BIOCHEMICAL EFFECTS OF CERTAIN BIOPESTICIDES ON PINK BOLLWORM (*Pectinophora gossypiella*)

By

ALSHIMAA MOHAMED SALAH EL-DEEN HASSAN
B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2003

THESIS
Submitted in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

In

Agricultural Sciences
(Agricultural Biochemistry)

Department of Biochemistry
Faculty of Agriculture
Cairo University
EGYPT

2014

SUPERVISION SHEET

BIOCHEMICAL EFFECTS OF CERTAIN BIOPESTICIDES ON PINK BOLLWORM (*Pectinophora gossypiella*)

M. Sc. Thesis
In
Agric. Sci. (Agricultural Biochemistry)

By

ALSHIMAA MOHAMED SALAH EL-DEEN HASSAN

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2003

SUPERVISION COMMITTEE

Dr. MOHAMED MAGDY RASHED
Professor of Biochemistry, Fac. Agric., Cairo University

Dr. NADIA MOHAMED ABD EL-MOEIN
Professor of Biochemistry, Fac. Agric., Cairo University

Dr. EMAN MOHAMED MOSTAFA RADWAN
Head Research of Insect Population Toxicology Department
Central Agriculture Pesticides Laboratory, A. R. C.

APPROVAL SHEET

BIOCHEMICAL EFFECTS OF CERTAIN BIOPESTICIDES ON PINK BOLLWORM (*Pectinophora gossypiella*)

M. Sc. Thesis
In
Agric. Sci. (Agricultural Biochemistry)

By

ALSHIMAA MOHAMED SALAH EL-DEEN HASSAN
B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2003

APPROVAL COMMITTEE

Dr. KHALED MAMOUN TAHA.....

Professor of Biochemistry, Fac. Agric., Menoufia University

Dr. EMAM ABDEL-MOBDY ABDEL-REHEEM .....

Professor of Biochemistry, Fac. Agric., Cairo University

Dr. MOHAMED MAGDY RASHED.....

Professor of Biochemistry, Fac. Agric., Cairo University

Date: 23 /9/2014

Name of Candidate: Alshimaa Mohamed Salah El-Din Hassan **Degree**: M. Sc.

Title of Thesis: Biochemical Effects of Certain Biopesticides on Pink bollworm *(Pectinophora gossypiella*).

Supervisors: Dr. Mohamed Magdy Rashed
Dr. Nadia Mohamed Abd El-Moein
Dr. Eman Mohamed Mostafa Radwan

Department: Agricultural Biochemistry Approval: 23/9/ 2014

ABSTRACT

The Pink bollworm, *Pectinophora gossypiella* is the most destructive pest of cotton crop in Egypt.

Chemical insecticides play a major role in controlling the Pink bollworm but they caused environmental pollution, this pollution can be reduced by using the safer biopesticides.

This study aims to contribute to the toxicological, biological and biochemical effects of three biopesticides (Dipel-2x, Abamectin and Spinosad) and one recommended pyrethroid insecticide (Esfenvalerate) on Pink bollworm insect, the four tested pesticides had high toxic effect on neonate and 3rd larval instar, neonate larvae were more susceptible to the effect of four tested insecticides than third instar larvae, the toxic effects of four pesticides were correlated with time of treatment. The high insecticidal effect was presented in long treatment interval (48 hrs.). The results revealed the following findings:-

Male moths were more susceptible to the effect of tested pesticides than females.

Abamectin was more effective pesticide against neonate, 3rd instar larvae and adults followed by Spinosad, Esfenvalerate and Dipel-2x.

The four pesticides produced high deleterious effects on the development of Pink bollworm treated on neonate larvae.

The treatment on 3rd instar larvae of Pink bollworm with LC_{50} of tested pesticides caused significant increase (25-48.9%) in total protein content of larvae gut tissue after 6 hrs. of treatment, significant decrease (39-26.3%) in total protein of larvae gut was detected with Abamectin and Dipel-2x after 24 and 48 hrs. of treatment.

Significant elevation of enzymes activity was present in protease (especially in 48 hrs. treatment of Dipel-2x), glutathione-S-transferaes (especially in 48 hrs. after treatment of Abametin), acid and alkaline phosphatases (especially in 6 and 24 hrs. after treatment of Esfenvalerate), significant inhibition of ATPase activity resulted from the four tested pesticides at 6 and 24 hrs. after treatment. Insignificant increase or decrease in acetylcholinesterase enzyme activity was detected in the four tested pesticides.

The proteins of 3rd instar larvae gut tissue were fractionated on SDS PAGE to 30 bands, total number of bands appeared in control larvae were 23, 22 and 22 at 6, 24 and 48 hrs. intervals, the treatment of four pesticides increased the protein bands in gut tissue of larvae, Abamectin and Dipel-2x treatment caused the appearance of three bands(No. 27, 28 and 29 with M.W.of 9.98, 9.62 and 9.06 KDa), Dipel-2x treatment caused the appearance of specific band No. 30 with M.W. of 8.12 KDa). The highest polymorphism (44%) in gut proteins was presented in Dipel-2x treated larvae at 48 hrs. intervals compared with control larvae.

Nine protease bands were common in control and treated larvae gut.

The insecticidal treatment caused appearance of three specific bands.

The classification of esterase isozyme revealed that bands No. 2, 5 and 9 act as cholinesterases, bands No. 4, 6, 7 and 8 carboxylesterases and band No. l and 3 arylesterases.

Key words: Abamectin, Spinosad, Dipel-2x, Esfenvalerate, *Pectinophora gossypiella*, Toxicological studies, Biological studies, Biochemical studies, Total protein content, Protease, Adinosine-tri-phosphatase, Glutathion-S-Transferase, Phosphatase, Esterases.

DEDICATION

Firstly and ultimate my thanks and praise to be great Allah most gracious who shined me way and supported me with patience and perseverance to fulfill this work.

Then, I dedicate this work to my Mother, to my Father, to my brothers Sameh and Hazem for their understanding, confidence in me and supported me from the day one and suffered with me a lot of troubles also, I dedicate this work to Prof. Dr. Mohamed, Prof. Dr. Emam, Prof. Dr. Eman, Dr. Ragab, to my friends Dr. Azza, Amal, Ghada and Dr. Reham for all the support and sincere, cooperation throughout the whole period of my post graduation.

ACKNOWLEDGEMENT

I would like to express my gratitude to **Dr. Mohamed Magdy Rashed** *Professor of Biochemistry, Faculty of Agriculture, Cairo University for his supervision, encouragement, valuable advice, constructive suggestions, help during this study and the development and revising of the thesis.*

I'm grateful to **Dr. Nadia Mohamed Abd El-Moein** *professor of Biochemistry, faculty of Agriculture, Cairo University for her supervision and great encouragement through the period of study.*

No words can adequately express my deepest gratitude to **Dr. Eman Mohamed Mostafa Radwan,** *Head Research of Insect Population Toxicology Department, Central Agricultural Pesticides laboratory, Agricultural Research Center for suggesting the problem, offering all the needed materials and equipment, for her continuous encouragement, close and valuable super vision and for revising the manuscript.*

Thanks alot are also forwarded to Central Agricultural pesticides laboratory, especially my colleagues in the Insect Population Toxicology Department for sincere cooperation and valuable help throughout the whole period of this study.

LIST OF ABBREVIATIONS

Abbreviation	
3rd	Third instar larvae
Ab	Abamectin
AChE	Acetylcholinesterase
ANSA	(1-amino-2-naphthyl-4-sulphonic acid)
ATP	Adenosine-5-triphosphate
ATPase	Adenosine-5-triphosphatase
BT	*Bacillus thuringiensis*
C	Control
CbE	Carboxylesterase
CDNB	1-chloro-2,4-dinitrobenzene
COBB	Coomassie brilliant blue stain
CONC	Concentration
D	Dipel 2X
Dw	Distilled water
EC	Emulsifiable concentrate
ES	Esfenvalerate
GABA	γ- amino butyric acid
Hrs.	Hours
ICPS	Insecticide Crystal Proteins
L	Liter
LC_{50}	Lethal concentration for 50% of population
M	Marker
Min(s)	Minute (s)
PAGE	Polyacrylamide gel electrophoresis
PBW	Pink bollworm
RH	Relative humidity
Rm	Relative mobility
Rpm	Rotation per minute
SD	Standard deviation
SDS	Sodium dodecyl sulphate
SE	Standard error
Sec	Second (s)
SL	Suspension liquid
SP	Spinosad
TEMED	(N,N,N',N'-tetramethyelthylenediamine)
WP	Wettable powder

CONTENTS

	Page
INTRODUCTION	**1**
REVIEW OF LITERATURE	**6**
1. Toxicological and biological studies	**6**
a. Toxicity of insecticides against Lepidopterous	**6**
b. Effect and mode of action of Esfenvalerate	**11**
c. Effect and mode of action of *Bacillus thuringiensis*	**13**
d. Effect and mode of action of Abamectin	**20**
e. Effect and mode of action of spinosad	**24**
f. The delayed effects of insecticides on some biological aspects of insects	**28**
2. Biochemical studies	**33**
a. protein	**34**
b. protease	**39**
c. ATPase	**40**
d. GST	**42**
e. Phosphatases	**47**
f. Non specific esterase	**51**
MATERIALS AND METHODS	**60**
1. MATERIALS	**60**
a.Tested insect	**60**
b. Insecticides used	**61**
2. METHODS	**64**
a. Toxicological studies	**64**
b. Biological studies	**66**
c. Biochemical studies	**68**
d. Biochemical analysis	**68**
(**1)** Determination of the total protein content	**69**
(2) Determination of protease activity	**70**
(3) Determination of Adenosinetriphosphatase (ATPase) activity	**71**
(4) Determination of Glutathione-S-Transferase activity	**72**
(5)Determination of phosphatases enzymes (AC-pase & ALK-pase) activity	**74**
(6) Determination of Acetylcholinesterase activity	**76**
(7) Fractionation of gut proteins by electrophoresis	**78**

(8) Zymogram analysis of midgut protease **83**
(9) Electrophoretic separation of esterases **84**
(10) Esterase classification according to inhibition studies **87**
(11) Statistical analysis **88**
RESULTS AND DISCUSSION **89**
1.Toxicological studies **89**
a. larvicidal activity **89**
b. Adulticidal activity **93**
2. Biological studies **95**
3. Biochemical studies **105**
a. Effect on total protein content **105**
b. Effect on protease activity **109**
c. Effect on ATPase activity **114**
d. Effect on GST activity **118**
e. Effect on Phosphatases enzymes activity **121**
f. Effect on Acetylcholinesterase activity **128**
g. Fractionation of gut proteins patterns by electrophoresis **131**
h. Fractionation of protease patterns by electrophoresis .. **142**
i. Fractionation of esterase patterns by electrophoresis ... **149**
j. Classification of esterase isozyme **156**
CONCLUSION **164**
SUMMARY **165**
REFERENCES **171**
ARABIC SUMMARY .....

LIST OF TABLES

No.	Title	Page
1.	Classification of esterase bands	87
2.	Toxicological effect of the tested pesticides on neonate larvae of the Pink bollworm, *Pectinophora gossypiella* (Saunders)	91
3.	Toxicological effect of the tested pesticides on 3rd larval instar of *P. gossypiella* (Saund)	93
4.	Toxicological effect of the tested pesticides on adult female and male moths of the Pink bollworm, *Pectinophora gossypiella* (Saunders) by residual thin film and feeding methods	95
5.	Latent effects of LC_{50} of Abamectin and Spinosad on the biotic potential of the pink bollworm, *P. gossypiella* treated as neonate larvae	101
6.	Latent effects of LC_{50} of Dipel 2x and Esfenvalerate on the biotic potential of the pink bollworm, *P. gossypie*lla treated as neonate larvae	102
7.	Change percent in biological aspects of *P. gossypiella* treated as neonate larvae with LC_{50} of Abamectin and Spinosad	103
8.	Change percent in biological aspects of *P. gossypiella* treated as neonate larvae with LC_{50} of Dipel 2x and Esfenvalerate	104
9.	Effect of treatment with the tested pesticides at LC_{50} on total protein content of Pink bollworm 3rd instar larvae gut tissue at different time intervals	108
10.	Effect of treatment with the tested pesticides at LC_{50} on protease activity of Pink bollworm 3rd instar larvae gut tissue at different time intervals	113
11.	Effect of treatment with the tested pesticides at LC_{50} on ATPase activity of Pink bollworm 3rd instar larvae gut tissue at different time intervals	117

12. Effect of treatment with the tested pesticides at LC_{50} on glutathione -S-transferase activity of Pink bollworm 3rd instar larvae gut tissue at different time intervals 120

13. Effect of treatment with the tested pesticides at LC_{50} on acid phosphatase activity of Pink bollworm 3rd instar larvae gut tissue at different time intervals 123

14. Effect of treatment with the tested pesticides at LC50 on alkaline phosphatase activity of Pink bollworm 3rd instar larvae gut tissue at different time intervals 127

15. Effect of treatment with the tested pesticides at LC_{50} on acetylch- olinesterase activity of Pink bollworm 3rd instar larvae gut tissue at different time intervals 130

16. Relative mobility and concentration of SDS protein bands in gut tissue untreated and treated 3rd larval instar of *P. gossypiella* 137

17. The similarity index, commonality band ratio and percentage of difference in gut tissues fractionated protein between the untreated and treated 3rd instar larvae of *P. gossypiella* 141

18. Relative mobility and concentration of protease bands in gut tissue of untreated and treated 3rd larval instar of *P. gossypiella*... 145

19. The similarity index, commonality band ratio and percentage of difference in gut tissues protease patterns between the untreated and treated 3rd instar larvae of *P. gossypiella* 148

20. Relative mobility and concentration of esterase bands in whole body tissues of untreated and treated 3rd larval instar of P. *gossypiella* 152

21. The similarity index, commonality band ratio and percentage of difference in whole body tissues esterase patterns between the untreated and treated 3rd instar larvae of *P. gossypiella* 155

22. Response of non specific esterase bands to specific inhibitors in the whole body tissues of untreated and treated 3rd larval instar of *P. gossypiella* using α-naphthyl acetate as substrate 161

23. Type of esterase enzyme bands in the whole body tissue third instar larvae of *P. gossypiella* detected by α-naph acetate as a substrate 163

LIST OF FIGURES

No.	Title	Page
1.	Mechanism of Cry protein toxicity. A: Ingestion of spores or recombinant protein by phytophagous larva. B: In the midgut, endotoxins are solubilized from *Bt* spores (s) and inclusions of crystallized protein. (Cp) . C: Cry toxins are proteolytically processed to active toxins in the midgut. Active toxin binds receptors on the surface of columnar epithelial cells. Bound toxin inserts into the cellular membrane. D: Cry toxins aggregate to form pores in the membrane. E: Pore formation leads to osmotic lysis. F: Heavy damage to midgut membranes leads to starvation or septicemia.....	19
2.	Effect of treatment with the tested pesticides at LC_{50} on total protein content of Pink bollworm 3rd instar larvae gut tissue at different time intervals.....	109
3.	Effect of treatment with the tested pesticides at LC_{50} on protease activity of Pink bollworm 3rd instar larvae gut tissue at different time intervals.....	114
4.	Effect of treatment with the tested pesticides at LC_{50} on ATPas activity of Pink bollworm 3rd instar larvae gut tissue at different time intervals.	118
5.	Effect of treatment with the tested pesticides at LC_{50} on glutathione-S-transferase activity of Pink bollworm 3rd instar larvae gut tissue at different time intervals.....	121
6.	Effect of treatment with the tested pesticides at LC_{50} on acid phosphatase activity of Pink bollworm 3rd instar larvae gut tissue at different time intervals..	124
7.	Effect of treatment with the tested pesticides at LC_{50} on alkaline phosphatase activity of Pink bollworm 3rd instar larvae gut tissue at different time intervals	128
8.	Effect of treatment with the tested pesticides at LC_{50} on acetylcholinesterase activity of Pink bollworm 3rd instar.	