<0.05).There is no significant differences are found on the activities of superoxide dismutase in culex pipiens larvae (p>0.05).

The genotoxic effect of tested aromatic substances was evaluated by comet assay as a measure of DNA strand-break damage. There was dose-dependent increase in DNA damage as evident by increase in the Comet parameters i.e. tail length (Px) and tail DNA (%), DNA % in head and % of DNA damage as compared with control.

The aromatic substances selected showed a promising potentiality to be used as green insecticides in controlling *Culex pipiens* adult and larvae.

Key words: *Culex pipiens* , eucalyptus oil , clove oil , rose water, GPx ,catalase and SOD.

List of Tables

Table No.	Title	Page No.
1	Chemical components of clove essential oil and their percentages by GC mass.	**73**
2	Chemical components of eucalyptus essential oil and their percentages by GC mass.	**74**
3	Chemical components of rose essential oil and their percentages by GC mass.	**75**
4	*Culex pipiens* adulticidal activity of tested aromatic substances by fumigation technique after 1h,6h and 24h.	**77**
5	*Culex pipiens* adulticidal activity of deltamethrine by fumigation technique after 1h,6h and 24h**.**	**78**
6	*Culex pipiens* larvicidal activity of tested aromatic substances after 24h and 48h.	**79**
7	*Culex pipiens* larvicidal activity of temephos after 24h and 48h.	**80**
8	Effect of the tested aromatic substances on some biological aspects after treatment of 3rd larval instars of *Culex pipiens.*	**80**
9	Effects of tested aromatic substances on the activities of some antioxidant enzymes in *Culex pipiens* adult after 1h.	**81**
10	Effects of tested aromatic substances on the activities of some antioxidant enzymes in *Culex pipiens* larvae after 24h.	**81**

List of Figures

Figure No.	Title	Page No.
1	**Anatomy of an adult mosquito.**	**5**
2	**Male and female *Culex pipiens* adult mouth part.**	**6**
3	**Anatomy of *culex pipiens* larva.**	**7**
4	***Culex pipiens* pupa.**	**8**
5	**Life cycle stages of *Culex pipiens.***	**9**
6	**Female *Culex pipiens*, ovipositing an egg raft.**	**10**
7	**Rose petals (*Rosa damascene).***	**49**
8	**Clove buds (*Syzygium aromaticum*).**	**49**
9	**Eucalyptus leaves (*Eucalyptus globulus*)**	**49**
10	**Clevenger apparatus**	**49**
11	**Schmadzu GC-MS-2010 plus (Germany).**	**51**
12	**Rearing media of *Culex pipiens* larvae.**	**52**
13	**Rearing cages of *Culex pipiens* adult.**	**53**
14	**Glass jar fitted with a screw cap with a 7 cm length of cotton yarn suspended from the center of the internal surface of the cap.**	**55**
15	**Dounce Tissue Grinders.**	**75**

16	**Axio fluorescence microscope (CD75V1A Zeiss, Germany).**	**69**
17	**Comet Assay Electrophoresis System (Horizontal Gel Electrophoresis Apparatus - GIBCO BRL, Life Technologies, power supply).**	**69**
18	**Mass spectrum of clove essential oil .**	**72**
19	**Mass spectrum of eucalyptus essential oil**	**72**
20	**Mass spectrum of rose essential oil**	**72**
21	**Probit dose-response curve of tested aromatic substances on *Culex pipiens* adults after 1h, 6h, 24h and their positive control.**	**76**
22	**Probit dose-response curve of tested aromatic substances on *Culex pipiens* larvae after 24h, 48h and their positive control.**	**78**

List of Abbreviations

BS	*Bacillus sphaericus*
BTI	*Bacillus thuringiensis israelensis*
DMSO	Dimethylsulfoxide
EDTA	Ethylene diamine tetra acetic acid
GC/MS	Gas chromatography-mass spectrometry
GPx	Glutathione peroxidase
IGRs	Insect Growth Regulators
IPM	Integrated pest management
JE	Japanese encephalitis
JHAs	juvenile hormone analogs
NBT	Nitroblue tetrazolium
Ops	Organophosphates
PMS	phenazine methosulphate
ROS	Reactive oxygen species
RVF	Rift Valley fever
RVFV	Rift Valley fever virus
SCGE	Single Cell Gel Electrophoresis

SLEV	Staint Louis encephalitis virus
SOD	superoxide dismutase
WNV	West Nile virus

PROTOCOL

Evaluation of Insecticidal and Genotoxic Activities of some Essential Oils Against *Culex pipiens*

A thesis protocol submitted for partial fulfillment of Master Degree in

Medical Sciences (Medical Parasitology)

By

ASMAA MUSTAFA ABD EL MOATY

Demonstrator of Medical Parasitology

Faculty of Medicine, Ain-Shams University

Supervised by

Prof. Dr. ELHAM ABBAS El ZAYYAT

Professor of Medical Parasitology

Faculty of Medicine, Ain-Shams University

PROF. Dr. AZZA ABDEL FATTAH MOSTAFA

Professor of Insecticides, Research Institute

Of Medical Entomology

Dr. NOHA ABDEL FATTAH ELLEBOUDY

Lecturer of Medical Parasitology

Faculty of Medicine,Ain-Shams University

2014