

Faculty of Science EntomologyDepartment

Biological and Biochemical Studies on *Culex pipiens* L. (Diptera:Culicidae) Exposed to *Allium sativum*, *Nigella sativa* Oil Extractsand *Bacillus sphaericus* (Neid)

A Thesis

Submitted to the Department of Entomology, Faculty of Science, Ain Shams University
In Partial Fulfillment for the award of M.Sc. Degree

Submitted by

Eslam Mohamed HosnyMostafa

Demonstrator, Entomology Department Faculty of Science, Ain Shams University B.Sc. Entomology (2010).

Supervisors

Magda Hassan Abdul Aziz Rady

Professor of Entomology (Molecular Biology), Entomology
Department,
Faculty of Science, Ain Shams University

Dalia Mohamad Mahmoud

Lecturer, Entomology Department, Faculty of Science, Ain Shams University

Mohamed Ali Mahmoud Abdou

Lecturer, Entomology Department, Faculty of Science, Ain Shams University

(2015)



Student Name: Eslam Mohamed HosnyMostafa

Thesis Title:Biological and Biochemical Studies on *Culex pipiens* L. (Diptera: Culicidae) Exposed to *Allium sativum,Nigella sativa* Oil Extractsand *Bacillus sphaericus*(Neid)

Degree: Master of Science (Entomology).

This thesis has been supervised by:

Prof. Magda Hassan Abdul Aziz Rady

Professorof Entomology (Molecular Biology), Entomology

Department, Faculty of Science, Ain Shams University

Dr. Dalia Mohamad Mahmoud

Lecturer, Entomology Department, Faculty of Science, Ain Shams University

Dr. Mohamed Ali Mahmoud Abdou

Lecturer, Entomology Department, Faculty of Science, Ain Shams University



Approval Sheet

Title of Thesis:

Biological and Biochemical Studies on *Culex pipiens* L. (Diptera: Culicidae) Exposed to *Allium sativum*, *Nigella sativa* Oil Extractsand *Bacillus sphaericus*(Neid)

Name of Student: Eslam Mohamed HosnyMostafa

Degree: Master of Science (Entomology).

This thesis has been approved by:

Prof.HussienSamer Abdel Rahman

Research Professor In National Research Center.

Prof.Naeem Mohamed Essa

Professor of Entomology, Cairo University.

Prof.Magda Hassan Abdul Aziz Rady

Professor of Entomology (Molecular Biology), Entomology Department, Faculty of Science, Ain Shams University.

Prof. Adel Kamal Elsaed

Head of Entomology Department Faculty of Science Ain Shams University

BIOGRAPHY

Name: Eslam Mohamed HosnyMoustafa.

Degree Award: B.Sc. (Entomology).

Department: Entomology.

Faculty: Science.

University: Ain Shams University.

Date of Graduation: June 2010.

Occupation: Demonstrator, Department of

Entomology, Faculty of science,

Ain-shams University.

Date of Appointment: April, 2011.

Date of Regestration: May, 2014.

Dedication

I am pleased to dedicate this work to my beloved parents, sister, wife, daughter and people who I missed them U. Yousr M. Abdel-Hady U. Khaled Moustafa Ammar

﴿ إِنَّ اللّهَ لَا يَسْتَخِيء أَن يَضْرِبُ مَثَلًا مَا يَعُوضَةُ فَمَا فَوْقَهَا فَا مَا الّذِينَ ء امنُوا فَيَعْلَمُونَ أَنَهُ الْحَقُ مِن وَقِهَا فَا مَا الّذِينَ عَلَمُولَ اللّهُ مَا الّذِينَ حَفَرُوا فَيَقُولُونَ مَا ذَا أَرَادَ اللّهُ وَيَهِمْ وَأَمَا الّذِينَ حَفَرُوا فَيَقُولُونَ مَا ذَا أَرَادَ اللّهُ وَيَهِمْ وَأَمَا الّذِينَ حَفَرُ اللّهُ عَلَيْ اللّهُ الللّهُ اللّهُ الللّهُ اللّهُ الللّهُ اللللّهُ اللّهُ اللّهُ اللّهُ اللّهُ اللللّهُ اللّهُ اللّهُ اللّهُ الللّهُ اللّهُ الللللّهُ الللّهُ الللّهُ الللّهُ اللللللّهُ اللّهُ اللللللّهُ اللللللّهُ الللللللّهُ اللللللللللللّهُ الللللللللللللللللللللللللللل

ددق الله العظيم

بسو الله الرحمن (سوره البقره الأية 26) الرحمن (عوره البقرة الأية 26)

AKNOWLEDGMENT

First of all, thanks to "ALLAH" to whom I relate any success in achieving any work in my life.

It is great pleasure to express my deep thanks and sincere gratitude to **Prof. Dr. Magda H. Rady**, Professor of Entomology, Faculty of Science, Ain Shams University for her continuous supervision, helpful suggestion, reading the manuscript, criticizing the results. Also, she was after every step by her enthusiastic guidance and her continuous encouragement which made this work possible.

My deep thanks and everlasting gratitude goes to **Dr. Dalia M. Mahmoud**, Doctor of Entomology, Faculty of science, Ain Shams University for her help, valuable advice, keen supervision, encouragement and all facilities offered during the investigation, the preparation of this manuscript, and completion of this study.

I am greatly thankful for **Dr. Mohamed Ali Abdou**, Doctor of Entomology, Faculty of science, Ain Shams University for his innovation, continuous supervision, helpful suggestions, constructive criticism, wise advises and his continuous encouragement.

ABSTRACT

Bacillus sphaericus has a larvicidal activity towards Culex pipiens larvae. Development of resistance in certain populations of Culex sp. is the most common disadvantage of B. sphaericus application. During application, pesticide, B. sphaericus formulations suffering from long exposure to sun light and UV So, the present study was which turned them impotent. designed to evaluate the larvicidal activity of Bacillus sphaericus toxin, Allium sativum and Nigella sativa oil extracts solitary and in combination against *Culex pipiens* 3rd larval instar. Data revealed that, Bacillus sphaericus was the most potent insecticide recording LC₅₀ value as (0.25ppm.) followed by Allium sativum and Nigella sativa recording (0.45 and 2.46) ppm.), respectively. Addition of *Allium sativum* generally increased the toxic effect of Bacillus sphaericus, where LC₅₀ decreased from (0.25 ppm to 0.12 ppm). Addition of Nigella sativa to Bacillus sphaericus decreased the toxicity of Bacillus sphaericus by increasing its LC₅₀ from (0.25 ppm to 0.39 ppm) .The biochemical studies using (SDS-PAGE), revealed that there was an increase in number of protein bands due to treatment with (Bacillus sphaericus combined with Allium sativumandBacillus sphaericus with Nigella These impressive results from combinations proved that when joint-action is considered, the application possibilities for vector control increase significantly.

Keywords: *Bacillus sphaericus*, Plant oil extracts, *Allium sativum*, *Nigella sativa*, *Culex pipiens*, Bioassay test, protein electrophoresis.

LIST OF ABBREVIATIONS

ITEM	Abbreviation
Bacillus sphaericus	B.sphaericus
Bacillus thuringiensis israelensis	B.t.i.
Culex	Cx.
Anopheles	An.
Aedes	Ae.
Allium sativum	A. sativum
Nigella sativa	N. sativa
Lethal Concentration	LC
Lethal Time	LT
Binary Toxin	Bin
World Health Organization	WHO
Part Per Million	ppm.
Kilo Dalton	KDa
Sodium Dodecyl Sulfate	SDS
Poly Acrylamide Gel Electrophoresis	PAGE
Commassie Brilliant Blue	CBB
N,N,N',N'Tetramethylethylenediamine	TEMED
Ploymerase Chain Reaction	PCR
High Performance Liquid Chromatography	HPLC
Thin Layer Chromatography	TLC
Culex pipiens membrane	Cpm
Anopheles gambiae membrane	Agm

Glycosyl-Phosphatidyl-Inositol	GPI
Genetically Engineered Organism	GEO
Brush Border Membrane Fraction	BBMF
Acetyl Choline Esterase	AChE
Hours	Hrs.
Resistance Ratio	RR
Internal transcribed spacer	ITS
Co-toxicity Factor	C.F
Potentiation	P
Additive	A

LIST OF TABLES

TABLE	PAGE

Table 1. Essential compounds of garlic essential oils	33
Table 2. Fatty acid composition of the fixed oil of <i>Nigella sativa</i> L.	34
Table 3. Chemical composition of the volatile constituents of <i>Nigella sativa</i> L.	34:35
Table 4: Percentage mortalities of mosquito larvae treated with <i>Bacillus sphaericus</i> toxin.	44
Table 5: Percentage mortalities of mosquito larvae treated with Allium sativum oil extracts.	46
Table 6: Percentage mortalities of mosquito larvae treated with Nigella sativa oil extract.	48
Table 7: Percentage mortalities of mosquito larvae treated with Allium sativum oil and Bacillus sphaericus toxin.	50
Table 8: Percentage mortalities of mosquito larvae treated with <i>Nigella sativa</i> oil and <i>Bacillus sphaericus</i> toxin.	52
Table 9: Lethal concentration values of <i>Allium</i> sativum, <i>Nigella sativa</i> oilsand Bacillus sphaericustoxinsolitary and in combinationused against Culex pipiens larvae.	54
Table 10: Joint action of <i>Bacillus sphaericus</i> mixtures against 3 rd instar <i>Culex pipiens</i> larvae.	55
Table 11: Toxicity of <i>Allium sativum</i> oil extract and <i>Bacillus sphaericus</i> (2362) toxin against 3 rd instar <i>Culex pipiens</i> larvae at different time intervals.	57
Table 12: Toxicity of <i>Allium sativum</i> oil extract against 3 rd instar <i>Culex pipiens</i> larvae at different time intervals.	59

Table 13: Toxicity of <i>Bacillus sphaericus</i> (2362) toxin against 3 rd instar <i>Culex pipiens</i> larvae at different time intervals.	61
Table 14:Toxicity of <i>Nigellasativa</i> oil extract against 3 rd instar <i>Culex pipiens</i> larvae at different time intervals.	63
Table 15: Effect of <i>Nigella sativa</i> oil extract and <i>Bacillus sphaericus</i> (2362) toxin against <i>Culex pipiens</i> third larval instar at different time interval.	65
Table 16: Values of LT ₅₀ of different treatments against mosquito larvae.	67
Table 17: Protein configuration of <i>Culex</i> pipiens larvae treated with <i>Bacillus</i> sphaericus toxin.	69
Table 18: Protein configuration of <i>Culex</i> pipiens larvae treated with <i>Allium</i> sativum oil.	71
Table 19: Protein configuration of <i>Culex</i> pipiens larvae treated with <i>Nigella</i> sativa oil.	73
Table 20: Protein configuration of <i>Culex</i> pipiens larvae treated with <i>Bacillus</i> sphaericus toxinand Allium sativum oil.	75
Table 21: Protein configuration of <i>Culex</i> pipiens larvae treated with <i>Bacillus</i> sphaericus toxin and <i>Nigella</i> sativaoil.	77

LIST OF FIGURES

Figures	Pages
Figure 1: Regression line of relation between <i>Bacillus sphaericus</i> application and percentage mortalities.	45
Figure 2: Regression line showing the relation between <i>Allium sativum</i> oil concentrations and percentage mortalities of <i>Culex pipiens</i> larvae.	47
Figure 3: Regression line showing the relation between <i>Nigella sativa</i> oil concentrations and percentage mortalities of <i>Culex pipiens</i> larvae.	49
Figure 4: Regression line showingthe relation between <i>Allium sativum</i> oil with <i>Bacillus sphaericus</i> concentrations and percentage mortalities of <i>Culex pipiens</i> larvae.	51
Figure 5:Regression line showing the relation between <i>Nigella sativa</i> oil with <i>Bacillus sphaericus</i> concentrations and percentage mortalities of <i>Culex pipiens</i> larvae.	53
Figure 6: Lethal concentration values of <i>Allium sativum</i> , <i>Nigella sativa</i> and <i>Bacillus sphaericus</i> solitary and in combination used against <i>Culex pipiens</i> larvae.	54
Figure 7: Mortality percentages of <i>Culex pipiens</i> larvae treated with <i>Bacillus sphaericus</i> at different time interval and different concentrations.	57
Figure 8: Mortality percentages of <i>Culex</i> pipiens larvae treated with	59

Allium sativum at different time intervals and different concentrations.	
Figure 9: Mortality percentages of mosquito larvae treated with <i>Nigella sativa</i> at different time intervals and different concentrations.	61
Figure 10: Mortality percentages of mosquito larvae treated with combination of <i>Bacillus sphaericus</i> and <i>Allium sativum</i> at different time interval and different concentrations.	63
Figure 11: Mortality percentages of mosquito larvae treated with combination of <i>Bacillus sphaericus</i> and <i>Nigella sativa</i> at different time interval and different concentrations.	65
Figure 12: Lethal time values of <i>Allium</i> sativum, <i>Nigella sativa</i> and <i>Bacillus</i> sphaericus solitary and in combination against <i>Culex pipiens</i> larvae used against <i>Culex pipiens</i> larvae.	67
Figure 13: SDS-PAGE of Protein patterns of <i>Culex pipiens</i> larvae treated with <i>Bacillus sphaericus</i> toxin.	68
Figure 14: Histogram illustrates different band frequancies in lanes (M,1,2)	68
Figure 15: SDS-PAGE of Protein patterns of <i>Culex pipiens</i> larvae treated with <i>Allium sativum</i> oil.	70
Figure 16: Histogram illustrates different band frequancies in lanes (M,1,2)	70
Figure 17: SDS-PAGE of Protein patterns of <i>Culex pipiens</i> larvae treated with <i>Nigella sativa</i> oil.	72
Figure 18: Histogram illustrates different band frequancies in lanes (M,1,2)	72

Figure 19: SDS-PAGE of Protein patterns of <i>Culex pipiens</i> larvae treated with <i>Bacillus sphaericus</i> toxin and <i>Allium sativum</i> oil.	74
Figure 20: Histogram illustrates different	74
band frequancies in lanes (M,1,2)	
Figure 21: SDS-PAGE of Protein patterns of <i>Culex pipiens</i> larvae treated with <i>Bacillus sphaericus</i> toxin and <i>Nigella sativa</i> oil.	76
Figure 22: Histogram illustrates different	76
band frequancies in lanes (M,1,2)	