DEVELOPMENT OF RESISTANCE TO CERTAIN INSECTICIDES IN THE PINK BOLLWORM PECTINOPHORA GOSSYPIELLA (Saund.)

BY

HEMAT ZAKARIA MOHAMED MOUSTAFA
B.Sc.Agric.Sc. (Pesticides), Ain Shams University, 1999

A thesis submitted in partial fulfillment

of

the requirements for the degree of

MASTER OF SCIENCE

In
Agricultural Science
(Pesticides)

Department of Plant Protection
Faculty of Agriculture
Ain Shams University

2005
DEVELOPMENT OF RESISTANCE TO CERTAIN INSECTICIDES IN THE PINK BOLLWORM
PECTINOPHORA GOSSYPIELLA (SAUND.)

BY

HEMAT ZAKARIA MOHAMED MOUSTAFA
B.Sc. Agric. Sc. (Pesticides), Ain Shams University, 1999

This thesis for M. Sc. degree has been approved by:

Prof. Dr. Adel Abd El-Hameed El Feshawy.........................
   Prof. of Pesticides, Faculty of Agriculture, Zagazig University

Prof. Dr. Mohamed Ibrahim Abdel-Megeed.........................
   Prof. Emeritus of Pesticides, Faculty of Agriculture, Ain
   Shams University

Prof. Dr. Mohamed El-Said Saleh El-Zemaity......................
   Prof. of Pesticides, Faculty of Agriculture, Ain Shams
   University

Date of Examination: 27 / 4 / 2005
DEVELOPMENT OF RESISTANCE TO CERTAIN INSECTICIDES IN THE PINK BOLLWORM PECTINOPHORA GOSSYPIELLA (Saund.)

BY

HEMAT ZAKARIA MOHAMED MOUSTAFA
B. Sc. Agric. Sc. (Pesticides), Ain Shams University, 1999

Under the supervision of:

Prof. Dr. Mohamed El-Said Saleh El-Zemaity
Prof. of Pesticides, Department of Plant Protection,
Faculty of Agriculture, Ain Shams University

Dr. Alaa El-Din Bayoumi Abdel Khalek
Associate Prof. of Pesticides, Department of Plant Protection, Faculty of Agriculture, Ain Shams University

Prof. Dr. Mona Fikri Rofail
Senior Researcher, Plant Protection Research Institute, Agricultural Research Center
ABSTRACT


Two field strains of pink bollworm *Pectinophora gossypiella* (Saund) collected from Sharkia governorate were exposed to the selection pressure of pyrethroid deltamethrin and to *Bacillus thuringiensis* subsp. *Kurstaki* (Dipel 2x) in artificial diet by using adequate method to each compound under laboratory condition. After 14 generations of selection pressure resistance of deltamethrin increased to 215.11-fold compared to susceptible strain. In Dipel 2x resistant strain was obtained after 7 generations. Resistance ratio attained 16-fold based on the susceptible strain after 14 generations of selection. Study the response of deltamethrin and Dipel 2x resistant strains to some insecticides indicated that there is cross resistance to esfenvalerate was 23.75-fold in deltamethrin strain compared to the susceptible strain and no cross resistance occurred to thiodicarb, chlorpyrifos and the bioinsecticides Ecotech and Agerin. In Dipel 2x resistant strain there is no cross resistance to the conventional insecticides, esfenvalerate, chlorpyrifos, thiodicarb or the bioinsecticides Ecotech and Agerin. These data may be emphasize the possibility of rotation Dipel 2x with these insecticides in pest control program of pink bollworm to manage resistance to *B.t.* products. Detoxication enzymes assay revealed that activity of glutathion S-transferase were higher in all selected generations than susceptible strain. In phosphatases activity, acid phosphatase increased than susceptible in all generations in Dipel 2x strain, alkaline phosphatase decreased in all generations than the susceptible strain. Study of protein electrophoresis in gut of resistant and susceptible larvae revealed that there are new bands appear in the
resistant strains and disappear in susceptible strain. Cross sections of larvae midgut of deltamethrin and Dipel 2x resistant strains showed histological changes in epithelium cells than susceptible, development of resistance resulted in thickness of epithelium cells.

**Key words:** pink bollworm, resistance, selection, pyrethroid, *Bacillus thuringiensis*, cross resistance.
ACKNOWLEDGEMENT

First of all ultimate thanks are to Allah

I wish to express my deep apperception and sincere gratitude to Prof. Dr. Mohamed Said Saleh El-Zemaity, Professor of Pesticides, Department of Plant Protection, Faculty of Agriculture, Ain Shams University, for his supervision, reading the manuscript, helpful suggestion and constructive criticism.

My deepest gratitude is extended to Dr. Alaa El-Din Bayoumi Abdel Khalek, Associate Professor of Pesticides, Department of Plant Protection, Faculty of Agriculture, Ain Shams University, for his helpful suggestion and advices throughout this study.

Deep gratitude is also due to Prof. Dr. Mona Fikri Rofail Senior Researcher, Plant Protection Research Institute, Agricultural Research Center, for her supervision, useful guidance and follow up the experiment.

Deep thanks, also to Prof. Dr. Amira M. Rashad and Prof. Dr. Alia Abdel Hafez, Senior Researcher, at Bollworm Research Division, Plant Protection Research Institute, Agriculture Research Center for her valuable help in conducting statistically analyses of the data.

Deep appreciation is also extended to all staff members and colleagues of the Bollworm Research Division, Plant Protection Research Institute, Agriculture Research Center, for their help and cooperation throughout the period of this study.
## CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. INTRODUCTION</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>II. REVIEW OF LITERATURE</strong></td>
<td>3</td>
</tr>
<tr>
<td>1- Development of resistance</td>
<td>3</td>
</tr>
<tr>
<td>1.1- Development of resistance to pyrethroid insecticides</td>
<td>3</td>
</tr>
<tr>
<td>1.2- Development of resistance to bioinsecticide</td>
<td>8</td>
</tr>
<tr>
<td>2- Cross resistance spectra of the resistant strains to various insecticides</td>
<td>17</td>
</tr>
<tr>
<td>2.1- Cross resistance to pyrethroids</td>
<td>17</td>
</tr>
<tr>
<td>2.2- Cross resistance to B. thuringiensis</td>
<td>20</td>
</tr>
<tr>
<td>3- Mechanisms of insects resistance</td>
<td>24</td>
</tr>
<tr>
<td>3.1- Biochemical aspects</td>
<td>24</td>
</tr>
<tr>
<td>3.1.1- Glutathione S-transferase</td>
<td>24</td>
</tr>
<tr>
<td>3.1.2- Phosphatases</td>
<td>26</td>
</tr>
<tr>
<td>3.2- Electrophoretic pattern</td>
<td>27</td>
</tr>
<tr>
<td>3.3- Histological changes</td>
<td>29</td>
</tr>
<tr>
<td><strong>III. MATERIALS AND METHODS</strong></td>
<td>31</td>
</tr>
<tr>
<td>1- Insect rearing technique</td>
<td>31</td>
</tr>
<tr>
<td>2- Tested materials</td>
<td>31</td>
</tr>
<tr>
<td>2.1- Chemical insecticides</td>
<td>31</td>
</tr>
<tr>
<td>2.1.1- Deltamethrin</td>
<td>31</td>
</tr>
<tr>
<td>2.1.2- Esfenvalerate</td>
<td>32</td>
</tr>
<tr>
<td>2.1.3- Chlorpyrifos</td>
<td>32</td>
</tr>
<tr>
<td>2.1.4- Thiodicarb</td>
<td>33</td>
</tr>
<tr>
<td>2.2- Bioinsecticides (Bt products)</td>
<td>33</td>
</tr>
<tr>
<td>2.2.1- Dipel 2x</td>
<td>33</td>
</tr>
<tr>
<td>2.2.2- Ecotech</td>
<td>33</td>
</tr>
<tr>
<td>2.2.3- Agerin</td>
<td>33</td>
</tr>
<tr>
<td>3- Bioassay and selection pressure procedures for resistance</td>
<td>33</td>
</tr>
<tr>
<td>to the tested insecticides</td>
<td>33</td>
</tr>
</tbody>
</table>
3.1- Deltamethrin

3.2- Dipel 2x

4- Cross resistance of the resistant strains to the tested insecticides

5- Biochemical studies

5.1- Enzyme assay

5.1.1-Determination of glutathione S- transferase activity

5.1.2-Determination of phosphatases activity (Ac-pase & Alk-pase)

5.1.3-Determination of total protein

5.2- Protein electrophoresis

6- Histological studies

IV. RESULTS AND DISCUSSION

1- Development of resistance

1.1- Development of resistance of pink bollworm to deltamethrin

1.2- Development of resistance of pink bollworm to bioinsecticide Dipel 2x

2- Cross resistance to certain insecticides

2.1- Cross resistance in deltamethrin resistant strain of P. gossypiella to certain insecticides

2.2- Cross resistance in Dipel 2x resistant strain of P. gossypiella to certain insecticides

3- Biochemical studies on resistance of P. gossypiella

3.1- Glutathione S-transferase activity

3.1.1-The specific activity of GST in deltamethrin resistant strain

3.1.2- The specific activity of GST in Dipel 2x resistant strain

3.2- Phosphatases activity

3.2.1- Acid and alkaline phosphatases activity in deltamethrin resistant strain

3.2.2- Acid and alkaline phosphatases activity in
Dipel 2x resistant strain………………………………. 59
4-Electrophoretic pattern ........................................... 64
5- Histopathological changes........................................ 67
V. SUMMARY .................................................................. 73
VI. REFERENCES ............................................................ 76
VII. ARABIC SUMMARY ....................................................
INTRODUCTION

Pink bollworm *Pectinophora gossypiella* (Saund.) is primarily a mid and late season pest and one of the most serious insect pest attacking cotton crop in Egypt as well as the most cotton producing countries which cause a great damage in the quality and quantity of cotton yield.

In the early 1980’s, pyrethroid insecticides were rapidly substituted for organophosphorus and organochlorine insecticides for control of the pink bollworm due to their wide spectrum, low dosage, high killing efficiency, low residue and low toxicity to humans and animals. Unfortunately, resistance by the bollworm to such insecticides became more and serious because of indiscriminate applications. (Wang 1992).

*Bacillus thuringeinsis* as a biopesticide is a valuable source of insecticidal proteins for use in conventional sprayable formulations, and in transgenic crops and it is the most promising alternative to synthetic insecticides (Ferre and Van Rie 2002). The benefits of using *B. thuringeinsis* include reduced environmental and worker exposure to conventional insecticides, reduced selection for resistance to conventional insecticides and improved conservation of natural enemies. However Lepidopteran resistance to *B.t.* has been known since 1985 but only in a few taxonomic families. Nonetheless these insects were susceptible to other *Bt* toxins. Resistance to *B. thuringeinsis* has documented for several insect species (Tabashnik 1994). Pink bollworm and more than a dozen other pests have been selected in the laboratory for resistance to *B. thuringeinsis* toxin (Frutos et al. 1999).

Since the resistance of such pests are expected, the aim of the present work is to investigate:

1-The development of resistance of *P. gossypiella* to the pyrethroid deltamethrin as well as a formulation of *B. thuringeinsis* (Dipel 2x).
2-Cross-resistance to other insecticides in resistant strains.
3- Biochemical mechanism of resistance.
4- Histopathological changes of gut of resistant strains larvae.
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Fig. No.</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Toxicity lines of deltamethrin to the tested strains during selected generations of <em>P. gossypiella</em></td>
<td>41</td>
</tr>
<tr>
<td>2</td>
<td>Toxicity lines of Dipel 2x to the tested strains during selected generations of <em>P. gossypiella</em></td>
<td>44</td>
</tr>
<tr>
<td>3</td>
<td>Toxicity lines of esfenvalerate to the tested strains of <em>P. gossypiella</em></td>
<td>47</td>
</tr>
<tr>
<td>4</td>
<td>Toxicity lines of chlorpyrifos to the tested strains of <em>P. gossypiella</em></td>
<td>48</td>
</tr>
<tr>
<td>5</td>
<td>Toxicity lines of thiodicarb to the tested strains of <em>P. gossypiella</em></td>
<td>49</td>
</tr>
<tr>
<td>6</td>
<td>Toxicity lines of Ecotech to the tested strains of <em>P. gossypiella</em></td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>Toxicity lines of Agerin to the tested strains of <em>P. gossypiella</em></td>
<td>51</td>
</tr>
<tr>
<td>8</td>
<td>GST activity of full grown larvae of <em>P. gossypiella</em> in susceptible, parent and different generations of deltamethrin resistant strain</td>
<td>56</td>
</tr>
<tr>
<td>9</td>
<td>GST activity of full grown larvae of <em>P. gossypiella</em> in susceptible, parent and different generations of Dipel 2x resistant strain</td>
<td>56</td>
</tr>
<tr>
<td>10</td>
<td>Acid and alkaline phosphatases activity of full grown larvae of <em>P. gossypiella</em> in susceptible, parent and different generations of deltamethrin resistant strain</td>
<td>62</td>
</tr>
<tr>
<td>11</td>
<td>Acid and alkaline phosphatases activity of full grown larvae of <em>P. gossypiella</em> in susceptible, parent and different generations of Dipel 2x resistant strain</td>
<td>62</td>
</tr>
</tbody>
</table>
12-SDS-PAGE gel of gut protein from full grown larvae of susceptible strain, G14 of Dipel 2x and G14 of deltamethrin resistant strains of *P. gossypiella*........... 66

13-Midgut epithelium cross section of normal untreated 4th instar larvae of *P. gossypiella*........................................ 68

14-Midgut epithelium cross section of treated 4th instar larvae with Dipel 2x ...................................................... 69

15-Midgut epithelium cross section of treated 4th instar larvae with deltamethrin........................................ 69

16-Midgut epithelium cross section of resistant larvae fed on Dipel2x................................................................. 70

17-Midgut epithelium cross section of resistant larvae fed on deltamethrin.............................................................. 70