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

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Histopathological effect of certain recent pesticides on The pink bollworm *Pactinophora gossypiella* (Saund)

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التأثير الهيستوپاثولوجي لبعض مبيدات الآفات الحديثة
على دودة اللوز القرنفلية

مقدمة من
سلمان رزق محارب محمد

 رسالة علمية مقدمة استيفاء لمتطلبات منح درجة
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(تخصص مبيدات الآفات)
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HISTOPATHOLOGICAL EFFECT OF CERTAIN RECENT PESTICIDES ON THE PINK BOLLWORM

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COTTON IS A PLANT THAT SEEMS TO BE DESIGNATED ESPECIALLY TO ATTRACT A WIDE RANGE 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 GOSypiELLA (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.


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: Gelechidae].

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°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 a piece 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: C₉ H₁₁ Cl₃ NO₃ 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: C₁₁ H₁₅ Cl Br S P O₃.
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:**

![Structural formula](image)

---

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 (3S, 3aR, 4S, 5S, 5aR, 5a1R, 7aS, 8R, 10S, 10aS) -8 - acetoxycyclo[6.1.1.03,7.09,11]difuran -3,7a-dicarboxylate. (IUPAC)

**Empirical formula**: C35H44O16

**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.
3.2.3. Bio insecticides

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

**Chemical group**: Avermectin

**Common name**: Emamectin benzoate

**Trade name**: Proclaim, Banlep, Denim.


A mixture of emamectin B\textsubscript{1a} (90%) and emamectin B\textsubscript{1b} (10%), as benzoate salts.

**Empirical formula**: C\textsubscript{56} H\textsubscript{81} NO\textsubscript{15} (B\textsubscript{1a}); C\textsubscript{55} H\textsubscript{79} NO\textsubscript{15} (B\textsubscript{1b}) as benzoate salts. C\textsubscript{49} H\textsubscript{75} NO\textsubscript{13} (B\textsubscript{1a}); C\textsubscript{48} H\textsubscript{73} NO\textsubscript{13} (B\textsubscript{1b}) as emamectin.

**Code No**: MK 244

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

**Formulation**: EC; SG

**Application rate**: from 5 – 25 g/ha.

**Biochemistry**: Acts by stimulating the release of \textbf{α}-aminobutyric acid, an inhibitory neurotransmitter, thus causing paralysis.

**Mode of action**: Non-systemic insecticide which penetrates leaf tissues by translaminar movement. Parasites 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:**

![Structural formula image]

**b) Spinosad (Spintor) ®**

**Chemical group**: Spinosyn  
**Common name**: Spinosad  
**Trade name**: Spintor  
**Chemical name**: A mixture of \((2R,3aR,5aR,5bS,9S,13S,14R,16aS,16bR)-2-(6-deoxy-2,3,4-tri-O-methyl-a-L mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetrae...-1H-8-oxacyclododeca[b] as-indacene-7,15-dione and \((2R,3aS,5aR,5bS,9S,13S,14R,16aS,16bR)-2-(6-deoxy-2,3,4-tri-O-methyl-a-L mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetrae...-1H-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/ha  
**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/ha. 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.