# CONTENTS

# CHAPTER 5

# SUMMARY

Cotton is a plant that seems to be designated specially to attract a wide rang of insect pests. Cotton, the world's most important fiber is grown on more than 33.9 million hectares in about 100 countries. 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. 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. 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.

Three different groups of insecticides; organophosphate, bio-insecticides and notionally origin insecticides; were compared against the newly hatched larvae of *Pectinophora gossypiella* using film residue method, while diet incorporating was utilized for *Bt* (Subsp. *Kurstaki*).

The present investigation aimed to study:

1- The toxicity of two modern insecticides (spinosad and emamectin benzoate), *Bt* (Subsp. *Kurstaki*) and neem - azadirachtin comparing with the intensively used conventional insecticides in controlling bollworms in Egypt ( chlorpyrifos and profenofos).
2- The effects of these insecticides on the survival and development of pink bollworm comparing with that conventionally used.
3- The histopathological effects of the tested insecticides.

The obtained results of this study could be summarized as follows:-

## 5.1. Bioassay of certain insecticides against *Pectinophora gossypiella*

### 5.1.1. Comparative toxicity study of certain pesticides against the newly hatched larvae of, *Pectinophora gossypiella* ($LC_{25}$):

The $LC_{25}$ values of chlorpyrifos, profenofos, spinosad, emamectin benzoate, *Bt* (Subsp. *Kurstaki*) and neem (azadirachtin) were 0.124, 0.328, 0.0006, 0.0001, 0.506 and 0.0022 ppm, respectively. The Ld-P line indicated that, the toxicities of the previous mentioned insecticides were in the following descending order: emamectin benzoate, spinosad, neem (azadirachtin), chlorpyrifos, profenofos and then *Bt* (Subsp. *Kurstaki*).

### 5.1.2. Comparative toxicity study of certain pesticides against the newly hatched larvae of *Pectinophora gossypiella* ($LC_{50}$).

The $LC_{50}$ values of the two organophosphate insecticides, chlorpyrifos was 0.369 ppm and profenofos was 0.645 ppm with, while the LC50 values of the three bio-origin insecticides were 0.0066 ppm for spinosad, 0.002 ppm for emamectin benzoate and 1.456 ppm for *Bt* (Subsp. *kurstaki*), also; the plant origin insecticide, neem (azadirachtin) $LC_{50}$ value was 0.036 ppm.

The Ld-P lines indicated the toxicity of these insecticides against the pink bollworm could be arranged in the following descending order: emamectin benzoate, spinosad, neem (azadirachtin), chlorpyrifos, profenofos and then *Bt* (Subsp. *Kurstaki*).

## 5.2. The latent effect of the certain pesticide treatments on the newly hatch larvae of *Pectinophora gossypiella*.

The calculated $LC_{50}$ and $LC_{25}$ of these insecticides were selected and tested against the 1st instar larvae. The percentages of the survival pink bollworm insects which were treated with $LC_{25}$ of the tested insecticides and also the untreated check throughout 26 days. The highest effect after the 8th day was observed by the treatment of profenofos followed by emamectin benzoate and then with the same values for both of chlorpyrifos, spinosad and *BT* Subsp. *Kurstaki*. While the lowest effect was recorded by neem-azadirachtin. The survival percentages after the 8th day post treatment were 48, 54, 60, 60, 60, and 68% respectively.

The final efficacy order after the 26th day post treatment for the tested insecticides was: profenofos > *BT* Subsp. *Kurstaki* > neem–azadirachtin and emamectin benzoate > spinosad > chlorpyrifos with survival insect percentages of 26%, 30%, 34%, 42% and 44%, respectively. The greatest loss in the survival insect percentage, 34%, was for neem–azadirachtin (from 68% after the 8th day post treatment to 34% after the 26th day post treatment) followed by *Bt* Subsp. *Kurstaki*, 30% loss in survival insect (from 60% after the 8th day post treatment to 30% after the 26th day post treatment) and then profenofos with 22% loss (from 48% after the 8th day post treatment to 26% after the 26th day post treatment) while emamectin benzoate has a lose value in survival insect of 20 % (from 54% after the 8th day post treatment to 34% after the 26th day post treatment); also, the spinosad insecticide has a lose value in survival insect of 18 % (from 60% after the 8th day post treatment to 42% after the 26th day post treatment).

Regarding the $LC_{50,}$ the results showed that, the highest effect after 8th day was observed by emamectin benzoate followed by profenofos, *Bt* Subsp. *Kurstaki,* and then with the same values both of chlorpyrifos, spinosad and neem- azadirachtin. The survival percentages were 34%, 38%, 48%, and 50%, respectively.

By the final inspection day, the 26th day, the efficacy order was: emamectin benzoate, profenofos and *Bt* Subsp. *kurstaki* > spinosad > neem – azadirachtin > chlorpyrifos with survival insect percentages of 20%, 20%, 20%, 24%, 30%, and 32%, respectively.

In conclusion, the effective insecticides on the biological parameters of the pink bollworm were emamectin benzoate and profenofos followed by *Bt* Subsp. *kurstaki,* neem – azadirachtin and spinosad. The least effective insecticide was chlorpyrifos in that manner. Among the $LC_{25}$, it could be showed that, profenofos and emamectin benzoate induced the superior effect resulting in 48 and 54% after 8 days post treatment, respectively. Both of profenofos and *Bt* Subsp. *kurstaki,* continued as the deleterious insecticides at the $20^{th}$ day (30 and 38%) and the $26^{th}$ day (26 and 30%).

However, there are insignificant differences between the $LC_{50}$ and $LC_{25}$ among most insecticides except the treatment of spinosad indicating the importance of applying the accurate dose in the field to induce the suitable control.

## 5.2. Biological parameters of the pink bollworms as affected by insecticidal and *Bt* Subsp. *kurstaki,* treatments.

It is obvious from the results throughout 10 days post treatment that profenofos was the most effective one on the larval stage at both $LC_{25}$ and $LC_{50}$. Also the least effective ones on the larval stage were neem – azadirachtin, chlorpyrifos and *Bt* Subsp. *kurstaki,* at both $LC_{25}$ and $LC_{50}$ and spinosad at the level of $LC_{25}$ on the larval stage.

Emamectin benzoate and profenofos with their $LC_{50}$ at the $8^{th}$ day post treatment were the most effective ones giving 34 and 38%, consequently. Profenofos was the most effective one against the larval stage at both $LC_{25}$ and $LC_{50}$. Also the least effective ones on the larval stage were neem – azadirachtin, chlorpyrifos and *Bt* Subsp. *Kurstaki,* at both $LC_{25}$ and $LC_{50}$ and spinosad at the level of $LC_{25}$ on the larval stage.

## 5.4. Effect of the tested insecticides on the biology parameters of the pink bollworm pupae.

At the $14^{th}$ day post treatment, emamectin benzoate ($LC_{50}$), neem - azadirachtin ($LC_{25}$) and spinosad ($LC_{25}$) highly reduced the developed pupae giving 6, 8 and 8% pupae, respectively. At the level of $LC_{25}$, *Bt* Subsp. *kurstaki,* and chlorpyrifos showed lower effect on the developed pupae 24 and 18%, consequently versus 32% in the untreated control. After 20 days from the treatment, profenofos and emamectin benzoate have continued their superior effect in reducing the developed pupae. Profenofos gave 26 and 22% of developed pupae for the $LC_{25}$ and $LC_{50}$. Both of emamectin benzoate ($LC_{50}$) and *Bt* Subsp. *kurstaki,* ($LC_{25}$) induced 24% developed pupae.

Generally, it is obvious that emamectin benzoate at its $LC_{50}$ and profenofos at both $LC_{25}$ and $LC_{50}$ were the most effective insecticides in reducing the percentage of the developed pupae along the pupation period. Vice versa, both spinosad and chlorpyrifos moderately reduced the percentage of the developed pupae indicating a comparative weak effect which could be referred to the resistance development of this insect toward them or and the development of pupae stage is not a target for both insecticides.

## 5.5. Effect of the tested insecticides on the emergency of the pink bollworm adults.

The perusal data after 22, 24 and 26 days post larval treatment revealed that emamectin benzoate ($LC_{50}$) induced the least emerged adults along the three inspection dates showing 2, 18 and 20%, respectively. Also, both of profenofos and *Bt* Subsp. *Kurstaki,* at $LC_{50}$ achieved the superior effect after 24 and 26 days giving only 16 and 20 % of emerged adults, respectively.

After 24 and 26 days, the most effective insecticides with their $LC_{25}$ in reducing the developed adults were profenofos that induced only 16 and 26% emerged adults, respectively. In contradiction with the above results, chlorpyrifos and spinosad at their $LC_{25}$ showed high percentages of developed adults compared with the other treatments showing 30 and 44% for chlorpyrifos and 38 and 42 % for spinosad, respectively.

## 5.6. Effect of the tested insecticides on failure % of the development stages of the *Pectinophora gossypiella*

All treatments highly increased the failure percentage of stages development of *P. gossypiella*.

According to the $LC_{50}$, the most effective insecticide was profenofos which gave (76%), followed by emamectin benzoate, spinosad, *Bt* Subsp. *kurstaki*, neem - azadirachtin and chlorpyrifos resulting in 72, 62, 60, 56 and 52% development failure, respectively. In pupae stage, *Bt* Subsp. *kurstaki,* (at $LC_{50}$) was the superior treatment (16%) followed by spinosad, chlorpyrifos, emamectin benzoate, neem - azadirachtin and profenofos that induced 10, 6, 4, 4 and 2% pupal development failure, respectively. Chlorpyrifos ($LC_{50}$) was the most effective treatment in the adult emergency failure (10%), while the following effective one was neem - azadirachtin (6%) then emamectin benzoate, spinosad and *Bt* Subsp. *Kurstaki,* (4%). On the other hand, the least effective treatment on adult development was profenofos giving (2%).

Concerning the $LC_{25}$, in larval stage, Profenofos was the effective treatment followed by emamectin benzoate, chlorpyrifos, *Bt* Subsp. *Kurstaki*, spinosad and neem - azadirachtin, respectively. While in the pupae stage, the superior one was neem - azadirachtin followed by *Bt* Subsp. *kurstaki*, chlorpyrifos, emamectin benzoate, profenofos and spinosad, respectively. In adult stage, the most effective treatment was spinosad followed by emamectin benzoate, neem - azadirachtin, *Bt* Subsp. *Kurstaki* and then chlorpyrifos showed the least effect.

## 5.7. Effect of the tested insecticides on the oviposition and the eggs hatchability.

Along total period of 8 days which the control females spent in their oviposition, the laid eggs/female/day were 8.2 and 2.27 eggs, as well as the actual laid eggs/female/day were 16.4 and 4.5 eggs, respectively.

Emamectin benzoate (Proclaim) decreased the actual oviposition period to four days for both $LC_{25}$ and $LC_{50}$ treatments. The total laid eggs / female were 65.7 eggs with hatching percentage of 35% and 18 eggs with hatching percentage of 38.9% for both $LC_{25}$ and $LC_{50}$ treatments, respectively.

In chlorpyrifos $LC_{25}$ treatment, the total laid eggs / female were 154.3 eggs and 80 with hatching percentage of 51.9%. Also the oviposited eggs / female/day were 19.29. For $LC_{50}$ treatment, the total oviposited eggs / female were 87.7 and only 32 eggs with hatching percentage of 36.5%, also, the mean oviposited eggs / female/day was 10.96.

Spinosad in its $LC_{25}$, the total oviposited eggs/female were declined into 16.3 eggs / female with hatching percentage of 34.97%. The effects of $LC_{50}$ were differed, the total laid eggs/female was 14 eggs and only one egg of them was hatched with hatching percentage of 7.14%. The mean oviposited eggs/female/day was 1.75.

The effect of the $LC_{25}$ treatment of neem - azadirachtin was completely differed than that of the $LC_{50}$ treatment. The oviposition period was 7 days for the $LC_{25}$ treatment and only one day for $LC_{50}$ treatment, the mean oviposited eggs/female/day was 9.95 eggs and 1.5 eggs for $LC_{25}$ treatment and $LC_{50}$ treatment, respectively. Another difference was appeared when the $LC_{25}$ treatment delayed the oviposition one day and the $LC_{50}$ treatment delayed it four days. The hatchability percentage also was differing where it was 45.64% and 66.67% for $LC_{25}$ treatment and $LC_{50}$ treatment, respectively.

The other commonly used insecticide, profenofos, had the strongest effect on the hatching% for both $LC_{25}$ and $LC_{50}$ treatments. The mean oviposited eggs / female/day was 10.96 for $LC_{25}$ treatment with a hatching percentage of 6.04% and 8.25 eggs with the lowest hatching percentage of 4.03% for $LC_{50}$ treatment.

The present data show a strange and strong effect of the bio-insecticide, *Bt* Subsp. *Kurstaki* which completely stopped the oviposition.

## 5.8. The efficacy of insecticide on reducing the oviposition period, total oviposited eggs and the total hatched eggs.

The treated female moths with the $LC_{25}$ of emamectin benzoate (Proclaim) or chlorpyrifos needed only five days as an oviposition period at $LC_{25}$ with reduction percentage value of 37.5%. The treatment $LC_{50}$ treatment of both gave 6 and 8 oviposition period with reduction percentages of 25%and 0%, respectively.

The two concentrations of spinosad (Spintor) have the same parallel way where the oviposition period was 6 days with reduction percentage of 25% for each.

In profenofos (Selecron), the oviposition period that recorded for $LC_{25}$ concentration was seven days with reduction percentage of 12.5% where the same period for $LC_{50}$ concentration was six days with reduction percentage of 25%.

The bio-insecticide *Bt* Subsp. *Kurstaki*, pointed to a sterile effect because no eggs were deposited in both concentrations so, the reduction percentages for all the tested aspects were 100%.

The modern insecticides were more effective than the conventional insecticides in reducing the total oviposited eggs/female. The commonly applied conventional insecticides in both $LC_{25}$ and $LC_{50,}$ concentrations strongly decreased the number of laid eggs with rates between 34.4% and 100%. *Bt* Subsp. *kurstaki,* profenofos and spinosad achieved the highest reduction % of the deposited eggs ranged between 95.92 and 100% for $LC_{25}$ or $LC_{50}$. The weakest insecticide in that respect was chlorpyrifos that induced only 42.7 and 77.1 reduction %.

## 5.9. Cytological procedures and electron microscopic investigation of the cerebral neurosecretory cells:

Some ultra microtomic preparations of the cerebral neurosecretory cells (C.N.S.C.) of treated and untreated 4$^{th}$ instar larvae were prepared and examined with the electronic microscope. The treated larvae showed some deviations of the resulted (C.N.S.C.) from the normal ones in their ultra structure.

Treated cells with emamectin benzoate showed irregular cell membrane (shape) and cell malformation and disorder of the neurosecretory cells.

Chlorpyrifos showed the hyper division of neurosecretory cells, chromatin accumulation and the presence of large spaces between the cells. Also, showed the numerous numbers of lysosomes inside the cell with and without autolytic active foci as well as cracks in the neural tissue.

Treated cells with spinosad indicated the nucleus shift to the cell wall, the beginning apolysis of the cell wall and as well apolysis of surrounded nucleus membrane accompanied with small in size of mitochondria.

Neem - azadirachtin revealed the irregular shape of the cerebral cells and the apolysis of many neurosecretory cells, the apolysis of mitochondria, apolysis of nucleus and the appearance of chromatin remains.

Profenofos showed separation of the endoplasmic reticulum (ER) and the presence of spaces between its parts. Also, it leaded to cracks in the cell and apolysis in the cell's microvilli, accumulation in cerebral tissue and the active and non active lusosomes.