Biological, histological and biochemical studies on the effect of different botanical products on the pink and spiny bollworms

By

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A thesis submitted in partial fulfillment

of

The Requirements for the degree of

Doctor of Philosophy

In

Agricultural Sciences

(Entomology)

Plant Protection Department,

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Benha University

2021

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I. Toxicological studies:

1. Toxicity of some bio-pesticides against some newly hatched larvae of pink bollworm and spiny bollworm:

A. Pink bollworm, *P. gossypiella* (Saunders):

The present study was carried out to evaluate the toxic effect of Jojoba oil, Flaxseed oil and Pasha compounds against newly hatched larvae of susceptible strain of the pink bollworm in the laboratory under constant conditions of 26 ± 1ºC and 75± 5% R.H. Both the LC_{50} and LC_{90} values of the tested compounds were estimated. The LC_{50s} values was very low for both Jojoba oil and Pasha (0.9770%and 0.0576 ppm respectively). On contrast there was more variation with LC_{50}value of Flaxseed oil, whereas it was 23.6195%. The present data detected that the newly hatched larvae of PBW is high susceptible to Jojoba oil and Pasha, then Flaxseed oil. According to LC_{50} and LC_{90} values, Jojoba oil and Pasha are considered the highest impact than Flaxseed oil. Also, the slope values of Pasha and Jojoba oil after one day post treatment**.** According to Sun's equation (1950) toxicity Index of the three tested compounds tested against the *P. gossypiella* 1^{st} larvae was calculated. At the LC_{50} and LC_{90} levels, chemical compound Pasha, taken as the standard compound and given the arbitrary value of 100 units, the toxicity index of Jojoba and Flaxseed oils at LC_{50} and LC_{90} level were (5.895 & 0.2438) and (23.283 & 0.7117%), respectively.

2. Effect of plant extracts on some biological aspects newly hatched larvae of *P. gossypiella*:

2.1. No. of dead larvae: Results showed that Jojoba oil (*S. chinensis*) have high effect on newly hatched larvae of *P. gossypiella.* Also,

highly significant difference between Jojoba oil, Flaxseed oil, Emamectin benzoate and control. But Emamectin benzoate has no difference compared to Jojoba oil

2.2. Total larval mortality: Tested Jojoba oil and Emamectin benzoate showed high effect on newly hatched larvae of *P. gossypiella* compared to control.

2.3. No. of malformed larvae: Results showed Jojoba oil and Flaxseed oil had significant effects on pink bollworm larvae malformations. As concerning Flaxseed oil, it recorded the highest number of malformed larvae followed by Emamectin benzoate and Jojoba oil (17.0,10.0 and 4.0, respectively) with significant differences between all the treatments and the control.

2.4. Mean % malformation larvae: The effect of treatment pink bollworm larvae with the tested Jojoba and Flax oils was extended to affect adults. The malformed larvae, resulted from treated larvae, was 14.16 % for Jojoba oil. In case of treated larvae with Flax oil was 12.02 % compared to 1.00 % in control. Meanwhile, treated pink bollworm larvae using Emamectin benzoate caused 10.00 % deformation in the tested larvae compared with control.

2.5. Larval duration: The three tested compounds (Jojoba and Flax and Emamectin benzoate) caused significant increase in larval period compared with untreated larvae.

2.6. Weight of the 4th instars larvae: Jojoba treatment affected larval weight of PBW significantly than the other two compounds and control. While *Flax* oil and Emamectin benzoate showed non significant decrease in larval weight in between, but differ significantly compared to control.

2.7. Pupal stage:

a. No. of formed pupae: The average number of formed pupae resulted from treated newly hatched larvae of pink bollworm with sub lethal concentrations of some plant oils and Emamectin benzoate were 16.33, 30.00 and 30.00 at 0.9770%, 23.6195% and 0.0576 ppm, respectively compared with 94.33 in control. Significant differences were found between the tested products and control.

b. Pupal mortality percentage: Present results indicated that the two tested plant oils and Emamectin compound increased pupal mortality percentage compared to control (8.57, 6.59, 18.99 and 4.3%).

c. Pupal period: The tested concentrations Jojoba and Flax oils significant affect pupal period than untreated control. The effect of sub-lethal concentration of Emamectin benzoate attained 13.04 day of the tested lepidopterous compared with control.

d. Pupation percentage: Results proved that the average of pupation percent decreased to 48.96% in case of treating PBW neonate with sub lethal concentration of Jojoba oil while it attained 88.34 % for Flax oil but in case of Emamectin benzoate treated larvae no effect was observed on pupation percent. Results indicated that feeding neonate larvae on diet treated with Emamectin benzoate caused highly pupation percentage 100.00 % compared with control.

e. Total duration of immature stage: Present results indicated that Jojoba and Flax oils and Emamectin benzoate treatments increased the immature duration of *P. gossypiella* in comparison to control. It recorded (30.00, 33.0,29.0 & 29.0 days, respectively) & 22.97 days for control.

2.8. Adult stage:

a. No. of adult emergence: The tested Jojoba and Flaxseed oils and Emamectin benzoate compound slightly reduced adult emergence. Significant difference between Jojoba and Flax oils and Emamectin benzoate compared with control.

b. Mean % of adult emergence: Results showed high pronounced effects on adult emergences for Jojoba and flaxseed oils at the tested concentrations. Percentages of adults' emergence recorded 91.85 and 93.33 %, respectively, while Emamectin benzoate attained 82.53% emergence compared to98.6% in control.

c. Effect of the number of male and female (sex ratio):

Present results show the effect of larval treatment with the tested compounds on the sex ratio of *P. gossypiella*. Obvouisly, the sex ratio in general was in the share of female and male. There are no significant difference between all treatments and control 2.9. Adult malformation:

a. • No. and percentage of adult malformed:

The treatment with Flaxseed oil caused malformation in the appeared adults (4.00±0.57 adult 14.28%)and more than the other treatments which were (1.33±0.33 and 5.39%) in the case of Emamectin treatment and (1.00 ±0.57adult and 6.67%) in Jojoba oil tretment.

2.10. Reproductive potential:

Oviposition periods, the total number of deposited eggs (fecundity) and (fertility):

The present results proved that Jojoba oil shortened pre-oviposition period of adult females resulted from treating newly hatched larvae of pink bollworm with sublethal concentration, but Flax

oil and Emamectin benzoate caused an elongation of this period than control. On the other hand, Jojoba and Flax oils elongated post-oviposition period of adult females compared with control. Significant difference was observed between the three tested compounds and the untreated check. Also, results cleared that Jojoba oil and Emamectin benzoate compound shortened female and male longevity periods significantly than control. Although, Flax oil elongated female longevity periods than control there was insignificant difference between both. In addition, the numbers of laid eggs were decreased in all the tested compounds compared with control. But the tested compounds caused moderately decrease hatchability % than untreated control with significant differences. Hatchability %attained 56.83, 60.46, 65.32 and 95.66 %, respectively.

3. Efficiency of the tested compounds on some biochemical aspects in *P. gossypiella* newly hatched larvae:

Biochemical assays were directed to evaluate the total soluble protein (TSP), total lipid (TL), Carbohydrate, the activities of transaminases; Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT), Lactate dehydrogenase catalyzes (LDH) and alkaline phosphatases in larvae of *P. gossypiella* treated as neonate with the LC_{50} concentration of Jojoba oil**,** Flaxseed oil and Pasha compound.

a. Total soluble protein and total lipid:

The previously studies cleared that proteins, lipids and carbohydrates are considered an essential biochemical component necessary for growth, development and reproduction. The concentration of (TSP), (TL) and total carbohydrates and the changes as a percentage from the control in larval supernatants of the pink bollworm treated with sublethal concentrations of Jojoba, Flaxseed oils

and Emamectin benzoate compound is evaluated. The (TSP) changes as a percentage in larval supernatants of the pink bollworm treated with LC_{50} concentrations of Jojoba oil and Flaxseed oil, and Emamectin benzoate is negatively changed than the control. In case of Jojoba oil treatment the total lipid (TL) was negatively changed (-44.82%), while it was positively changed in Flaxseed oil, (45.86%). Emamectin benzoate treatment achieved highly positive effect (620.68 %) compared with untreated larvae.

b. Total carbohydrate: The results cleared that the highest effect was recorded in larvae treated with Pasha 13.23 % compared with untreated larvae while it was decreased in Flax oil treatment **(**6.95 %). The highest decrease in carbohydrate level was recorded in Jojoba treatment (-34.19 %) compared to untreated larvae.

c. Transaminase activities (ALT and AST): The changes in (AST&ALT) activities as the concentration of the formed pyruvate and the relative activity as a percentage from the control of larval supernatants of *P.gossypiella*10 days after treatment treated as newly hatched larvae with LC_{50} concentration of the tested compounds (Jojoba, Flax oils and Pasha compounds. The results showed greatly increase at AST(102.33µg pyruvate /ml) and recorded 60.74% with Jojoba oil treatment following by moderate increase recorded in Flaxseed oil **(**43.46%) compared with (63.66µg pyruvate /ml) in the control treatment. On the other hand, Pasha treatment caused reduction in AST activity (-21.61%) compared with control. The effect of tested compounds on ALT activities in treated and untreated larvae were greatly high in Flaxseed oil treatment following by Jojoba oil recorded. Also, data reported reduction in ALT activity of *P. gossypiella* larvae treated with Pasha (-46.35%) compared with 228.66 in the control.

d. Lactate dehydrogenises catalyzes (LDH) and alkaline phosphates (A P) activities:

Present data showed the changes in (LDH & AP) in the supernatant of the homogenated larvae of pink bollworm treated with sublethal concentrations of Jojoba, Flaxseed oils and Emamectin benzoate compound. After ten days from treatment LDH were decreased in all treatments and reached(-56.80%; -37.05% & -29.85%) in Pasha, Flaxseed oil and in Jojoba oil. Also, (AP) enzyme activity acchived highly decreasein Pasha treatment (-73.63%) following by (-35.09%) in Flaxseed oil, while the lowest decrease (-1.31%) recorded in Jojoba oil compared to control.

B. *Earias insulana* newly hatched larvae:

1. Toxicological studied:

Calculation of LC_{50} and LC_{90}values resulted from treated newly hatched larvae of spiny bollworm, *E. insulana* with Jojoba, Flaxseed oils and Pasha compound cleared that the LC_{50} value was very low for Pasha followed by Jojoba oil (0.0147ppm, and 8.5236%ppm respectively). On contrast there was more variation with LC_{50} values of Flaxseed oil, whereas it had value of 4.88 ppm. The present data detected that the newly hatched larvae high susceptible to Pasha than Jojoba oil then Flaxseed oil. According to LC_{50} and LC_{90} values, Pasha is considered the highest impact than Flaxseed oil. To calculate Toxicity Index at the LC_{50} and LC_{90} levels, chemical compound Pasha is taken as the standard compound (which resulted in the least LC_{50} of all tested chemical compounds) and given the arbitrary value of 100 units, the toxicity index of Jojoba and Flaxseed oils at LC_{50} and LC_{90} level were 0.1724 &0.1421% and 0.1083 & 0.1652%, respectively. Results revealed that the newly hatched larvae high susceptible to Emamectin benzoate than Jojoba and Flaxseed oils. According to LC_{50}

and LC_{90} values, Pasha are considered the highest impact than Jojoba and Flaxseed oils.

2. Effect of plant oils and Emamectin benzoate on some biological aspects of Spiny bollworm, *Earia sinsulana* newly hatched larvae:

2.1. No. of dead larvae:

Results clearly showed that Jojoba, Flaxseed oils and Emamectin benzoate highly affected the newly hatched larvae of *E. insulana* when treated by sublethal concentration. The larval mortality progressively increased Emamectin benzoate (80.68; 75.67 &56.33, respectively) compared to untreated check (6.67%). These results indicated that there was significant difference between Jojoba, Flax oils & Emamectin benzoate and control. On the other hand, there was no significant difference between Jojoba, Flax oils treatments.

2.2. Total larval mortality percent: results showed that Jojoba oil have high effect on percentage mortality of newly hatched larvae of *E. insulana* and recorded 68.90% followed by Flax oil with 63.06% then Emamectin benzoate which attained 63.06% larval mortality compared to control. Significant difference between Jojoba and Flax oils and control (6.67%) was found. But there was no significant difference between Flax oil and Emamectin benzoate treatments.

2.3. Mean malformed larvae of *E. insulana*: Data showed that Jojba and Flax oils had nosignificant effects on malformed larvae of spiny bollworm compared with control. The tested Flax oil don't record malformed larvae. Meanwhile, treated spiny bollworm newly hatched larvae using Emamectin benzoate at 0.0147 ppm resulted significant deformed larvae (2.63) compared with control (0.0).

2.4. % malformed larvae of *E. insulana*: The effect of treating spiny bollworm neonate larvae with the tested Jojoba oil was extended

slightly to larval malformation (1.13%). While, Flax oil under study did not record any malformed values in the alive larvae as control. In case of treated larvae with Emamectin benzoate at 0.0147 ppm it caused 2.63% deformed adults compared with control (0.0%).

2.5. Larval duration: Data showed that the tested concentrations of Jojoba and Flax oils caused significant prolongation in larval period compared with untreated larvae. Also, Emamectin benzoate elongated larval duration of *E. insulana* with significant difference between Emamectin benzoate and untreated check.

2.6. Weight of the 4th instars larvae: The latent effects of the natural product compounds, Jojoba and Flax oils on larval weight of *E. insulana* treated with sublethal concentrations were checked. The average weight was decreased insignificantly to 0.0097 g / larva of Jojoba tested compound. Meanwhile, Flax product recorded 0.0086 g/ larvae. Also, results cleared that Emamectin benzoate decreased larval weight significantly where it attained 0.0132g/ larvae at 0.0147 ppm compared to control (0.1031g/ larvae).

2.7. Pupal stage:

a. No. of pupa formed: Results showed that the average number of formed pupae resulted from treated newly hatched larvae of spiny bollworm with sublethal concentrations of the two tested plant oils and Emamectin benzoate were 30.04, 44.33 at and 39.00, respectively compared with 93.33 in control. Significant differences were found between the tested products and control.

b. Pupal mortality percent:

Results of the two tested plant oils and Emamectin compound showed moderately effect on pupal mortality percentage of *E. insulana*. The highest was 36.62% in followed by14.58 % and 13.53% for

Emamectin benzoate compound and Flaxseed oil , respectively compared with 4.63 % in control.

c. Pupal duration: Results show that the pupal period of *E. insulana* treated with sublethal concentrations as neonate elongated to reach 12.00; 10.33 &9.00 days in Jojoba , Flax oils & Emamectin benzoate compound with non significant effect than control.

d. Pupation percentage: The results showed that the average percent of pupation was decresed to reach (89.31%, 76.4% and 73.48%) for Emamectin Jojoba and Flax , respectively compared to 100% in control.

e. Total mortality of immature stage: results clearly obvious that Jojoba oil have high effect on newly hatched larvae of *E. insulana* when fed on treated diet by LC_{50} concentration it recorded 29.67. The larval mortality decreased to 26.00 at Jojoba oil while it was 22.63 for untreated check. These results indicated that there was no significant difference between the three tested compounds. On the other hand, there was significant difference between these compounds and control.

2.8. Adult stage:

a. No. of adult emergence: Results indicated that, the Jojoba and Flax tested extracts and Emamectin benzoate reduced adult emergence 21.38, 38.33 and 33.33 with Jojoba and Flax oils and Emamectin benzoate compared with 89.00 in control. On the other hand, all tested compounds have significant difference with control adult emergence.

b. Mean % of adult emergence: Data showed high pronounced effects on adult emergences % of *E. insulana* treated with Jojoba , Flax oils and Emamectin benzoate which recorded 71.18 %, 86.47% &85.46 % compared to 95.36% in control.

c. .Effect of *E. insulana* larval treatment on the number of male and

female (sex ratio):

Present results cleared the effect of larval treatment with the tested compounds on the sex ratio of *E. insulana*. these data observed that the sex ratio in general was in adequate to females and there is significant difference between all treatments and also with untreated ones.

9. Adult malformation:

Present results showed that the treatment with flaxseed oil caused malformation in the appeared adults(11adults and 28.69%) more than other treatments which were (4.33 adult and 13.41%) in the case of Jojoba oil treatment and 3.33 adult and 9.73%)after the treatment with Emamectin**.**

2.10. Reproductive potential:

Oviposition periods, the total number of deposited eggs (fecundity) and (fertility):

It is clearly obvious that treating newly hatched larvae of spiny bollworm with the Jojoba & Flax oil elongated pre-oviposition period, but in case of Emamectin benzoate treatment this period was shortened than control. Meanwhile, Flax oil elongated significantly post-oviposition period compared with control. Also, results cleared that Jojoba and Flax tested oils shortened female and male longevity periods than control. Male longevity was in par in all tested compounds and control. The total numbers of laid eggs/♀ were inspected in all the tested compounds compared with control as 118.0, 48.27, 102.0 and 197.92/♀ eggs/female, respectively. But the tested compounds caused moderately decrease of hatchability % in all tested compounds and untreated check (56.83, 60.46,65.32 and 95.66 %, respectively). Also, data cleared significant differences between the tested compounds and control**.**

3. Efficiency of the tested compounds on some biochemical aspects in *Earias insulana* larvae:

It is important to evaluate Total proteins, total lipids and total carbohydrates in the 4th larval instar of *E. insulana* treated as newly hatched larvae with LC_{50} of the tested compounds because these biochemical components are considered essential necessary for growth and reproduction. Data showed the concentration of (TSP), (TSL) and the changes as a percentage in treated larvae compared with untreated larvae. The protein level increased in Jojoba oil and Flaxseed oil treated larvae in comparison to the untreated control. It recorded (7.93%) and (4.50%), respectively compared to control. But in Pasha treated obtained protein level decreased to reach (-15.57%) compared to control.

3.1. Total proteins, total lipids:

Total proteins, total lipids and total carbohydrates are evaluated in the 4th larval instar of *E.insulana* treated as newly hatched larvae with LC50 of the tested compounds. The protein level increased in Jojoba oil and Flaxseed oil treated larvae in comparison to the untreated control. It recorded 96.53 (7.93%) and 93.46 (4.50%), respectively compared to 89.43 in control, but in Pasha treated larvae obtained protein decreased to reach to75.5 (-15.57%). compared to control. In the same trend, results of total lipid were evaluated in larvae treated as newly hatched larvae with LC_{50} of Pasha compound its value recorded 20.11 mg/ml (862.2%) compared to 2.09 mg/ml in untreated larvae, while lipids level in jojoba, Flaxseed oil treatments decreased to reash1.98 mg/ml (-5.26%) and1.85 mg/ml (-11.48%) compared with control.

3.2. Total Carbohydrate enzymes:

Data revealed that the carbohydrate level increased in both Jojoba and Flaxseed oil treatment in comparison to the untreated control. It recorded 23.13 mg/ml (39.33%), 25.13 mg/ml (51.38 %), respectively. but in Pasha compound the level was the lowest and reach 8.40 mg/ml (-49.39 %) compared to control (16.60 mg/ml).

3.3. Transaminase enzymes (ALT) and (AST):

Treated and un treated *E. insulana* larvae samples of larvae were chosen after 10 days of treatment expressed as (mg/ml)) and (Change %) in treated larvae with the three tested compounds at LC_{50} and untreated ones. The highest effect was recorded in larvae treated with Jojoba oil 9237.66 and (359.81 %) following by 2588.66 and (28.85%) in Flaxseed oil treatment while in case of Pasha treatment massive drop occurred to reach 6.33 (-99.68%) compared to 2009 in control. ALT activities in treated and untreated larvae of *E. insulana* to clarify the effect of the tested compounds was recordedas follows: 4043 and (17.73%) in Jojoba oil following by 3464.66 and (0.89 %) in Flaxseed oil, but it was 1178 and (-65.69%) of Pasha compound.

3.4. Lactate dehydrogenises catalyzes (LDH) and alkaline phosphates activities in *E. insulana* larvae.

The obtained results showed that LDH were decreased in all treated larvae after ten days from treatment with Jojoba oil, Flaxseed oil and pasha compounds to reach (-22.14%), (-4.62%) and (-34.37 %), respectively than untreated larvae. In addition, the results cleared that the same trend was noticed in the Alkaline phosphatase levels which decreased in all treatments and reached (-41.57 %) in Pasha, (-34.94 %) in Flaxseed oil and (-19.19 %) in Jojoba oil compared with 4.95 in control.

d. Lactate dehydrogenises catalyzes (LDH) and alkaline phosphates (A P) activities.

The changes in (LDH and AP) in the supernatant of the homogenated larvae of spiny bollworm treated with sub lethal concentrations of Jojoba, Flaxseed oils and Emamectin benzoate were evaluated. After ten days from treatment of newly hatched larvae with Jojoba oil, Flaxseed oil and pasha compounds LDH were decreased in all treated to reach355.9 µg pyruvate /ml (-56.80%) in Pasha,518.66 µg pyruvate /ml (-37.05%) in Flaxseed oil and578 µg pyruvate /ml (-29.85%) in Jojoba oil and also alkaline phosphates enzyme activity highly decreased to reach 2.6 µg pyruvate /ml (-73.63%) in Pasha compound followed by 6.4 µg pyruvate /ml (-35.09%) in Flaxseed oil. The lowest decrease was recorded in Jojoba oil 9.73 µg pyruvate /ml (-1.31%) compared to (9.86 µg pyruvate /ml) in the control.

II- Histological studies:

histological procedures were done for both *Pectinophora gossypiella* and *Earias insulana* larvae to show the histopathological changes occurred in structure of cells and tissues, as a result of jojoba oil, flaxseed oil and pasha compound treatments for the two insects in comparison to untreated check.

1. Effect on cuticle:

1.1. Untreated larvae:

The cuticle layer of both *P. gossypiella* and *E. insulana* untreated larvae indicated normal histological structure with no histopathological alteration of the outer corrugated surface and the underlying epidermis, with protruded spines.

1.2. Jojoba oil treatment:

Treating *P. gossypiella* larvae with Jojoba oil showed necrosis with dark basophilic structure in the cuticle layer in addition, a loss of spines as well as the corrugated outline were observed. The effect was obvious also in *E. insulana* treated larvae as the outer layer of cuticle was thin compared to control and showed protruded spines.

1.3. Flaxseed oil treatment:

Treating *P. gossypiella* larvae with Flaxseed oil showed necrosis and the cuticle layers appeared thin with lose of the spines on contrast, there was no effect on *E. insulana* larvae.

1.4. Pasha Treatment:

Data cleared that necrosis were detected in the outer layer of the cuticle for *P. gossypiella* larvae after Pasha compound treatment. Also, auscultating showed the same effect in *E. insulana* treated larvae in addition to much loss of the spines.

2. Effect on glandular Structure:

2.1. Untreated larvae:

A normal histological structure with normal histological structure with no glandular structure alteration was recorded for both *P. gossypiell*a and *E. insulana* untreated larvae. Normal cells with centrally located nuclei and eosinophilic cytoplasm were also detected.

2.2. Jojoba oil treatment:

Treating *P. gossypiella* larvae with Jojoba oil showed laceration in the cells with lose of the nuclei. Also, the effect was obviously clear in *E. insulana* treated larvae as a vacuolization in the cytoplasm of the cells.

2.3. Flaxseed oil treatment:

Treating *P. gossypiella* larvae with Flaxseed oil resulted in cellular swelling associated with cytoplasmic vacuolization and lose of the nuclei. But in case of *E. insulana* treated larvae, the cytoplasmic vacuolization was greatly obvious.

2.4. Pasha Treatment:

Treating ***P. gossypiella*** larvae with Pasha compound showed granular eosinophilic cytoplasm as well as fat vacuoles. However, in case of *E. insulana* treated larvae Pasha compound showed intact histological structure of the nuclei and cytoplasm of the cells.

3. Effect on mid gut:

3.1. Untreated larvae:

The mid gut histological structure of untreated larva indicated normal the lining mucosal epithelium with eosinophilic cytoplasm and tall basophilic nuclei for both *P. gossypiella* and *E. insulana*, respectively.

3.2. Jojoba oil treatment:

Treating *P. gossypiella* larvae with Jojoba oil caused diffuse necrosis and lose of histological details of the lining epithelium. The effect was visible also in *E. insulana* treated larva as atrophy in the lining mucosal epithelium.

3.3. Flaxseed oil treatment:

Treating *P. gossypiella* larvae with flaxseed oil was noticed as mild vacuolization of the hypertrophied mid gut lining epithelial cells. Similar effect was observed in *E. insulana* treated larvae for the same treatment.

3.4. Pasha treatment:

Vacuolization in the cytoplasm of the tall hypertrophied lining mucosal epithelial were detected associated with thick outer cuticle layer when *P. gossypiella* treated as larvae in addition to the basophilic cytoplasm of the atrophied lining epithelium in case of *E. insulana* treated larvae.