



Ain Shams University
Faculty of Science
Department of Entomology

**TOXICOLOGICAL AND BIOCHEMICAL CHANGES
INDUCED BY SOME WASTE PRODUCTS AS
INSECTICIDAL AGENTS AGAINST THE COTTON
LEAF WORM,
SPUDOPTERA LITTORALIS (BIOSD)**

THESIS
SUBMITTED TO THE FACULTY OF SCIENCE- AIN
SHAMS UNIVERSITY FOR AWARD OF PH.D. DEGREE
(IN ENTOMOLOGY)

BY

HEBA MAHMOUD SAID ELBANNA

B.Sc & M.Sc. (in Entomology)

SUPERVISED BY

Dr. Reda Fadeel Ali Bakr *Bakr*
Professor of Entomology
Faculty of Science
Ain Shams University

Dr. Galal Abd El-mouen Nouar
Professor of organic chemistry
National Research Center

Galal Nouar

Dr. Heba Abd-Elwahab Hassan
Assistant professor of Entomology
Plant Protection Research Institute

Heba

Dr. Marah Mohammad Abd El-Bar
Lecturer of Entomology
Faculty of Science
Ain Shams University

M.M.A

(٢٠١٣)

ACKNOWLEDGMENTS

First of all, ultimate thanks to **ALLAH** for the support in every step and for enabling me to overcome all the problems that faced me throughout the work.

I owe more than what can be expressed to **Dr. Reda Fadeel Ali Bakr**, Professor of Entomology, Faculty of Science, Ain Shams University, for suggesting the plan of this work and for his unlimited help; he generously did his best to help me, solving the problem by providing the valuable facilities, throughout the period of this study and for reading and revising the manuscript.

I would like to express my deep gratitude to **Dr. Galal Abd El-mouen Nouar**, Professor of organic chemistry, Head of Industrial Chemistry Division, National Research Center, for the fruitful supervision, useful advice, for reading and correcting the manuscript and great help offered during this study.

I wish to express my appreciation to **Dr. Heba Abd-Elwahab Hassan**, Assistant professor of Entomology, Plant Protection Research Institute, for her useful suggestions, for reading and correcting the manuscript and for the great help offered throughout the investigation period.

I wish also to express my sincere appreciation to **Dr. Marah Mohammad Abd El-Bar**, Lecturer of Entomology, Faculty of Science, Ain Shams University, for reading and correcting the manuscript.

I would like, as well, to thank all staff members in the Department of Entomology, Faculty of Science, Ain Shams University and special thanks to **Dr. Nadia Abu Gabal**, professor of Entomology and Head of Entomology Department.

Many great appreciations are due to **Mr. Mohsen Ameen** for his great effort, encouragement and authentic help throughout this study.

Finally, I am grateful to all members who have helped me or have contributed with me in one way or another throughout the period of this work.

Dedication

There are always great people in our
life who give love, support, success and
progress, I dedicate this work,

To the spirit of my father

To the greatest woman in my life,
my mother

To my Husband

To my children, Ahmed and Sarah

To my lovely sisters and my brother

ABSTRACT

This study was conducted under laboratory conditions to evaluate the insecticidal activity of three newly compounds extracted from wastes from natural origin, Cyano acetyl urea (CAU), Benzimidazolyl acetyl urea (BAU) from urea and Cyano acetyl hydrolyzate (CAH) from rice straw, on 2nd and 4th instar larvae of *Spodoptera littoralis*. For example, LC₅₀ ranged from 0.0001 to 0.0002%, from 0.0001 to 0.0002% and ranged from 0.0001 to 0.0002% for 4th instar larvae treated with CAU, BAU and CAH, respectively. Hence, the present data declared that (CAH) was the most efficient extract followed by (CAU), and then (BAU). Results demonstrated that the percentage of larval mortality was increased with increasing the concentrations of all tested compounds. It also showed that the three tested compounds caused reduction in percentage of pupation and in pupal weight and an increase in pupal duration compared with control. Treatment with these compounds also affected the adult emergence, fecundity and fertility of both sexes. Morphogenic abnormalities were also recorded and many aberrations have been induced in larvae, pupae and adults. Changes in the total haemocytes and differential haemocytes counts were observed as a result of CAU, BAU, and CAH treatments of 4th instar larvae.

Biochemical studies showed distinguishable patterns between treated samples and the control by the appearance and disappearance of certain protein fractions and differences in the activities of tested enzymes.

The other objective of this study was to evaluate the potential of the random amplified polymorphic DNA (RAPD) assay for the detection of genetic polymorphism between control and treated *S. littoralis* larvae, which have been exposed to the tested compounds at both LC₅₀ and LC₉₀. Five primers namely: OP-01, OP-02, OP-03, OP-04, and OP-05 were used in this study. These primers generated a maximum of 26, 24, 20, 13 and 23 bands, respectively. RAPD profiles generated by these primers revealed differences between control and treated samples with visible changes in number and size of amplified DNA fragments. Polymorphism ranged from 44.4 to 100% as screened by the five primers among all samples. Based on LC₅₀, the highest polymorphism (86.4%) was observed in those treated with CAH comparing those either treated with CAU (77.8%) or with BAU (84.4%). Definitely, RAPD data confirmed the susceptibility test as well as the morphological study, and suggest that DNA damage and the possible occurred mutations may be appeared as the main factor influencing the evident polymorphism between control and treated larvae.

Key words: *Spodoptera littoralis*- urea derivatives - rice straw- insecticidal activity – haemocytes- isozymes- protein electrophoresis- RAPD (PCR).

CONTENTS

	Page
Acknowledgment	
Abstract	
I- Introduction	۱
II- Literature review	
۱- Toxicological and Biological studies:	۷
۱.۱. Insecticidal and biological effects of urea- derivative on insects with special reference to <i>Spodoptera littoralis</i>	۷
۱.۲. Insecticidal and biological effects of agri- cultural wast products on insects with special reference to <i>Spodoptera littoralis</i>	۳۷
۲- Haematological studies:	۴۲
۲,۱. Effect of agricultural waste products and urea- derivatives on haematocyte of insects with special reference to <i>Spodoptera</i> <i>littoralis</i>	
۳- Biochemical studies:	۴۵
۳,۱ Effect of agricultural waste products and urea-derivatives on protein of insects with special reference to <i>Spodoptera littoralis</i>	
۳,۲ Effect of agricultural waste products and urea-derivatives on enzymes of insects with special reference to <i>Spodoptera littoralis</i>	۵۰
۴- Molecular studies:	۵۴
۴,۱ RAPD PCR technique for detecting dam- age occurred in DNA of different organisms by different physical or chemical agents.	
III- Materials and Methods.	
۱- Toxicological and biological studies	۶۷
۱,۱ Laboratory maintenance of <i>Spodoptera littoralis</i> .	۶۷

	Page
1,2 Experimental compounds.	68
1,3. Insecticidal and biological effects.	68
1,4. Calculations and data analysis.	69
2- Heamatological studies	
2,1 Collection of haemolymph	70
2,2 Total haemocytes counts	70
2,3 Different haemocytes count	70
3- Biochemical studies	
3,1 Sample preparation	71
3,2 Polyacrelamide gel electrophoresis	71
3,2,1 Non denaturing polyacrelamide gel elec- trophoresis	72
3,2,1,1 Native protein	76
3,2,1,2 Glycoprotein	77
3,2,1,3 Lipo-protein	78
3,2,2 Fraction protein	78
3,2,3 Isozyme analysis	81
3,2,3,1 α -esterase	83
3,2,3,2 β -esterase	83
3,2,3,3 Malic enzyme	83
3,2,3,4 Malate dehydrogenase	83
3,2,3,5 Alcoholic dehydrogenase	84
3,2,3,6 Aldehyde oxidase	84
3,2,3,7 Peroxidase	84
3,2,3,8 Acid phosphatase	84
4- Molecular studies	85
4,1 DNA extraction	86
4,2 RAPD analysis	86
IV- Experimental results	
1 Toxicological and biological studies	89
1,1 Insecticidal activity:	89

	Page
१,२ Biological studies	१२
१,२.१ Biological activities of cyano acetyl urea against २ nd instar larvae of <i>S. littoralis</i> .	१३
१,२,२ Biological activities of cyano acetyl urea against ६ th instar larvae of <i>S. littoralis</i> .	१६
१,२,३. Biological activities of benzimidazolyl acetyl urea against २ nd instar larvae of <i>S. littoralis</i> .	११
१,२,६. Biological activities of benzimidazolyl acetyl urea against ६ th instar larvae of <i>S. littoralis</i>	११
१२,०. Biological activities of cyano acetyl hydrolyzate against २ nd instar larvae of <i>S. littoralis</i> .	१००
१,२,६. Biological activities of cyano acetyl hydrolyzate against ६ th instar larvae of <i>S. littoralis</i> .	१०८
१,३ Morphogenic abnormalities	१११
२. Haematological studies	११६
२,१ Changes in total haemocytes counts	११६
२,२ Changes in differential haemocytes counts	११०
३ Biochemical studies	११६
३,१ Separation of protein bands by electrophoresis	११६
३,१,१ Native protein pattern.	११६
३,१,२ Glycoprotein:	१२२
३,१,३ Lipoprotein:	१२७
३,१,६ Fraction protein pattern (SDS-PAGE)	१३२
३,१,० ISOZYME ANALYSIS	१३७
३,१,०,१ Esterase detected by α -naphthyl acetate :	१३७
३,१,०,२ Esterase detected by β -naphthyl acetate :	१६३
३,१,०,३ Malic enzyme (<i>Mal</i>):	१६८

	Page
٣,١,٥,٤ Malate dehydrogenase (<i>Mdh</i>)	١٥٣
٣,١,٥,٥ Alcohol dehydrogenase (<i>Adh</i>)	١٥٩
٣,١,٥,٦ Aldehyde oxidase (<i>Alo</i>)	١٦٤
٣,١,٥,٧ Peroxidase isozyme (<i>Px</i>):	١٦٩
٣,١,٥,٨ Acid phosphatase (<i>Acph</i>):	١٧٤
٤. Molecular studies	١٧٩
٤,١ RAPD ANALYSIS	١٧٩
٤,١,١ Primer OP-٠١	١٧٩
٤,١,٢ primer OP-٠٢.	١٨٤
٤,١,٣ primer OP-٠٣	١٨٩
٤,١,٤ Primer OP-٠٤	١٩٤
٤,١,٥ Primer OP-٠٥	١٩٩
V- Discussion	٢٠٧
VI-Summary	٢٣١
VII-Reference	٢٤٠
Arabic Summary	

LIST OF TABLES

No.	Contents	Page
١	The code and sequences of five RAPD primers.	٨٧
٢	Insecticidal activity of ٢ nd larval instars of <i>Spodoptera littoralis</i> toward tested compounds.	٨٩
٣	Insecticidal activity of ٤ th larval instars of <i>Spodoptera littoralis</i> toward tested compounds.	٩١
٤	Biological influences of CAU on ٢ nd instar larvae of <i>S. littoralis</i> .	٩٤
٥	Biological influences of CAU on ٤ th instar larvae of <i>S. littoralis</i> .	٩٧
٦	Biological influences of BAU on ٢ nd instar larvae of <i>S. littoralis</i> .	١٠١
٧	Biological influences of BAU on ٤ th instar larvae of <i>S. littoralis</i> .	١٠٣
٨	Biological influences of CAH on ٢ nd instar larvae of <i>S. littoralis</i> .	١٠٦
٩	Biological influences of CAH on ٤ th instar larvae of <i>S. littoralis</i> .	١٠٩
١٠	Changes in total haemocytes counts of the ٦ th larval instar of <i>S. littoralis</i> after treatment of ٤ th larval instar with sub-lethal concentrations of CAU, BAU and CAH.	١١٤
١١	Mean percentage of differential haemocytes of the ٦ th larval instar of <i>S. littoralis</i> after treatment of ٤ th larval instar with sub-lethal concentrations of CAU, BAU and CAH.	١١٦
١٢	Relative fragmentation (Rf) and % amount of larval tissues native protein pattern for CAU, BAU, CAH and control samples of ٤ th larval instar of <i>S. littoralis</i> .	١١٩
١٣	Similarity index and genetic distance between treated and untreated samples in native protein larval tissue of <i>S. littoralis</i> .	١٢٢
١٤	Relative fragmentation (Rf) and % amount of larval tissues Glycoprotein pattern for CAU, BAU, CAH and control samples of ٤ th larval instar of <i>S. littoralis</i> .	١٢٤
١٥	Similarity index and genetic distance between treated and untreated samples in glycoprotein larval tissue of <i>S. littoralis</i> .	١٢٧

No.	Contents	Page
۱۶	Relative fragmentation (Rf) and % amount of larval tissues lipoprotein pattern for CAU, BAU, CAH and control samples of ξ^{th} larval instar of <i>S. littoralis</i> .	۱۲۹
۱۷	Similarity index and genetic distance between treated and untreated samples lipoprotein larval tissue of <i>S. littoralis</i>	۱۳۲
۱۸	Molecular weight (M wt.) and % amount of larval tissues refractionation protein pattern for CAU, BAU, CAH and control samples of ξ^{th} larval instar of <i>S. littoralis</i> .	۱۳۴
۱۹	Similarity index and genetic distance between treated and untreated samples in protein larval tissue of <i>S. littoralis</i>	۱۳۷
۲۰	Relative fragmentation (Rf) and % amount of larval tissues α -esterase pattern for CAU, BAU, CAH and control samples of ξ^{th} larval instar of <i>S. littoralis</i> .	۱۴۰
۲۱	Similarity index and genetic distance between treated and untreated samples α -esterase larval tissue of <i>S. littoralis</i>	۱۴۳
۲۲	Relative fragmentation (Rf) and % amount of larval tissues β -esterase pattern for CAU, BAU, CAH and control samples of ξ^{th} larval instar of <i>S. littoralis</i> .	۱۴۵
۲۳	Similarity index and genetic distance between treated and untreated samples β -esterase larval tissue of <i>S. littoralis</i>	۱۴۸
۲۴	Relative fragmentation (Rf) and % amount of larval tissues malic enzyme pattern for CAU, BAU, CAH and control samples of ξ^{th} larval instar of <i>S. littoralis</i>	۱۵۰
۲۵	Similarity index and genetic distance between treated and untreated samples malic enzyme larval tissue of <i>S. littoralis</i> .	۱۵۳
۲۶	Relative fragmentation (Rf) and % amount of larval tissues malate dehydrogenase pattern for CAU, BAU, CAH and control samples of ξ^{th} larval instar of <i>S. littoralis</i> .	۱۵۶
۲۷	Similarity index and genetic distance between treated and untreated samples malate dehydrogenase larval tissue of <i>S. littoralis</i>	۱۵۹
۲۸	Relative fragmentation (Rf) and % amount of larval tissues alcohol dehydrogenase pattern for CAU, BAU, CAH and control samples of ξ^{th} larval instar of <i>S. littoralis</i> .	۱۶۱

No.	Contents	Page
୨୭	Similarity index and genetic distance between treated and untreated samples alcohol dehydrogenase larval tissue of <i>S. littoralis</i>	୧୬୧
୩୦	Relative fragmentation (Rf) and % amount of larval tissues aldehyde oxidase pattern for CAU, BAU, CAH and control samples of ୫ th larval instar of <i>S. littoralis</i> .	୧୬୬
୩୧	Similarity index and genetic distance between treated and untreated samples aldehyde oxidase larval tissue of <i>S. littoralis</i> .	୧୬୭
୩୨	Relative fragmentation (Rf) and % amount of larval tissues peroxidase pattern for CAU, BAU, CAH and control samples of ୫ th larval instar of <i>S. littoralis</i> .	୧୭୧
୩୩	Similarity index and genetic distance between treated and untreated samples peroxidase larval tissue of <i>S. littoralis</i>	୧୭୧
୩୪	Relative fragmentation (Rf) and % amount of larval tissues acid phosphatase pattern for CAU, BAU, CAH and control samples of ୫ th larval instar of <i>S. littoralis</i> .	୧୭୬
୩୫	Similarity index and genetic distance between treated and untreated samples acid phosphatase larval tissue of <i>S. littoralis</i>	୧୭୭
୩୬	RAPD-PCR profile for <i>S. littoralis</i> ୩ th instar larvae treated by newly-extracted compounds from waste and amplified by primer OP-୦୧.	୧୮୧
୩୭	Similarity index and genetic distance between treated and untreated samples of ୩ th larval tissue of <i>S. littoralis</i> using primer OP-୦୧.	୧୮୧
୩୮	RAPD-PCR profile for <i>S. littoralis</i> ୩ th instar larvae treated by newly-extracted compounds from waste and amplified by primer OP-୦୨.	୧୮୬
୩୯	Similarity index and genetic distance between treated and untreated samples of ୩ th larval tissue of <i>S. littoralis</i> using primer OP-୦୨	୧୮୭
୪୦	RAPD-PCR profile for <i>S. littoralis</i> ୩ th instar larvae treated by newly-extracted compounds from waste and amplified by primer OP-୦୩.	୧୯୦
୪୧	Similarity index and genetic distance between treated and untreated samples of ୩ th larval tissue of <i>S. littoralis</i> using primer OP-୦୩	୧୯୧

No.	Contents	Page
xi	RAPD-PCR profile for <i>S.littoralis</i> 7 th instar larvae treated by newly-extracted compounds from waste and amplified by primer OP-13.	196
xii	Similarity index and genetic distance between treated and untreated samples of 7 th larval tissue of <i>S. littoralis</i> using primer OP-13.	199
xiii	RAPD-PCR profile for <i>S.littoralis</i> 7 th instar larvae treated by newly-extracted compounds from waste and amplified by primer OP-14.	201
xiv	Similarity index and genetic distance between treated and untreated samples of 7 th larval tissue of <i>S. littoralis</i> using primer OP-14.	204
xv	Genotype polymorphism (% polymorphic)* among <i>S. littoralis</i> 7 th instar larvae treated with newly-extracted compounds from waste and assayed by RAPD-PCR.	205

LIST OF FIGURES

No.	Contents	Page
١	Insecticidal activity of ٢ nd larval instars of <i>S. littoralis</i> toward CAU	٩٠
٢	Insecticidal activity of ٢ nd larval instars of <i>S. littoralis</i> toward BAU	٩٠
٣	Insecticidal activity of ٢ nd larval instars of <i>S. littoralis</i> toward CAH	٩٠
٤	Insecticidal activity of ٤ th larval instars of <i>S. littoralis</i> toward CAU	٩١
٥	Insecticidal activity of ٤ th larval instars of <i>S. littoralis</i> toward BAU	٩٢
٦	Insecticidal activity of ٤ th larval instars of <i>S. littoralis</i> toward CAH	٩٢
٧	Effect of Cyano acetyl urea on some biological influences of <i>S. littoralis</i> treated as ٢ nd instar larvae	٩٥
٨	Effect of Cyano acetyl urea on some biological influences of <i>S. littoralis</i> treated as ٤ th instar larvae	٩٨
٩	Effect of Benzimidazolyl acetyl urea on some biological influences of <i>S. littoralis</i> treated as ٢ nd instar larvae	١٠٢
١٠	Effect of Benzimidazolyl acetyl urea on some biological influences of <i>S. littoralis</i> treated as ٤ th instar larvae	١٠٤
١١	Effect of Cyano acetyl hydrolyzate on some biological influences of <i>S. littoralis</i> treated as ٢ nd instar larvae	١٠٧
١٢	Effect of Cyano acetyl hydrolyzate on some biological influences of <i>S. littoralis</i> treated as ٤ th instar larvae	١١٠
١٣	Normal morphology of <i>S. littoralis</i> larva, pupa and adult.	١١٢
١٤	Morphological malformation of <i>S. littoralis</i> induced by tested compounds.	١١٣
١٥	Electrophoretic native protein pattern of larval tissues of <i>S. littoralis</i> as control and treated samples with tested compounds.	١١٨
١٦	Graph illustration of native protein pattern of larval tissues of <i>S. littoralis</i> as control and treated samples with tested compounds.	١٢٠

No.	Contents	Page
۱۷	Electrophoretic glycoprotein pattern of larval tissues of <i>S. littoralis</i> as control and treated samples with tested compounds.	۱۲۳
۱۸	Graph illustration of glyoprotein pattern of larval tissues of <i>S. littoralis</i> as control and treated samples with tested compounds.	۱۲۵
۱۹	Electrophoretic lipoprotein pattern of larval tissues of <i>S. littoralis</i> as control and treated samples with tested compounds.	۱۲۸
۲۰	Graph illustration of lipoprotein pattern of larval tissues of <i>S. littoralis</i> as control and treated samples with tested compounds.	۱۳۰
۲۱	Electrophoretic SDS protein pattern of larval tissues of <i>S. littoralis</i> as control and treated samples with tested compounds.	۱۳۳
۲۲	Graph illustration of SDS protein pattern of larval tissues of <i>S. littoralis</i> as control and treated samples with tested compounds.	۱۳۵
۲۳	Electrophoretic α - esterase pattern of larval tissues of <i>S. littoralis</i> as control and treated samples with tested compounds.	۱۳۹
۲۴	Graph illustration of α - esterase pattern of larval tissues of <i>S. littoralis</i> as control and treated samples with tested compounds.	۱۴۱
۲۵	Electrophoretic β -esterase pattern of larval tissues of <i>S. littoralis</i> as control and treated samples with tested compounds.	۱۴۴
۲۶	Graph illustration of β -esterase pattern of larval tissues of <i>S. littoralis</i> as control and treated samples with tested compounds.	۱۴۶
۲۷	Electrophoretic malic enzyme pattern of larval tissues of <i>S. littoralis</i> as control and treated samples with tested compounds.	۱۴۹
۲۸	Graph illustration of malic enzyme pattern of larval tissues of <i>S. littoralis</i> as control and treated samples with tested compounds.	۱۵۱
۲۹	Electrophoretic malate dehydrogenase pattern of larval tissues of <i>S. littoralis</i> as control and treated samples with tested compounds.	۱۵۵