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Biochemical and Histological Effects of some Natural Plant Essential Oils on Pink Bollworm, *Pectinophora gossypiella* (Saund.) and Spiny Bollworm, *Earias insulana* (Boisd.) Larvae

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ABSTRACT

Biochemical and histological studies were executed on 4th larval instar larvae of the Pink bollworm (PBW) and the spiny bollworm (SBW) treated as newly hatched larvae with LC₅₀ for two tested oils (Jojoba oil, and flaxseed oil). Results noticed that Jojoba oil and flaxseed oil decreased protein levels in *Pectinophora gossypiella* by 35.92 and 20.73% as compared with control, respectively. On the other hand, they caused increases in protein levels in *Earias insulana*, by 7.93% for Jojoba and by 4.50% for Flax. The total soluble lipids were estimated by -44.82 and 45.86%, respectively for *P. gossypiella*, while they were -5.26 % in Jojoba and -11.48 in Flaxseed oil for *E. insulana*. Carbohydrate hydrolyzing enzymes activity was clearly increased by both treatments on *E. insulana*, while variations impacts on *P. gossypiella* were recorded (-34.19 % for Jojoba oil and 6.95% for Flaxseed oil). General increases in GOT and GPT transaminases activity were recorded in *P. gossypiella* and *E. insulana* by due to the used oil treatments, but reduction percentages were found in alkaline phosphates as well as for Lactate dehydrogenase catalyzes (LDH) when the investigated pests treated with Jojoba and Flaxseed oils. Also, the histopathological disturbance was estimated in this study when neonate larvae fed on diet treated with LC₅₀ value of Jojoba oil and flaxseed oil. The cuticle and mid-gut tissues of the 4th instar larvae showed vacuolization with hypertrophied lining mucosal epithelial, and the epithelial cells are blurred and Necrosis of nuclei.

Keywords: *Pectinophora gossypiella*, PBW; *Earias insulana*, SBW; Jojoba oil, Flaxseed oil, biochemical studies and histopathological studies.

INTRODUCTION

The pink bollworm, *Pectinophora gossypiella* (Sound.) and the spiny bollworm, *Earias insulana* (Boisd.) are considered the most important economic insect pests in Egypt and most cotton producing countries over the world. (Abdel-Salam, and Negm 2009). The PBW and SBW larvae damage squares, flower buds, flowers and bolls and cause marked reductions in the quality and quantity of the lint and oil of the obtained yield.

Essential oils are volatile, natural, complex compounds with strong odor and are usually formed by plants as secondary metabolites. In nature, essential oils play an important role in protection of the plants against harmful insects through reducing their appetite for such plants. They also, may attract some insects to errand the dispersion of pollens and seeds or repel undesirable others (Bakkali *et al.*, 2008).

The aim of this work is to assess some biochemical changes and to observe the histopathological effects on the cuticle and mid-gut of Pink bollworm (PBW), *Pectinophora gossypiella* (Saund.) and spiny bollworm (SBW), *Earias insulana* (Boisd.) 4th instar larvae after using LC₅₀ of Jojoba oil and Flaxoil against neonate.

The authors are highly interested to answer the question of whether the tested compounds have marked effects on PBW & SBW metabolites and structures or not to find new natural materials to apply them in biological control of these dangerous cotton pests.

MATERIALS AND METHODS

1- Tested insects:

The newly hatched larvae of pink bollworm used in this study obtained from a susceptible laboratory colony established in Bollworms Department, Plant Protection Research Institute, Dokki, Giza. This colony was reared on a semi artificial diet as described by Rashad and Ammar (1985). Rearing conditions were controlled at 27±1°C and 65-75% R.H. Spiny bollworm larvae were obtained from susceptible strain established in Bollworms Department, Plant Protection Research Institute, AlSharkya, Zagazig. This strain was reared on a modified artificial diet as described by Amer (2015) under laboratory conditions of 26 ± 1 °C and 70 ± 5 % R.H.

2-Tested oils:

The jojoba oil is liquid wax ester oil extracted from its seed (S.N: *Simmondsiac hūnensis*, family: Simmondsiaceae). It makes up approximately 50% of the jojoba seed by weight.

Flaxseed oil is a colorless to yellowish oil obtained from the ripened seeds of the flax plant (*Linum usitatissimum*: Fam Linaceae.) by cold mechanical pressing seeds, sometimes followed by solvent extraction. The used oil was obtained from Oils Press Unit in National Research Center. The oils were obtained by cold mechanical pressing.

3-Toxicity tests:

Trial experiment was assumed to calculate LC₅₀ for the tested oils using the probit software program according to Finney (1971). Serial concentration dilutions (0.5, 1.0, 2.0, 4.0, 7.0 %) for jojoba and (10, 20, 30, 40, 50%) for flax oil on *P. Gossypiella* &

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(3.75,7.53,15.30 %) for jojoba and (10,20,30,40,50%) for flax oil on *E. insulana* were prepared from the stock solution of Jojoba oil and Flax oil using droplets of Triton asan emulsifying agent. In addition, untreated replicates were used as control. The LC₅₀ was 0.9770% for Jojoba oil and 23.6195% for Flax oil on *P. gossypiella*. In case of *E. insulana*, LC₅₀ was 8.2059% and 13.5692%, respectively.

4-Biochemical Studies:

For the biochemical studies, newly hatched larvae of *P. gossypiella* and *E. insulana* were treated with LC₅₀ of the tested oil safter10 days larvae/each treatment as the untreated larvae (control) were transferred individually to glass tubes (2 x 7 cm) and kept in a refrigerator (7±1°C) for chemical analysis. Larvae were chemically analyzed for each compound with untreated check(control) in Physiological Dept. of plant Protection Researches Institute, (P.P.R.I.).Total protein, total lipids, total carbohydrate, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), Lactate dehydrogenase catalyzes (LDH) and alkaline phosphatases were determined colorimetrically according to Reitman and Frankle (1957), Knight et al. (1972), Bradford, (1976), Ishaaya and Swirski (1976), &DGCK (Deutsche Gesellschaftfür Klinischechemie,(1972).

5-Histological Studies:

The histological studies were conducted on newly hatched larvae of *P. gossypiella* and *E. insulana* using the feeding technique method at the LC₅₀ concentrations of Jojoba oil and Flaxseed oil. After ten days from treatments, samples were taken and fixed in 10% formolsaline for 12 hours followed by the rest of

the histological stages of making sectors. The obtained tissue sections were collected on glass slides and then deparaffinized, stained by hematoxylinand eosin stain for routine examination through the light electric microscope (Banchroft et al., 1996). To compare the histological changes, specimens from the control of the treatment were sampled.

RESULTS AND DISCUSSION

Only newly hatched larvae of *P. gossypiella* and *E. insulana* were treated with the LC₅₀ concentration of Jojoba oil and Flaxseed oil for bio-chemical assays to evaluate the total soluble protein, total lipid, Carbohydrate, the activities of transaminases; Aspartate aminotransferase (AST or GOT) and Alanine aminotransferase (ALT or GPT) , Lactate dehydrogenase catalyzes(LDH) and alkaline phosphatases.

Latent effects of Jojoba oil and Flaxseed oil on the activity of some enzymes of *P. gossypiella* are shown in Table (1). The protein levels were decreased in both transactions' comparison to the untreated control. They ranged from 118.5 mg/ml in the control to 75.93mg/ml for Jojoba oil and 93.93 mg/ml for Flaxoil. Total lipid was increased significantly in Flaxseed oil treatment (4.23±0.14 mg/ml) than control (2.90±0.26 mg/ml), whereas it was decreased in Jojoba oil treated than control (1.6±0.067 mg/ml). TotalCarbohydrate was recorded moderate increased inFlaxseedoil treatment (42.033±1.31 mg/ml) while the effect ofJojoba oil was decreased to (25.86±0.45 mg/ml) compared with for untreated larvae (39.3±0.96 mg/ml).

Table 1. Enzyme activities of *Pectinophora gossypiella* 4th instar larvae after treatment with Jojoba and Flaxseed oil.

Enzymes	Control Mean±S.E	Jojoba oil Mean±S.E	C %	Flax oil Mean±S.E	C%
Total soluble protein	118.5±2.02	75.93±1.51	-35.92	93.93±0.74	-20.73
Total lipid	2.90±0.26	1.6±0.067	-44.82	4.23±0.14	45.86
Carbohydrate	39.3±0.96	25.86±0.45	-34.19	42.033±1.31	6.95
GPT	63.66±1.85	102.33±3.38	60.74	91.33±1.21	43.46
GOT	228.66±145.9	303.66±67.9	32.79	368.66±34.16	61.22
LHD	824.0±12.22	578.0±11.135	-29.85	518.66±8.74	-37.05
Alkaline phosphatase	9.86±0.18	9.73±0.436	-1.31	6.4±0.241	-35.09

SA (Specific activity) as (µg pyruvate /ml), Concentration expressed as (mg/ml) and $C\% = \frac{\text{Change } \%}{\text{Control}} = \frac{\text{Treatment} - \text{Control}}{\text{Control}} \times 100$

GPT activities were significantly increased and were 102.33 µg pyruvate /ml when under Jojoba oil following by 91.33 µg pyruvate /mlin Flaxseed oil treatment compared with in the control (63.66µg pyruvate /ml). GOT activities were greatly high in Flaxseed oil treatment following by Jojoba oil (368.66 and 303.66µg pyruvate /ml), respectively than control (228.66µg pyruvate /ml).LDH enzyme decreased significantly in Jojoba, Flaxseed oil treatment (578 and 518.66mU/g.b.wt) compared to control (824mU/g.b.wt). Alkaline phosphatase was increased in control and Jojoba oil treatments but decreased in Flaxseed oil treatment. These results regarded with Dahi et al.2009, who revealed that alkaline phosphatase enzymes decreased significantly when 4 instar of *S. littoralis* larvae after 24 hours were treated with the LC50 of radiant. Hunge et al. (2004) reported that the protein level was significantly impacted when *S. littoralis* larvae treated with azadirachtin. This episode was due to the freedom of proteins to free amino acids. Elbarky et al.2008indicated that the total protein level in *S. littoralis* was decreased compared to control when it was treated with the LC₅₀ of radiant. Reda et al. (2013) indicated that the use of Tar oil at 95% against 4th instar *P. gossypiella* larvae caused marked changes in total protein, carbohydrate and lipids. Results showed that total protein and carbohydrate in larvae was equally match with those from the control but total lipids in treatments were un-significantly differed from the control. Kawakibetal., (2017) evaluated four potential plants seed extracts (*Latana camara*, *Sapindus trifoliatus*, *Solanum trilobatum* and *Ceiba*

pentandra) on two lepidopteran larvae species (*Trichoplusia ni* & *Pieris brassicae*) results showed high effect on phytochemicals like alkaloids, flavonoids and saponins in *apindus trifoliatus* and *Solanum trilobatum*. Also, Ali et al. (2017) evaluated the effect of garlic and lemone oils on some biochemical aspects of *S. littoralis* larvae. They found significantly decrease in total lipids while the carbohydrate levels increased. Their result is consistent with the present existing study.

In the same trend, and according to Table (2) some enzyme activity in *E. insulana* are shown the total protein level were significantly increased in Jojoba oil treatment following by Flaxseed oil (96.53 &93.46mg/ml) compared to control (89.43 mg/ml). in addition, total lipid level showed decrease in both treatment which recorded (1.98 and1.85 mg/ml) compared to control. On the contrary, the carbohydrate activates greatly increased in treatments than untreated larvae as indicated in table (2). The highest effect of Jojoba oil(9237.66 µg pyruvate /ml) and go down to reach 2588.66 µg pyruvate /ml) in Flaxseed oil treatment compared to in control (2009 µg pyruvate /ml). As for the effect on GOT activities, it was (4043 µg pyruvate /ml) in Jojoba oil treatment following by Flax oil (3464.66 µg pyruvate /ml). LDH enzyme was significantly increased in control (137mU/g.b.wt) compared to Jojoba and Flaxseed oil treatments. However, equal effect was recorded in Alkaline phosphatase activities. These results are in agreement with the findings of Hemat (2016) who tested two plant extracts against ¹⁵larvaeof *P. gossypiella* and *E. insulana* with LC₅₀ values to evaluate some

biological aspects. Yuan et al (2020) studied tannic acid which was used as the standard of plant tannins to determine the effects on nutritional indices and activities of glutathione S-transferases (GSTs), and acetyl choline esterase (A Ch E) in fourth-instar larvae of *Hyphantria cunea* (Drury) by feeding on an artificial diet containing tannic acid under different treatments. Inhibitory effects have been observed at high concentrations (>2.5%) and

the results proved that tannic acid had a significant effect on the activity of detoxification enzymes and A Ch E in the tested larvae. Also, Suzan and Sara (2018) evaluated the biochemical effects of four aromatic oils; garlic, mint, eucalyptus, and lavender oils against 2nd and 4th instar larvae. Results provide that the tested oils have inhibitory effects enzymatic activity.

Table 2. Enzyme activities of *Eariasinsulana* larvae after treatment with Jojoba and Flaxseed oil.

Enzymes	Control Mean±S.E	Jojoba oil Mean±S.E	C%	Flaxseed oil Mean±S.E	C%
Total soluble protein	89.43±2.29	96.53 ±2.18	7.93	93.46±2.19	4.50
Total lipid	2.09±0.073	1.98±0.05	-5.26	1.85±0.76	-11.48
Carbohydrate	16.6±0.78	23.13±0.41	39.33	25.13±1.04	51.38
GPT	2009.0±22.11	9237.66±288.6	359.81	2588.66±50.5	28.85
GOT	3434.0±145.96	4043.0±34.16	17.73	3464.66±67.9	0.89
LHD	137.0±6.55	106.66±5.36	-22.14	130.66±3.39	-4.62
Alkaline phosphatase	4.95±0.11	4.0±0.11	-19.19	3.22±0.076	-34.94

SA (Specific activity) as (µg pyruvate/ml), Concentration expressed as (mg/ml) and $C\% = \frac{(\text{Change } \%) - \text{Treatment} - \text{Control} \times 100}{\text{Control}}$

Histopathological results

The midgut is the area of most active digestion (Chapman, 1985). The digested food absorbed through the midgut tissue into the surrounding haemocoel then diffused to the different body parts. Histological sections were checked up to display the histopathological changes on cuticle and mid gut of *P. gossypiella* and *E. insulana* larvae after 10 days from their feeding on diet treated with the LC₅₀ of Jojoba oil and Flaxseed oil.

Histological changes in cuticle and Mid Gut of *Pectinophora gossypiella* and *Earias insulana* larvae

Cuticle and spines of untreated *P. gossypiella* and *E. insulana* larvae showed that normal histological structure with spines and Corrugated surface and underlying epidermis (Figs. 1 and 2). On the other hand, Jojoba oil treatment showed lost in spines, the outline was crimp and Necrosis with dark basophilic structure in the cuticle layer of *P. gossypiella* larvae. In addition, a thin cuticle layer with protruded spines on *E. insulana* was

shown in Figs (3&4). Also, showed the cuticle appeared thin with lost in the spines, the cells are blurred and Necrosis when *P. gossypiella* larvae treated with Flaxseed oil (Fig5), while, *E. insulana* larvae treated with Flaxseed oil showed a normal histological structure of the corrugated surface with osmophilic spines (Fig. 6).

The midgut of untreated *P. gossypiella* larvae cleared that, normal histological structure of the lining epithelium eosinophilic cytoplasm and tall basophilic nuclei (Fig. 7). The untreated larvae of *E. insulana* (Fig. 8) showed mucosal tall lining epithelium with eosinophilic cytoplasm and enlarged tall nuclei. The effect of jojoba oil on *P. gossypiella* larvae had widespread necrosis and lost in the lining epithelium (Fig. 9). *E. insulana* larvae were visual as atrophy in the lining mucosal epithelium (Fig. 10). On the same trend, treating by Flaxseed oil had equal effect on both pests, which observed in simple vacuolization with hypertrophied lining mucosal epithelial (Figs. 11 and 12).

Histological Changes on cuticle of *Pectinophora gossypiella* and *Earias insulana* Larvae.



Fig. 1. control of *Pectinophora gossypiella* larvae

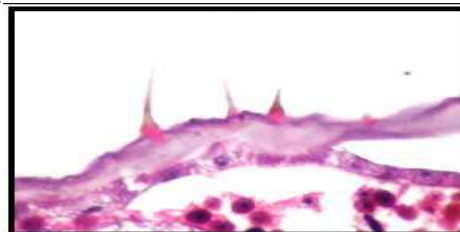


Fig. 2. control of *Earias insulana* larvae

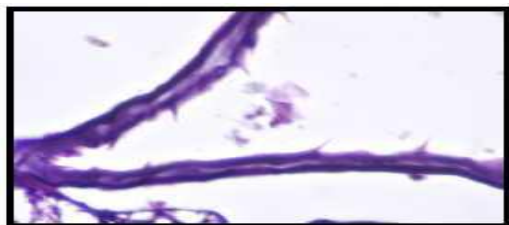


Fig. 3. cuticle of *Pectinophora gossypiella* larvae treated with jojoba oil.



Fig. 4. cuticle of *Earias insulana* larvae treated with jojoba oil

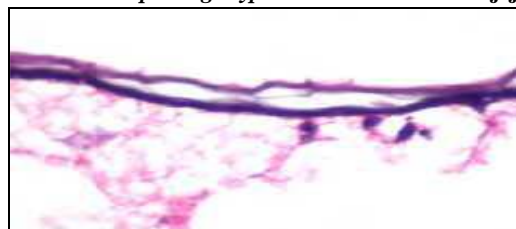


Fig. 5. cuticle of *Pectinophora gossypiella* larvae treated with Flaxseed oil.



Fig. 6. cuticle *Earias insulana* larvae treated with Flaxseed oil.

Histological Changes on Mid Gut of *Pectinophora gossypiella* and *Earias insulana* Larvae.



Fig. 7. The mid gut of untreated *Pectinophora gossypiella* larvae showing normal histological structure (control)

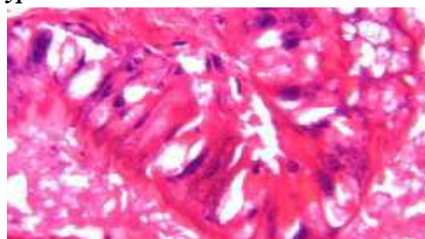


Fig. 8. The mid gut of *Earias insulana* untreated larvae showing normal histological structure (control)

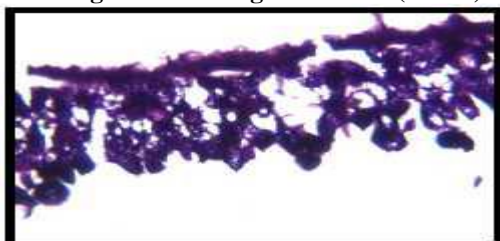


Fig. 9. The mid gut of *Pectinophora Gossypiella* larvae treated with jojoba oil.

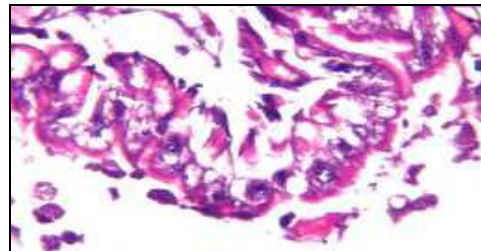


Fig. 10. The mid gut of *Earias insulana* larvae treated with jojoba oil.



Fig. 11. The midgut of *P.gossypiella* larvae treated with Flaxseed oil.



Fig. 12. The mid gut of *E. insulana* larvae treated with Flaxseed oil

Younes *et al.* (1999) noticed degradation in the epithelial cells and decay of its boundaries when *S. littoralis* larvae treated with extracts of both *Clerodendroinerm*e and *Conyzadioscoridis* and they caused fading of the boundaries of epithelial cells, and split of epithelium. Abd-El Wahed *et al.* (2011) studied the mid gut histological sectors of 4th or 6th instar of *S. littoralis* larvae and found efficient deviations in their mid-gut layers when they treated with LC50 of protecto, *B. thuringiensis*. Riawet *et al.* (2011) declared histological changes evidence (vacuolated and necrosis the epithelial cells) in midgut of *S. littoralis* larvae when they treated with *Azadirachtinindica* and *Citrus*. Sharaby *et al.* (2012) tested the toxic effect of three different natural essential oils of medicinal plants, namely Garlic (*Allium sativum*), Mint (*Minthapipereta*) and Eucalyptus (*Eucalyptus globulus*) on 1st nymphal instar of the grasshopper (*Heteracris littoralis*). Their results indicated that Garlic oil affected the epithelium of the treated nymphs. In addition, they recorded histological changes on the alimentary canal and fat bodies. Abd El-Mohsen *et al.* (2013) found some histopathological changes after testing efficiency of certain insecticidal sequences against pink and spiny bollworms larvae like Azadirachtin and *Bacillus thuringiensis*. With regard to the histopathological effects of the tested biocides, midgut histological sections were executed on the 2nd and 4th instar larvae of Pink bollworm. Mossad *et al.* (2016) showed severe histological changes in livers of rats when the rats were fed on low dose of Jojoba seed extract. These findings are consistent with those reported by El-Shewy (2018) who found histological changes in livers, kidney and stomach when 4th instar larvae of *Agrotis ipsilon* treated with LC50 of (crude - Nano) Jojoba oil.

In general, the present study proved that the two tested essential oils decreased the total soluble protein of the

4th larvae of PBW and/or SBW. Carbohydrate enzymes were inhibited in SBW treated with Jojoba, while the activity of GOT and GPT was increased and reduction in alkaline phosphates & Lactate LDH was recorded in both tested insects. It was clearly obvious shown the different enzymes was agreement with their effect on midgut membrane. The disturbances of the enzyme's activities in the treated larvae due to the damage of midgut epithelial tissues.

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دراسات بيوكيميائية وهستولوجية لبعض مستخلصات الزيوت النباتية وتأثيرها على يرقات دودة اللوز القرنفلية ودودة اللوز الشوكية

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أجريت بعض الدراسات البيوكيميائية على يرقات طور الرابع لدودة اللوز القرنفلية (PBW) ودودة اللوز الشوكية (SBW) التي عوملت كيرقات حديثة القفص بالتركيز القتال ل50% من الزيوت المختبرة (زيت الجوجوبا وزيتبذرة الكتان). أشارت النتائج إلى أن المعاملة بزيت الجوجوبا وزيتبذرة الكتان أدى إلى انخفاض مستويات البروتين في حشرة دودة اللوز القرنفلية بنسبة 35.92 و 20.73% مقارنة بالكونترول على التوالي. من ناحية أخرى، تسببت الزيوت المختبرة في زيادة مستويات البروتين في حشرة دودة اللوز الشوكية بنسبة 7.93% للجوجوبا و 4.50% للكتان. كما تم ملاحظة انخفاض في مجموع الدهون الذاتية بنسبة 44.82% للجوجوبا وزيادة بنسبة 45.86% عند المعاملة بالكتان بالنسبة لحشرة دودة اللوز القرنفلية بينما لوحظ انخفاض في كلا المعاملتين بنسبة 5.26% و 11.48% لدودة اللوز الشوكية للجوجوبا والكتان على التوالي. وقد زادت أنشطة إنزيمات التحلل المائي للكاربوهدرات بشكل واضح عن طريق كلا المعاملتين على حشرة دودة اللوز الشوكية، بينما سجلت تأثيرات مختلفة على حشرة دودة اللوز القرنفلية (بنسبة 34.19% لزيت الجوجوبا و 6.95% لزيت الكتان). كما تم تسجيل زيادة عامة في نشاط الإنزيمات الترانس أمينيز GOT و GPT في دودة اللوز القرنفلية والشوكية عند معامتهم بالزيوت المستخدمة، ولكن وجد نسبة انخفاض في إنزيم الفوسفاتيز القلوي وكذلك في محفزات اللاكتات ديهيدروجينيز (LDH) عند معاملة الحشرتين موضوع الدراسة بزيت الجوجوبا والكتان. وبالإضافة لذلك فقد تم تقدير الإضطراب في التركيب الهستولوجي في هذه الدراسة عند تغذية اليرقات حديثة القفص على بيئة معاملة بالتركيز القتال ل50% من اليرقات لزيت الجوجوبا وزيت الكتان. وأظهرت النتائج أن أنسجة البشرة والأمعاء الوسطى ليرقات طور الرابع لكلا الحشرتين قد حدث فيها فجوات و تضخم في الغشاء المخاطي الطلائي، والخلايا الطلائية غير واضحة ونخر في نواتها.