



Cairo University  
Faculty of Science

**Biochemical and Molecular Biological Studies to  
Resistance Mechanism in Whitefly *Bemisia  
tabaci* (Gennadius)**

Presented by

**Raghda Mohsen Hassan Mohamed**  
**Faculty of Science**  
**Cairo University**

A Thesis Submitted to Faculty of Science

In Partial Fulfillment of the  
Requirements for  
Ph.D. degree in  
Biochemistry

Chemistry Department  
Faculty of Science  
Cairo University

**2021**

# Abstract

**Student Name: Raghda Mohsen Hassan Mohamed**

**Title of the thesis: Biochemical and molecular biological studies to resistance mechanism in whitefly *Bemisia tabaci* (Gennadius).**

**Degree: Ph.D. in Biochemistry**

Because of excessive application of different classes of insecticides for controlling whitefly *Bemisia tabaci* (Gennadius) [Hemiptera: Aleyrodidae], the development of resistance in laboratory strain of *B.tabaci* against organophosphours insecticide (primiphos- methyl) were studied for ten generations under laboratory conditions. The resistance ratio (RR) was 22.23 fold in G<sub>10</sub> compared with susceptible strain (SS). Also, the development of resistance in seven field populations were collected from Egypt governorates, during season 2018 against different classes of insecticides (pyrethroids, organophosphorus, carbamates and neonicotinoids) were studied. The activity levels of detoxifying enzymes [acetylcholinesterase (AChE), glutathione -S- transferase (GST) and Carboxylesterase (CarE)] were determined in resistance strain and field populations of different governorates, compared to the control. Results showed significant changes in levels of enzymes activity. Applying molecular biological technique to identification the resistance mechanism by studying the role of sodium channel (Sc) and acetylcholinesterase gene (*Ace* gene) in field populations and resistance strain, the sequences were aligned using Clustal W program, the results showed changes at the level of the nucleotide bases, it was significant, that led to a change in the sequence of the resulting amino acids.

**Keywords:** *Bemisia tabaci*, insecticides resistance, detoxifying enzymes, sodium channel and acetylcholinesterase gene.

**Supervisors:**

- 1- Prof. Dr. Amr Saad Mohamed**
- 2- Prof. Dr. Bahia Yehia Reyad**
- 3- Prof. Dr. Hala Mohamed Abou Yousef**
- 4- Prof. Dr. Karima Hamdy Eissa Haggag**

**Signature:**

**Prof. Dr. Khaled M. Ismail**  
**Chairman of Chemistry Department**  
**Faculty of Science – Cairo University**

---

---

# Contents

---

---

	Pages
<b>AIM OF THE WORK</b>	1
<b>INTRODUCTION</b>	2
<b>REVIEW OF LITERATURE</b>	4
<b>MATERIALS AND METHODS</b>	21
1- Cotton plants	21
2- The used insecticides	21
A- Pyrethroids insecticides	21
B- Organophosphates insecticides	22
C- Carbamates insecticides	23
D- Neonicotinoids insecticides	24
3- Insect strains	25
3.1- Susceptible strain (SS)	25
3.2- Resistance strain	26
3.3- Field populations	26
4- Bioassays	27
4.1- Leaf-dip technique	27
4.2- Data analysis	27
5- Biochemical studies	28
5.1- Total protein contents	28
5.2- Detoxifying enzymes activity	30
5.2.1- Acetylcholinesterase (AChE)	30
5.2.2- Carboxyl esterase activity (CarE)	31
5.2.3- Glutathione-S-transferase (GST)	33
6- Molecular biological studies	34
6.1- <i>B. tabaci</i> samples	34
6.2- Resistance mutation diagnostic assays	35

6.2.1-	DNA extraction buffer	35
6.2.2-	DNA solutions	35
6.3-	Methods	36
6.4-	PCR reactions and electrophoresis	37
6.4.1-	<i>B. tabaci</i> voltage-gated sodium channel para type (Sc) gene amplification	37
6.4.2-	<i>B. tabaci</i> Acetylcholinesterase ( <i>Ace</i> ) gene amplification	37
6.5-	Electrophoresis	38
6.6-	PCR product purification	38
<b>RESULTS</b>		39
I-	Bioassays Studies	39
1-	Development of resistance to primiphos –methyl	39
2-	Evaluation of tested insecticides against field populations	39
2.1.	Pyrethroids	41
2.2.	Organophosphates	41
2.3.	Carbamates	46
2.4.	Neonicotinoids	49
II-	Enzymatic activities	55
1-	Resistance strain	55
1.1.	Acetylcholinesterase (AChE)	55
1.2.	Glutathione-S-transferase (GST)	55
1.3.	Carboxylesterase (CarE)	58
2-	Field populations	58
2.1.	Acetylcholinesterase (AChE)	58
2.2.	Glutathione-S-transferase (GST)	58
2.3.	Carboxylesterase (CarE)	62
III-	Molecular biological studies	64
1-	Field populations	64
2-	Resistance strain	73

<b>DISCUSSION</b>	82
<b>SUMMARY AND CONCLUSION</b>	92
<b>REFERENCES</b>	98
<b>ARABIC SUMMARY</b>	127

---

---

## List of Tables

---

---

	Pages
Table (1) Development of resistance in generations of <i>B. tabaci</i> to primiphos-methyl, under laboratory conditions.	40
Table (2) Evaluation of toxicity of pyrethroids insecticides against Egyptian field populations of <i>Bemisia tabaci</i> during season 2018.	42
Table (3) Evaluation of toxicity of organophosphours against Egyptian field populations of <i>Bemisia tabaci</i> during season 2018.	44
Table (4) Evaluation of toxicity of carbamates against Egyptian field populations of <i>Bemisia tabaci</i> during season 2018.	47
Table (5) Evaluation of toxicity of neonicotinoids against Egyptian field populations of <i>Bemisia tabaci</i> during season 2018.	50
Table (6) LC <sub>50</sub> of tested insecticides recorded with Egyptian field populations of <i>B. tabaci</i> .	53
Table (7) Resistance ratio recorded with Egyptian field populations of <i>B. tabaci</i> compared to Gharbia population.	54
Table (8) Detoxifying enzymes activity <i>i.e.</i> acetylcholinesterase (AChE), glutathione-S-transferase (GST) and Carboxylesterase (CarE) in resistance strain of <i>B. tabaci</i> .	56
Table (9) Detoxifying enzymes activity <i>i.e.</i> acetylcholinesterase (AChE), glutathione-S-transferase (GST) and Carboxylesterase (CarE) in <i>B. tabaci</i> field populations.	60

---

---

## List of Figures

---

---

	Pages
Fig. (1) Toxicity line of primiphos- methyl against generations of <i>B.tabaci</i> adults, compared with parent strain (PS) and susceptible strain (SS) in laboratory conditions.	40
Fig. (2) Toxicity line of $\alpha$ -Cypermethrin against Egyptian field populations of <i>B. tabaci</i> adults, compared with Gharbia population during season 2018.	43
Fig. (3) Toxicity line of etofenprox against Egyptian field populations of <i>B. tabaci</i> adults, compared with Gharbia population during season 2018.	43
Fig. (4) Toxicity line of primiphos- methyl populations Egyptian field populations of <i>B. tabaci</i> adults, compared with Gharbia population during season 2018.	45
Fig. (5) Toxicity line of malathion against Egyptian field populations of <i>B. tabaci</i> adults, compared with Gharbia population during season 2018	45
Fig. (6) Toxicity line of carbosulfan against Egyptian field populations of <i>B. tabaci</i> adults, compared with Gharbia population during season 2018	48
Fig. (7) Toxicity line of methomyl against Egyptian field populations of <i>B. tabaci</i> adults, compared with Gharbia population during season 2018	48
Fig. (8) Toxicity line of acetamiprid against Egyptian field populations of <i>B.tabaci</i> adults, compared with Gharbia population during season 2018.	51
Fig. (9) Toxicity line of thiamethoxam against Egyptian field populations of <i>B.tabaci</i> adults, compared with Gharbia population during season 2018.	51
Fig. (10) Percentage change of acetylcholinesterase (AChE) enzyme activity in resistance strain of <i>B. tabaci</i> .	57
Fig. (11) Percentage change of glutathione-S-transferase (GST) enzyme activity in resistance strain of <i>B. t1qabaci</i> .	57
Fig. (12) Percentage change of Carboxylesterase (CarE) enzyme activity in resistance strain of <i>B. tabaci</i> .	59

Fig. (13)	Percentage change of acetylcholinesterase (AChE) enzyme activity in field populations of <i>B. tabaci</i> .	61
Fig. (14)	Percentage change of glutathione-S-transferase (GST) enzyme activity in field populations of <i>B. tabaci</i> .	61
Fig. (15)	Percentage change of Carboxylesterase (CarE) enzyme activity in field populations of <i>B. tabaci</i> .	63
Fig. (16)	Agarose gel electrophoreses of the sodium channel (Sc) mutation gene. PCR products were obtained using primer Sc-F and Sc-R. and the (Ace) mutation gene in <i>B. tabaci</i> by using primer <i>Ace</i> -F and <i>Ace</i> -R. M: 1kb DNA ladder lane1 Gharbia, lane2 Behira, lane3 Dakahlia, lane4, Monofia, lane5 Sharkia, lane6 Qalubia, lane7 Kafr El- Sheikh.	65
Fig. (17)	Nucleotide sequence multiple sequence alignment of stander Sc gene with mutant Sc gene to seven Governorates using Clustalw program.	66-67
Fig. (18)	Protein multiple sequence alignment of stander Sc gene with mutant Sc gene to seven Governorates using Clustalw program.	69
Fig. (19)	Nucleotide sequence multiple sequence alignment of stander <i>Ace</i> gene with mutant <i>Ace</i> gene to seven Governorates using Clustalw program in using Clustalw program.	70-72
Fig. (20)	Protein multiple sequence alignment of stander <i>Ace</i> gene with mutant <i>Ace</i> gene to seven Governorates using Clustalw program.	74-75
Fig. (21)	PCR detection of the <i>Ace</i> mutation gene in <i>B. tabaci</i> were obtained using primer <i>Ace</i> -F and <i>Ace</i> -R.	76
Fig. (22)	Nucleotide sequence multiple sequence alignment of susceptible strain (SS) with mutant <i>Ace</i> gene to resistant strain using Clustalw program.	77-79
Fig. (23)	Protein multiple sequence alignment of stander susceptible strain (SS) with mutant <i>Ace</i> gene to resistant strain using Clustalw program.	81