

BIOCIDAL ACTIVITY OF SELECTED PLANT EXTRACTS AGAINST CULEX PIPIENS (Linn.)

A Thesis

Presented to the Faculty of Science
Ain Shams University

For the Award of the Ph.D. Degree Entomology

 $\mathbf{B}_{\mathbf{V}}$

Samia Shoukry Messeha

B.Sc., M.Sc. (Entomology)

Supervisors

Prof. Dr. Mohamed Saad Hamed

Professor of Toxicology

Faculty of Science Ain Shams Universit

Prof. Dr.

Moheb Sadek Ishak Professor of Chemistry National Research Centre Prof. Dr.

Sameeh Abdel- Kader Mansour

Professor of Environmental

Toxicology of Pesticides
National Research Centre

Prof. Dr.

El-Sayed Ibrahim El-Saxed

Professor of Pesticides

National Research Centre

Department of Entomology Faculty of Science Ain Shams University

1997



BIOCIDAL ACTIVITY OF SELECTED PLANT EXTRACTS AGAINST CULEX PIPIENS (Linn.)

A Thesis
Presented to the Faculty of Science
Ain Shams University

For the Award of the Ph.D. Degree Entomology

By

Samia Shoukry Messeha

B.Sc., M.Sc. (Entomology)

Supervisors

Prof. Dr.
Mohamed Saad Hamed
Professor of Toxicology
Faculty of Science
Ain Shams University

Prof. Dr.
Moheb Sadek Ishak
Professor of Chemistry
National Research Centre

Prof. Dr.
Sameeh Abdel- Kader Mansour
Professor of Environmental
Toxicology of Pesticides
National Research Centre

Prof. Dr.
El-Sayed Ibrahim El-Sayed
Professor of Pesticides
National Research Centre

Department of Entomology Faculty of Science Ain Shams University

1997



SUPERVISORS

- Prof. Dr. Mohamed Sand Hamed, Professor of Toxicology, Faculty of Science, Ain Shams University.
- Prof. Dr. Sameeh Abdel- Kader Mansour, Professor of Environmental Toxicology of Pesticides, National Research Centre.
- Prof. Dr. Moheb Sadek Ishak, Professor of Chemistry, National Research Centre
- Prof. Dr. El-Sayed Ibrahim El-Sayed, Professor of Pesticides, National Research Centre.

ACKNOWLEDGEMENT

I am deeply thankful to GOD, by the grace of whom, the present work was realised

I wish to express my sincere gratitude to

Prof. Dr. Mohamed S. Hamed
Professor of Toxicology
Faculty of Science
Ain Shams University

Prof. Dr. Sameeh A. Mansour Professor of Environmental Toxicology of Pesticides National Research Centre

for suggesting the subject, supervising this work, for their continuous encouragement, kind help, indispensible advice and valuable comments they display during the course of this study and for reading and correcting the manuscript.

I am profoundly grateful to Prof. Dr. Moheb Sadek Ishak, Professor of Chemistry, Department of the Chemistry of Tanning Materials and Protein, National Research Centre, for his unlimited kind help and for reading and correcting the manuscript.

Deep gratitude to Prof. Dr. El-Sayed Ibrahim El-Sayed, Professor of Pesticides, National Research Centre for his invaluable advices and facilities offered during this work.

The author is indebted with sincerest gratitude to Prof. Dr. Souad E. EL-Gengaihi, Prof. Dr. Hemaya M. Motawe and Dr. Samy M. Mohamed, Pharmaceutical Sciences Department, National Research Centre, for their keen interest to follow the progress of the phytochemical studies.

I am gratefull to Prof. Dr. Mohamed Refaat Mahran, Professor of Chemistry, Department of Pesticides Chemistry, National Research Centre, for his advice, kind help and facilities offered during this study.

The author is indebted with sincerest gratitude to the Chairman of National Research Centre for the facilities provided for such an accomplishment.

Thanks are also due to the Head and Staff Members of Flora Department, National Research Centre, for their valuable assistance in identifying some plants used in this study.

Finally, the author wishes to thank all of her colleagues in the National Research Centre, Dokki, Egypt as well as the Entomology Department, Ain shams University, for their assistances.

ABSTRACT

Out of 40 different crude extracts (obtained by extraction with pet ether, chloroform, acetone and ethanol), a sum of 21 extracts showed high mortality percentages against the fourth instar Cx. pipiens larvae at 1000 ppm concentration level

The promising extracts were classified into four groups according to their LC₅₀ values as criteria of potency. Pet ether, chloroform and acetone extracts of *Piper nigrum* were the most potent extracts showing LC₅₀ <100 ppm. (group 1). Group 4 (LC₅₀ >300 ppm) includes seven plant extracts (e.g. *Matricaria chamomilla* chloroformic extract and *Melia azedarach* pet. ether extract)

In combining the different plant extracts with Malathion, potentiation was achieved in all cases Different degrees of joint action were resulted from combining with Permethrin or Pirimiphos - methyl.

Out of ten pet, ether extracts tested for their adulticidal potency, against the female mosquitoes, three of them (C. rotundus, T. capitatus and C. cyminum) showed high potency. Their LC₅₀ values were 0.05, 0.06 and 0.068 mg/cm², respectively.

Fourty plant extracts were evaluated as repellent or antifeeding agents. All pet ether extracts of the tested plants, except that of L termis, showed repellency of 50% or more. C cyminum extract caused 100% repellency.

Some of the studied plant extracts showed considerable decrease in egg hatching (e.g. Acetone extract of P. nigrum, chloroform extracts of N. sativa & P. nigrum, pet. ether extract of C. rotundus& M. azedarach and ethanol extract of P. nigrum).

Thymus capitatus and Nigella sativa were subjected to further phytochemical and biological studies. Thymol was the main constituent of T. capitatus volatile oil, while ρ -Cymene was the major constituent of N. sativa volatile oil. The volatile oil of T. capitatus was more effective than N. sativa volatile oil either against larvae or adults of the tested insect.

α-Amyrin, Thymol, Carvacrol and β- Caryophyllene were isolated

from T. capitatus, while 3 Sterols (Campesterol, α -Spinasterol, β -Sitosterol) and Hydrocarbons were isolated from N. sativa. All the above mentioned compounds were evaluated biologically against the larvae and adults of Culex pipiens.

In conclusion, the results obtained in the present study may encourage further research of practical nature for mosquito control.

Key words:

Plant extracts - insecticides - Culex pipiens - larvicidal action - adulticidal action.

LIST OF TABLES

l'able		Page
1	Plants investigated for biological activity against mosquito, Culex pipiens.	63
2	Percent yield and mortality of different plant extracts against 4th instar Culex pipiens larvae at 1000 ppm concentration.	79
3	Mortalities resulted from exposing 4th instar ('x. pipiens larvae to different concentrations of some plant extracts.	81
4	Mortalities resulted from exposing 4 th instar Cx. pipiens larvae to different concentrations of Cuminum cyminum extracts	83
5	Mortalities resulted from exposing 4th instar Cx. pipiens larvae to different concentrations of some plant extracts.	85
6	Mortalities resulted from exposing 4th instar Cx. pipiens larvae to different concentrations of Nigella sativa extracts.	87
7	Mortalities resulted from exposing 4th instar Cx. pipiens larvae to different concentrations of Piper nigrum extracts.	89
8	Mortalities resulted from exposing 4th instar Cx. pipiens larvae to different concentrations of Thymus capitatus extracts.	91
9	Mortalities resulted from exposing 4 th instar Cx. pipiens larvae to different concentrations of some insecticides.	93
10	LC ₂₅ , LC ₅₀ , LC ₉₅ and slope values for certain plant extracts and insecticides tested against 4 th instar Cx. pipiens larvae.	95
11	Joint action analysis for plant extracts with Malathion mixed at LC25 levels of each as tested against 4 th instar Cx. pipiens larvae.	101

Table		Page
12	Joint action analysis for plant extracts with Permethrin mixed at LC ₂₅ levels of each as tested against ^{4th} instar Cx. pipiens larvae.	102
13	Joint action analysis for plant extracts with Pirimiphos - methyl mixed at LC ₂₅ levels of each as tested against 4 th instar Cx. pipiens larvae.	104
14	Synergistic / antagonistic effects resulted from combining plant extracts with Malathion against Cx. pipiens larvae.	107
15	Synergistic / antagonistic effects resulted from combining plant extracts with Permethrin against Cx. pipiens larvae.	108
16	Synergistic / antagonistic effects resulted from combining plant extracts with Pirimiphos - methyl against Cx. pipiens larvae.	109
17	Adulticidal effects of petroleum ether extracts tested at 0.16 mg/cm ² , each (12 mg/filter paper strip of 75 cm ² area) against Cx. pipiens females.	112
18	Mortalities resulted from exposing Cx. pipiens female adults to different concentrations of petroleum ether extracts of certain plants.	113
19	Percent of unfeeded female adults of Cx. pipiens and % of repellency / antifeedant effect in tests conducted at 0.5 g. of each plant extract (e.g. 15.73 mg/cm ²).	116
20	Percent of unfeeded female adults of Cx. pipiens and % of repellency / antifeedant effect in tests conducted at certain concentrations of selected petroleum ether extracts.	120
21	Effect of different plant extracts on egg hatchability of Cx. pipiens females developed from the treated larvae with certain concentrations of the tested candidates.	121

l'able		Page
22	Gas chromatographic analysis of Thymus capitatus volatile oil	126
23	Mortalities resulted from exposing 4th instar Cx. pipiens larvae to different concentrations of Thymus capitatus isolates	137
24	Joint action analysis for T. capitatus isolates with Malathion mixed at LC ₂₅ levels of each as tested against 4 th instar Cx. pipiens larvae	140
25	Joint action analysis for T. capitatus isolates with Permethrin mixed at LC ₂₅ levels of each as tested against 4 th instar ('x. pipiens larvae	140
26	Joint action analysis for T . capitatus isolates with Pirimiphos - methyl mixed at LC_{25} levels of each as tested against 4^{th} instar Cx . pipiens larvae.	140
27	Synergistic / antagonistic effects resulted from combining <i>Thymus capitatus</i> isolates with Malathion against 4 th instar Cx. pipiens larvae.	141
28	Synergistic / antagonistic effects resulted from combining <i>Thymus capitatus</i> isolates with Permethrin against 4 th instar Cx. pipiens larvae.	141
29	Synergistic / antagonistic effects resulted from combining <i>Thymus capitatus</i> isolates with Pirimiphos-methyl against 4th instar Cx. pipiens larvae.	141
30	Adulticidal effects of <i>T. capitatus</i> isolates tested at 0.16 mg/cm ² , each (12 mg/filter paper strip of 75 cm ² area) against <i>Cx. pipiens</i> females.	144
31	Mortalities resulted from exposing Cx. pipiens female adults to different concentrations of T. capitatus isolates.	145

LIST OF FIGURES

Fig.		Page
1	Flow diagram of extraction and separation of active substances from <i>Thymus capitatus</i> .	70
2	Flow diagram of extraction and separation of active substances from. Nigella sativa.	71
3	Concentration - mortality lines for some plant extracts tested against 4 th instar Cx. pipiens larvae.	82
4	Concentration - mortality lines for some extracts of Cuminum cyminum tested against 4 th instar Cx. pipiens larvae.	84
5	Concentration - mortality lines for some plant extracts tested against 4 th instar Cx. pipiens larvae.	86
6	Concentration - mortality lines for some extracts of Nigella sativa tested against 4 th instar Cx. pipiens larvae.	88
7	Concentration - mortality lines for some extracts of <i>Piper nigrum</i> tested against 4th instar Cx. pipiens larvae.	90
8	Concentration - mortality lines for some extracts of <i>Thymus</i> capitatus tested against 4 th instar Cx. pipiens larvae.	92
9	Concentration - mortality lines for some insecticides tested against 4 th instar Cx. pipiens larvae.	94
10	Concentration - mortality lines for pet. ether extracts of certain plants against Cx. pipiens female adults.	114
11	Outlines larvicidal tests conducted on 4 th instar Cx. pipiens larvae using different isolates from Thymus capitatus.	123
12	Outlines larvicidal tests conducted on 4 th instar Cx. pipiens larvae using different isolates from Nigella sativa	124

Fig.		Page
13	GLC analysis of Thymus capitatus volatile oil	125
14	TLC of isolated compounds of unsaponifiable portion of <i>Thymus capitatus</i> .	128
15	Mass spectrum analysis of α- Amytin	129
16	Mass fragmentation of α- Amyrin	130
17	Mass spectrum analysis of Thymol	132
18	Mass spectrum analysis of Carvacrol.	133
19	Mass spectrum analysis of β- Caryophyllene	133
20	Mass fragmentation of β- Caryophyllene	135
21	Concentration - mortality lines for some fractions of <i>Thymus capitatus</i> tested against 4 th instar Cx. pipiens larvae.	138
22	Concentration - mortality lines for some fractions of T. capitatus tested against Cx. pipiens female adults	146
23	GLC analysis of N. sativa volatile oil.	154
24	TLC of isolated compounds of unsaponifiable portion of N. sativa.	156
25	Mass spectrum analysis of peak no. 1 of sterol (campesterol)	160
26	Mass fragmentation of Campesterol.	161
27 28	Mass spectrum analysis of peak no. 2 of sterol (α-Spinasterol)	162
	Mass fragmentation of (α-Spinasterol).	163
29	Mass spectrum analysis of peak no. 3 of sterol (β- sitosterol)	164

	Page
4.2.2. Separation of unsaponifiable constituents using	
preparative TLC.	64
4.2.3. Purification of selected bands.	65
4.2.4. Fatty acids.	66
4.2.5. Volatile oils	67
4.2.6. Fractionation of chloroform extract of Nigella sativa	
and Thymus capitatus	68
4.2.7. Fractionation of acetone extract of Nigella sativa.	68
4.2.8. Instrumental analyses	68
5. Test Procedures	72
5.1. Larvicidal Activity	72
5.1.1. Efficacy of Plant Extracts	72
5.1.2. Efficacy of the Tested Insecticides.	73
5.1.3. Interaction Between Plant Extracts and Insecticides	73
(A) Joint Action	73
(B) Synergistic / Antagonistic Action	74
5.2. Adulticidal Activity.	74
5.2.1. Toxicity Screening.	74
5.2.2. Repellency / Antifeedant Action.	75
5.2.3. Effect on Egg Hatchability (Sterility Action).	76
RESULTS & DISCUSSION	77
PART I. LARVICIDAL ACTIVITY OF PLANT EXTRACTS	78
1. Preliminary Toxicity Evaluation of Different plant Extracts Against	
Mosquito Larvae.	78
2. Toxicity Screening of Selected Plant Extracts Against Cx. pipiens	80
Larvae. 3. Interaction Between Plant Extracts and Certain Insecticides.	100
A. Joint Action.	100
B. Synergistic / Antagonistic Action.	105
PART II. ADULTICIDAL ACTIVITY OF PLANT	
EXTRACTS.	111
1. Adulticidal Potency of Plant Extracts.	111
2. Repellency / Antifeeding Effects of Plant Extracts.	115
3. Chemosterillant Effects of Plant Extracts.	118
PART III. PHYTOCHEMICAL AND BIOLOGICAL STUDIES	
ON CERTAIN PLANTS.	122
A. Thymus capitatus	122
1. Phytochemical Studies.	122

	Page
1 1 Volatile Oil	122
1.2 Unsaponifiable Portion	127
2 Biological Studies	136
2 I. Larvicidal Potency.	136
2.2 Interaction of T. capitatus Isolates and Insecticides	139
2.3. Toxicity of T. capitatus Isolates to Mosquito Adults.	142
2.4. Repellency / Antifeedant Effects of T. capitatus Isolates.	147
2.5. Effect of T. capitatus Isolates on Egg Hatchability	147
3 Comparison of Biological Activities of Crude Extracts and	
Isolated Constituents.	151
B. Nigella sativa	152
1 Phytochemical Studies	152
1 1 Volatile Oil	152
1.2. Unsaponifiable Portion	153
1.3. Saponifiable Portion	184
2. Biological Studies	187
2.1. Larvicidal Potency.	187
2.2. Interaction of N. sativa Isolates and Insecticides.	190
2.3. Toxicity of N. sativa Isolates to Mosquito Adults.	193
2.4. Effect of N. sativa Isolates on Egg Hatchability	195
3. Comparison of Biological Activities of Crude Extracts and	
Isolated Constituents	195
SUMMARY	200
LITERATURE CITED	206
ADADIC CUMMADV	