ALEXANDRIA UNIVERSITY
Faculty of Agriculture
(Saba Basha)
Plant protection Department

Histopathological Effect Of Certain Recent Pesticides On The Pink Bollworm *Pectinophora gossypiella* (Saund.)

A Thesis Submitted In Partial Fulfillment Of The Requirements

Governing The Award Of The Degree Of

MASTER OF AGRICULTURAL SCIENCES

(PESTICIDES)

Department of Plant Protection

Alexandria University

BY

SULTAN RIZK MOUHARIB MOHAMED

2009

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Presented by
SULTAN RIZK MOUHARIB MOHAMED

For the degree of
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Plant Protection Department

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جامعة الإسكندرية
كلية الزراعة – سابا باشا

التأثير الهستوپاثولوجى لبعض مبيدات الآفات الحديثة على دودة اللوز القرنفلية

مقدمة من

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CONTENTS

CHAPTER 1

INTRODUCTION

Cotton is a plant that seems to be designated specially to attract a wide rang of insect pests. It is green, succulent leaves, open flowers, nectaries on every leaf and flower, and a vast amount of fruit. All these characters are variable for various insects i.e. pink bollworm, spiny bollworm, the tobacco budworm, cotton leaf worm, cotton aphid, boll weevil, cotton fleahopper, spider mites, grass-hoppers, white fly, thrips and many other insects. Cotton, the world's most important fiber is grown on more than 33.9 million hectares in about 100 countries. Four countries alone (China, USA, India, and Pakistan) account for approximately two thirds of world output. If we added Uzbekistan and Egypt, six countries would account for three fourths of world cotton production, (Anonymous, 2004).

The pink bollworm (PBW) *Pectinophora gossypiella* (Saunders) is a worldwide pest of cotton and in some regions of the world is the key cotton pest. Like the boll weevil, the PBW is a well-adapted herbivore of cotton, feeding throughout the growing season on the cotton fruit system (square, flowers and bolls) and burrowing habits. It has been caused loss in yield and costs of insect control, substantial indirect losses occur as result of the destruction of beneficial insects and the development of insecticides resistance in cotton. It has been extremely difficult to control using insecticides but considerable success has been achieved using alternative control tactics.

Formulated insecticides are used in a large scale through the world as a major mean for cotton insects management and control. Although insecticides provide numerous benefits in terms of increased production and quality of the cotton product but their efficacy may be not often good because it affected with the development of insecticide resistance. Therefore it is important to study efficacy of insecticides against pink bollworm and the other bollworms in Egypt to establish a program to control and reduce resistance values. Such program must have in its sequence the modern insecticides (*Bt*, Spintor, Neem extract,…etc.) which proved its possibility to alternate or replace the conventional insecticides could be efficiently used to reduce number of insecticide sprays; cost of insect control, delay resistance build up and increases the production of cotton per unit.

Relative suitability of different rearing environments and the actual increasing rate of pink bollworm under different conditions are given by the life table parameters (total number of the laid eggs, hatching %, survival ratio of the immature stages, rate of development and the sex ratio), so the life tables are considered as the basic parameter which may be established for an insect population under specific physical conditions (El-Metwally *et al*., 2007).

The life tables are considered a powerful tool to clarify and understand the impact of any external factor on the growth, survival, reproduction and rate of the population increase (Wittmeyer and Coudron 2001 and El-Gemeiy 2002).

The histopathological studies were done to understand how the insecticide do its action in controlling the target inset and to obtain more information on the mechanism of the tested insecticides, especially the modern insecticides on the cell activity and micro organelles.

The present investigation aimed to study:

1- The toxicity of some modern insecticides comparing with the intensively used conventional insecticides.
2- The effects of these modern insecticides on the life tables of pink bollworm comparing with that conventionally used.
3- The histopathological effects of the tested insecticides upon the treated cells.

CHAPTER 3

MATERIALS AND METHODS

3.1. Insect Used:

Pink bollworm (PBW) *Pectinophora gossypiella* (Saunders) [Lepidoptera: Gelechudae].

3.1.1. The Pink Bollworm Susceptible Strain

The stock culture of PBW susceptible strain was supplied by the Bollworm Research Department, Plant Protection Institute, Agriculture Research Center, El-Dokki, Gize, Egypt, where it has been mass reared for several years in conditioned laboratory without exposure to insecticides. The rearing procedure was adopted as that described by Abdel – Hafez *et al* (1982).

3.1.2. Diet Ingredients and Preparation:

3.1.2.1. The diet ingredients: The used diet is composed of the following ingredients:

Dry Kidney bean	215gm
Yeast extract powder	32.5gm
Agar agar	11.5gm
Methyl-p-hydroxy benzoate (Methyl paraben)	1.2gm
Sorbic acid	1.2gm
Ascorbic acid	2.5gm
Formaldehyde	2.5ml
Water	734ml

3.1.2.2. The diet preparation:

The dry kidney beans were soaked in water for 18-20 hours. The soaked kidney beans with the remainder water were cooked until they became soft and all the water disappeared, then they were blended with 25ml water. Agar first dissolved in 150ml cool water and heated until boiling .The boiled agar was added to the soaked kidney beans and other ingredients which had been blended until it became homogeneous. The diet was kept in refrigerator until it is needed. When used it was dispensed to the rearing vials (2 X 7.5 cm) by a plastic "squeeze" bottle. The vials were filled to about one – third of their volume.

3.1.3. Insect Rearing Method:

The eggs were incubated at 27 ± 1^{o}C, and 80 ± 5% relative humidity (RH) in ISCO – FTD - 250 incubators, with 14 hours light and 10 hours dark. After hatching, the newly hatched larvae were transferred individually into the rearing glass vials of 2 X 7.5 cm using a camel's hair brush. The vials which were filled to one-third with the above prepared diet were covered with absorbent cotton wool. Vials were incubated at the same conditions until larvae complete their development. Then larvae were sexed, the brown gonads are conspicuous externally in the male and appear as spots on the dorsum of the fifth abdominal such structures. After pupation, the pupae were transferred individually into clean vials and kept in the same conditions until the emergence of moths.

Ten pairs of newly emerged moths were confined in glass oviposition cage of one liter size. A piece of cotton wool soaked in 10% sugar solution was suspended in each cage for feeding. The cotton piece was changed every 48 h. The cages were covered with muslin cloth, secured with rubber bands and their bottoms were covered with screening mesh on apiece of paper placed under the cage in Petri dish that served as oviposition site. The cages were maintained at the mentioned conditions and were examined daily for collecting eggs. Paper and muslin containing eggs were kept in glass vials of 6.5 X 12 cm. and covered with pieces of muslin cloth until hatching again and then repeated the above mentioned rearing steps.

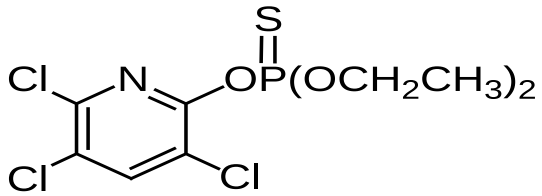
3.2. Insecticides Used:

3.2.1. Organophosphorus Insecticides

a) Chlorpyrifos

Common name : Chlorpyrifos
Trade name : Dursban, Lorsban, Tafaban, Priban
Chemical name : O, O-diethyl O-[3, 5, 6-trichloro-2-pyridyl] phosphorothioate. (IUPAC)
Empirical formula: $C_9 H_{11} Cl_3 NO_3 PS$.
Code No : Dowco 179
Molecular weight: 350.6
Formulation : 48%E.C
Application rate : 1 liter / feddan
Biochemistry : Cholinesterase inhibitor.
Mode of action : Non-systemic with contact, stomach, and respiratory action.
Insecticidal uses : Control of Coleoptera, Diptera, Homoptera and Lepidoptera in soil, on foliage in over 100 crops, glasshouse and outdoor ornamentals, turf, and in forestry. Also used for control of household pests (Blattellidae, Muscidae, Isoptera), mosquitoes (larvae and adults) and in animal houses.

Structural formula:



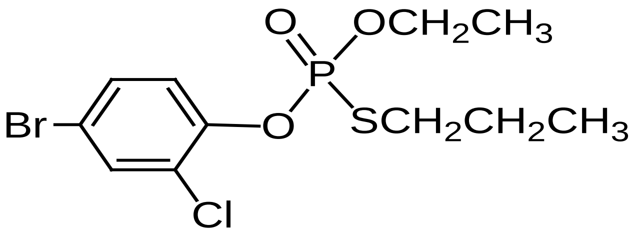
b) Profenofos

Common name : Profenofos
Trade name : Selecron, Curacron, Seliton , Teliton, Celcron.
Chemical name : O-4-bromo-2-chlorophenyl O-ethyl *S*-propyl phosphorothioate. (IUPAC)
Empirical formula: $C_{11} H_{15} Cl\ Br\ S\ P\ O_3$.
Code No : CGA 15 324
Molecular weight : 373.6
Formulation : 72 %E.C
Application rate : 1 liter / feddan
Biochemistry : Cholinesterase inhibitor.

Mode of action : Non-systemic insecticide and acaricide with contact and stomach action. Exhibits a translaminar effect. Has ovicidal properties.

Insecticidal uses : Control of insects (particularly Lepidoptera) and mites on cotton, maize, sugar beet, Soya beans, potatoes, vegetables, tobacco, and other crops, at 250-1000 g/ha.

Structural formula:



3.2.2. Botanical insecticides

Azadirachtin (Achook 0.15 % EC) ®

Azadirachtin is the principle insecticidal ingredient of neem seed extracts (Extracted from the neem tree, *Azadirachia indica*.): these extracts also contain a variety of limonoids, such as nimbolide, nimbin and salannin.

Chemical group :Terpenoids

Common name : Azadirachtin

Trade name : Achook , azad, Azatin, Ecozin, Kayneem (neem oil), NeemAzal Neememulsion , Neemix, Neemolin (seed extract), Vineem. Neemazad.

Chemical name : dimethyl (3*S*, 3a*R*, 4*S*, 5*S*, 5a*R*, 5a^1*R*, 7a*S*, 8*R*, 10*S*, 10a*S*) -8 - acetoxy-3,3a, 4,5,5a, 5a^1, 7a, 8,9,10 – decahydro -3,5 – dihydroxy -4-{(1*S*, 3*S*, 7*S*, 8*R*, 9*S*, 11*R*) – 7 – hydroxy – 9 – methyl - 2, 4,10 - trioxatetracyclo [6.3.1.0^{3,7} .0^{9,11}] dodec – 5 – en - 11-yl} – 4 – methyl – 10 [(*E*)-2-methylbut – 2 - enoyloxy] - 1H, 7H – naphtha [1, 8a, 8 – *bc* : 4, 4a-c′] difuran -3, 7a - dicarboxylate. (IUPAC)

Empirical formula : $C_{35}H_{44}O_{16}$

Code No : N-3101 (Cyclo)

Molecular weight : 720.7

Formulation : EC

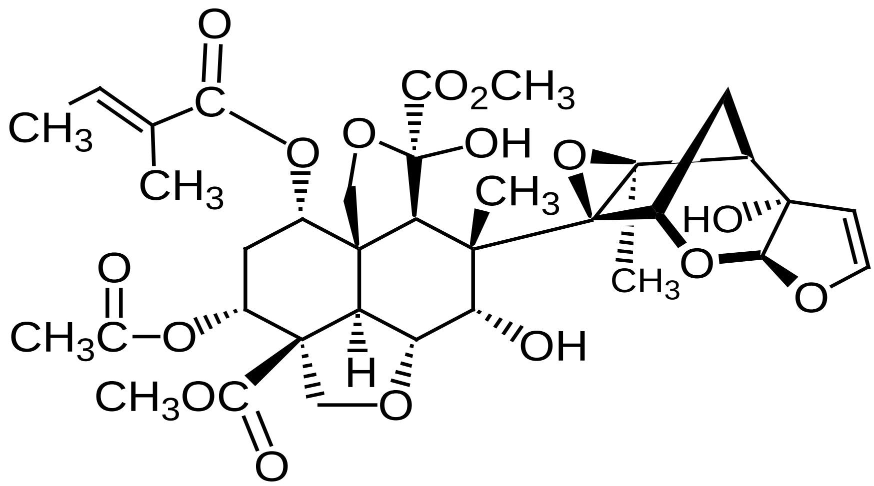
Application rate :

Biochemistry : Ecdysone antagonist

Mode of action : Disrupts insect moulting. Fungicidal and miticidal properties of the hydrophobic extract derive from physical smothering and desiccation.

Insecticidal uses: used for control of whitefly, leaf miners and other pests including pear psylla. Neem extracts also show anti-feedant and repellent properties, which have been shown to be due to other chemicals such as salannin. A hydrophobic extract shows nematicidal and fungicidal activity.

Structural formula:



3.2.3. Bio insecticides

a) Emamectin benzoate (Proclaim 5% S.G) ®.

Chemical group : Avermectin

Common name : Emamectin benzoate

Trade name : Proclaim, Banlep, Denim.

Chemical name : A mixture containing 90% of (10*E*,14*E*,16*E*,22*Z*)-(1*R*,4*S*, 5' *S* ,6*S* ,6'*R*,8*R*,12*S*,13*S*,20*R*,21*R*,24*S*)-6'-[(*S*)-*sec*-butyl]-21,24-dihydroxy-5',11,13,22-tetramethyl-2-oxo-3,7,19- trioxatetracyclo [15.6.1. $1^{4,8}$.$0^{20,24}$] pentacosa-10,14,16,22-tetraene-6-spiro-2'-(5',6'-dihydro-2'*H*-pyran)-12-yl 2,6-dideoxy-3-*O*-methyl-4-*O*-(2,4,6-trideoxy-3-*O*-methyl-4-methylamino-□-L-*lyxo*-hexopyranosyl)-□-L-*arabino*-hexopyranoside and 10% of (10*E*, 14*E*, 16*E*, 22*Z*) - (1*R*, 4*S*, 5'*S* ,6*S* ,6' *R*, 8*R*, 12*S*, 13*S*, 20 *R*, 21*R*, 24*S*) -21, 24 - dihydroxy - 6'-isopropyl -5',11,13, 22 – tetramethyl - 2-oxo-3,7,19-trioxatetracyclo[15.6.1.$1^{4,8}$.$0^{20,24}$] pentacosa-10,14, 16,22-tetraene – 6 – spiro - 2'-(5',6'-dihydro-2'*H*-pyran) -12-yl 2,6 - dideoxy -3-*O*-methyl – 4 – *O* - (2,4,6-trideoxy-3 –*O* -methyl- 4- methyl amino - □-L - *lyxo* – hexopyranosyl)-□-L- *arabino* - hexopyranoside . (IUPAC).
A mixture of emamectin B_{1a} (90%) and emamectin B_{1b} (10%),as benzoate salts.

Empirical formula: C_{56} H_{81} NO_{15} (B_{1a}); C_{55} H_{79} NO_{15} (B_{1b}) as benzoate salts. C_{49} H_{75} NO_{13} (B_{1a}); C_{48} H_{73} NO_{13} (B_{1b}) as emamectin.

Code No : MK 244

Molecular weight : 1008.3 (B_{1a}); 994.2 (B_{1b}) as benzoate salts. 886.1 (B_{1a}); 872.1 (B_{1b}) as emamectin

Formulation : EC; SG

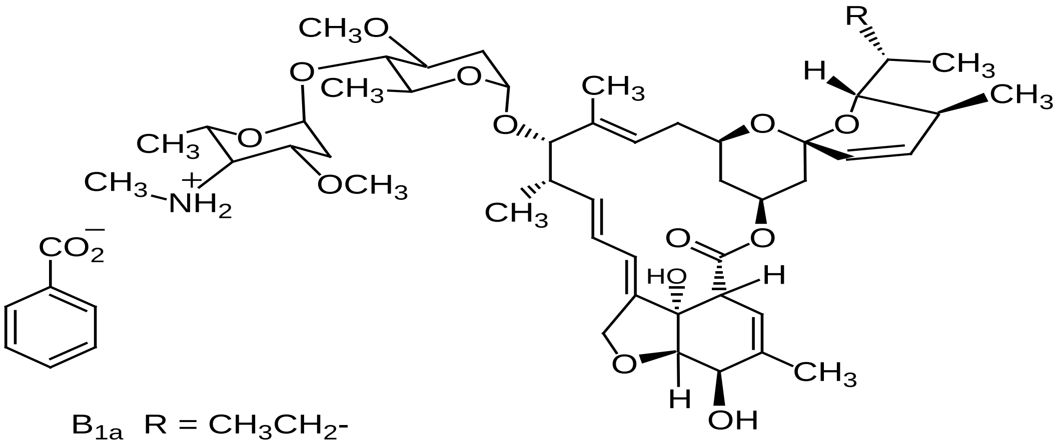
Application rate : from 5 – 25 g/ha.

Biochemistry : Acts by stimulating the release of □aminobutyric acid, an inhibitory neurotransmitter, thus causing paralysis.

Mode of action : Non-systemic insecticide which penetrates leaf tissues by translaminar movement. Paralyses the Lepidoptera, which stop feeding within hours of ingestion, and die 2-4 date.

Insecticidal uses : For control of Lepidoptera on vegetables, brassicas and cotton, at up to 16 g/ha, and in pine trees, at 5-25 g/ha.

Structural formula:



B_{1a} R = CH_3CH_2-

B_{1b} R = CH_3-

b) Spinosad (Spintor) ®

Chemical group : Spinosyn

Common name : Spinosad

Trade name : Spintor

Chemical name: A mixture of (2*R*,3a*R*,5a*R*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*R*)-2-(6-deoxy-2,3,4-tri-*O*-methyl-a-L mannopyranosyloxy)- 13-(4-dimethylamino-2,3,4,6-tetradeoxy-b-D-erythopyranosyloxy)-9-ethyl-2,3,3a,5a,5b,6,7,9,10,11, 12,13,14, 15, 16a,16b-hexadecahydro-14-methyl-1*H*-8-oxacyclododeca[*b*]*as*-indacene-7,15-dione and (2*R*,3a*S*,5a*R*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*R*)-2-(6-deoxy-2,3,4-tri-*O*-methyl-□-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-□-D-erythopyrano syloxy) -9-ethyl-2,3,3a,5a,5b,6,7,9,10,11,12,13, 14, 15, 16a, 16b-hexadeca hydro-4,14-dimethyl-1*H*-8-oxacyclododeca[*b*] *as*-indacene-7,15-dione. In the proportion 50-95% to 50-5% (IUPAC).

Empirical formula: $C_{41}H_{65}NO_{10}$ (spinosyn A); $C_{42}H_{67}NO_{10}$ (spinosyn D)

Code No : XDE-105; DE-105 (both Dow)

Molecular weight: 732.0 (spinosyn A); 746.0 (spinosyn D)

Formulation : SC; WG

Application rate : 4.8-36 g/hl

Biochemistry : Activation of the nicotinic acetylcholine receptor, but at a different site from nicotine or imidacloprid.

Mode of action : Active by contact and ingestion; causes paralysis.

Insecticidal uses : For control of pest Lepidoptera, thrips, flies, beetles and grass hoppers in cotton, row crops, vegetables, and fruits at 4.8-36 g/hl. Also used for urban pest control in turf and ornamentals, for structural control of dry wood termites and for fire ant control. Effective as bait for fruit flies and some ants