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Evaluation of Physiological and Biochemical Impacts of Some Essential Oils on *Galleria Mellonella* (Lepidoptera: Pyralidae)

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Abstract

In this study, the toxicity of three concentrations 2, 4 and 6 % of three natural essential oils: Garlic (*Allium sativum*: Alliaceae) , Neem (*Azadirachta indica*: Meliaceae) and Jojoba (*Simmondsia chinensis*: Simmondsiaceae) against the fourth instar larvae of the greater wax worm, *Galleria mellonella* L. (Lepidoptera : Pyralidae) were evaluated as well as the mortality percentages were computed. Mortality percentages were high at Garlic oil treatments, followed by neem and Jojoba compared to control. In addition, the values of LC_{50} were 1.983, 2.842 and 3.958% for Garlic, Neem and Jojoba essential oils, respectively. Regarding enzyme activites was Lactate Dehydrogenase activity (LDH), Glutathione S-Transferase (GST), ATPase, acetyl cholinesterase and Alpha esterase's were measured in the 4th instar larvae treated with the forementioned essential oils. The results indicated an increase in the LDH activity in all treatments compared with control. Also increase in the activity of GST and Alpha esterase's activity were recorded after treatment with jojoba and Garlic oils and decrease after treatment with Neem. Also, the results showed a decrease in ATPase activity after treatment with Neem and Jojoba oils and increase after treatment with Garlic oils. Results revealed decrease in the activity of acetylcholinesterase enzymes in all treatments. The garlic oil come first followed by neem oil and jojoba come last.

Keywords*:* Waxworm, *Galleria menonella*, Essential oils, enzymes, histological, biochemical Studies.

Introduction

One of the honeybees' most beneficial products is wax. Cosmetics, dentistry, and the pharmaceutical industry all use it. Wax is attacked by a variety of pests because it contains a lot of nutrients, pollen, and honey **(Nurullahoglu and Susurluk, 2001).** The greater wax worm, *G. mellonella*, is recognized as the most harmful and economically significant pests affecting wax on a global scale. **(Burges, 1978; Cantwell and Smith, 1970).**

Afew insects, including *Galleria mellonella*, is a common annoyance of *Apis mellifera* Linnaeus and *Apis cerana* Fabricius are honeybees. Larvae of greater wax moths burrow into the edge of cells that are not sealed. through the bee comb's midrib with pollen, bee brood and honey.

Currently, chemical pesticides are the most practical approach to insect pest control because of its ease of use and efficacy. But the intense and careless application of these pesticides has regrettably resulted in detrimental impacts on humans and environmental well-being. Therefore, more research is required to identify bee-safe control methods. In Africa, vegetation and Veterinary ethnomedicine makes extensive use of derivatives. **(Adenile, *et al*.2022).**

Natural essential oils show strong insecticidal effects against wax moths while posing low toxicity risks to honeybees. This approach facilitates their incorporation into integrated pest control programs to combat wax worm infestations in the colonies of honey bee and storage areas, ensuring honeybee products remain free from pesticide residues. Essential oils are distinguished by their regional accessibility. Furthermore, take note that these plants are commonly used for their insect-repelling properties, larvicide, and nematicide qualities, but it's also used to treat skin disorders and other illnesses. **(Sarah and Ourida, 2019); Olanipekun *et al*., 2018 and Ouraini *et al*., 2005)** Furthermore, bees in particular are not at risk of toxicity from these essential oils, **(Chenni.2016 and Chemat *et al*., 2012).** Therefore, this work focused on assessing how three essential oils influence the physiological and biochemical parameters of the greater waxworm.

Materials and methods

1. The tested materials show that in table (1)

English name	Scientific name	Family	Used part
Garlic	***Allium sativum***	**Alliaceae**	**Oil**
Jojoba	***Simmondsia chinensis***	**Simmondsiaceae**	**Oil**
Neem	***Azadirachta indica***	**Meliaceae**	**Oil**

2. Rearing Techniques for *Galleria mellonella:*

The strain of the greater wax moth, *Galleria mellonella* was obtained from the domestic hives in Faculty of Agriculture, Benha University and reared according to (**Hussein, 2004**). The larvae of the greater wax moth, *G. mellonella* were reared on a semi-synthetic diet composed of 22% wheat-flour, 22% corn groats, 11% honey, 11% milk powder, 11% glycerol, 5.5% yeast powder (**Wiesner, 1993**). The larvae were obtained from beehives and transferred to transparent plastic rearing jars (17 ×17 × 27 cm); containing 250 gm. from the previous prepared the media, closed with a lid of muslin for aeration and incubated at 28 ± 2°C and 65 ± 5% R.H. when larvae grown to the pupal stages and then to the adult moths, apiece (15 ×15 cm) of paper tissue was folded and placed in the container to promote egg laying. Eggs were laid on the lid and a paper tissue. These eggs were gently removed and transferred to another rearing jar containing 250 gm. The media closed tightly with a double muslin layer to prevent the escape of neonatal larvae then add fresh food frequently (1-2) times per week.

3. Preparation of Essential oils:

Three commercially Essential oils were tested in this study. Garlic oil (*Allium sativum*), Neem oil (*Azadirachta indica)* and Jojoba Oil (*Simmondsia chinensis*). The tested oils were purchased as pure oil from National Research Centre. The oils were extracted from the dried plants by steam distillation. Concentrations of 2,4 and 6 % from the three essential oils were prepared for application **Experimental Technique**: 5 grams of the semi artificial diet are mixed with 1 ml of the various concentrations previously prepared from the three essential oils. and another 5 grams of the semi artificial diet was treated only by Tween as control. The treated diet was placed in a glass jar and incubated at 30°C and 65% R.H. three replicates from each concentration and control (tween as control) were used, each containing 10 4th instar larvae of *G. mellonella*. Post-treatment numbers of dead and alive larvae were daily counted for 7 days.

4. Toxicological studies:

Larval mortality and determination of LC_{50} of the tested three essential oils were recorded at three concentrations 2, 4, 6% which dissolved on Tween and dispersed by shaking, then added to the artificial diet as one cm^3 / 5 g diet /containers, while control ones had Tween only. There were three replicates of each treatment with 10 larvae of each replicate. The fourth instar larvae were used for these treatments and determination of LC_{50}. The corrected mortality percentage of each material was statistically computed **according to finney (1971).**

5. Biochemical studies:

5.1. Preparation of samples for biochemical assays:

Larval specimens intended for biochemical assays were collected 48 hours after exposing the 4th instar *G. mellonella* larvae to the LC_{50} concentration of the all compound. Untreated caterpillar served as the control group. During each trial, the larvae were maintained in a sterile jars. Samples were blended in distilled water using a Teflon homogenizer, followed by centrifugation at 600 rpm for 15 minutes at 5°C. Enzyme activities, including AChE, LDH, GST, ATPase, and α-esterases, were measured in the freshly obtained supernatants

5.1.1. ATPase activity.

ATPase activity was quantified following the protocol described by **Amaral *et al.* (2001).**

5.1.2. Lactate dehydrogenase (LDH) determination.

The method outlined here is based on the formulation endorsed by the German Society for Clinical Chemistry **(DGKC, 1972).**

5.1.3. Nonspecific esterases.

Alpha-esterase (α-esterase) and beta-esterase (β-esterase) activities were assessed following the method described by **Van Asperen (1962)**.

5.1.4. Acetylcholinesterase (AChE) activity.

Measured using the method outlined by **Simpson *et.al. (*1964**).

5.1.5. Glutathione S-transferase(GST).

Glutathione S-transferase (GST) catalyzes the conjugation of reduced glutathione (GSH) with 1-chloro-2, 4-dinitrobenzene (CDNB) through the sulfhydryl (-SH) group of glutathione. The resulting conjugate, S-(2,4-dinitrophenyl)-L-glutathione, was quantified following the procedure described by **Habig *et al.*(I974)**.

6. Histological studies:

After treatment the tested insects with LC_{50} concentration the larvae sampled after 48 hours post treatment and the larvae were placed in bouin's solution, serving as a fixative to aid in dehydration and eliminate its yellow coloration. Following this, the larvae were washed using ethanol solutions of varying concentration. Afterwards, they were placed in a 50% ethyl alcohol solution for one hour and subsequently left undisturbed for 24 hours. The caterpillars were then subjected to a series of alcoholic treatments at room temperature over a period of two hours, beginning with 80% ethyl alcohol, followed by 90%, 96%, and finally 100%. Following dehydration, the catebller were immersed in a mixture of amyl acetate solution and soft paraffin wax and left at 50°C for 24 hours. Subsequently, the caterpillars were transferred into fresh soft paraffin wax three times, each at 48-hour intervals, again maintaining a temperature of 50°C. In the final step, one part of hard paraffin wax was incorporated into the process. The larvae were embedded in the wax mixture prepared during the last stage. Using a microtome, serial sections of 5 microns were carefully prepared and mounted onto Sterile slides covered with Mayer's albumin. The slides were then stained with hematoxylin, counterstained in an alcoholic solution, and made ready for examination and photomicroscopy**.**

7. Statistical analysis:

The statistical analysis was carried out using two-way ANOVA using SPSS, ver. 27 **(IBM Corp. Released 2013)**.

Data were treated as a complete randomization design according to **Steel *et al.* (1997)**. Multiple comparisons were carried out applying **Duncun test** The significance level was set at < 0.05.

Results and Discussion

1. Insecticidal activities of the tested plant essential oils against 4th instar larvae of the Greater wax worm, *Galleria mellonella* in laboratory:

1.1. Neem essential oil.

Results in Table (2) and Figure(1) cleared that after 1,2,3,5, and 7 days the mortality percentage were 6.67, 13.33, 33.33, 50.00 and 76.67% for concentration 2% of the plant essential oil Neem and 16.67, 23.33, 40.00, 63.33and 73.33% for the 2nd concentration 4% while for the third concentration 6% give 16.67, 23.33, 53.33,60. 83.33 and 96.67% mortality compared with control which were 0.00, 0.00, 0.00, 0.00 and 3.33% after 1, 2, 3, 5, and 7 days respectively. From the same table the means of treatments for the three plant essential oils and the untreated one control, it was clear that concentration 6% recorded the highest mean mortality percentage resulting 96.67% comparing with the other two concentrations and control.

Table 2. Mortality percentages of 4th instar larvae of *G. mellonella* after treatment with 3 different concentrations of the tested plant Essential oils in the laboratory at 26Cand 60% RH

Concentration (%)	Accumulative larval mortality (%) after indicated days.				
	1	2	3	5	7
Neem oil					
2	6.67 ± 3.33^{bE}	13.33 ± 3.33^{bD}	33.33 ± 3.33^{cC}	50.00 ± 0.00^{Cb}	76.67 ± 3.33^{cA}
4	16.67 ± 3.33^{aE}	23.33 ± 3.33^{aD}	40.00 ± 5.77^{bC}	63.33 ± 6.67^{bB}	73.33 ± 6.67^{bA}
6	16.67 ± 3.33^{aE}	23.33 ± 3.33^{aD}	53.33 ± 3.33^{aC}	83.33 ± 3.33^{aB}	96.67 ± 3.33^{aA}
Control	0 ± 0^{cA}	0 ± 0^{cA}	0 ± 0^{dA}	0 ± 0^{dA}	0 ± 0^{dA}
Garlic oil					
2	20.00 ± 0.00^{bE}	36.67 ± 3.33^{bD}	46.67 ± 3.33^{bC}	53.33 ± 3.33^{cB}	73.33 ± 3.33^{bA}
4	23.33 ± 3.33^{bE}	36.67 ± 3.33^{bD}	46.67 ± 8.82^{bC}	66.67 ± 3.33^{bB}	96.67 ± 3.33^{aA}
6	36.67 ± 3.33^{aE}	43.33 ± 3.33^{aD}	56.67 ± 3.33^{aC}	86.67 ± 3.33^{aB}	100 ± 0.00^{aA}
Control	0 ± 0^{cA}	0 ± 0^{cA}	0 ± 0^{cA}	0 ± 0^{dA}	0 ± 0^{cA}
Jojoba oil					
2	6.67 ± 3.33^{cE}	16.67 ± 3.33^{cD}	33.33 ± 3.33^{cC}	43.33 ± 3.33^{cB}	53.33 ± 3.33^{cA}
4	16.67 ± 3.33^{bE}	23.33 ± 3.33^{bD}	33.33 ± 3.33^{bC}	46.67 ± 3.33^{bB}	70.00 ± 0.00^{bA}
6	23.33 ± 3.33^{aE}	33.33 ± 3.33^{aD}	53.33 ± 3.33^{aC}	73.33 ± 6.67^{aB}	90.00 ± 5.77^{aA}
Control	0 ± 0^{dE}	0 ± 0^{dD}	0 ± 0^{dC}	0 ± 0^{Db}	0 ± 0^{Da}

Values in the same column bearing identical superscript letters do not differ significantly at P > 0.05.

1.2. Garlic essential oil.

Data in Table (2) and Figure(2) showed that after 1, 2, 3, 5, and 7 days post treatment, the mortality % were 20.00, 36.67, 46.67, 53.33and 73.33% for concentration 2% of The plant essential oil Garlic, and 23.33, 36.67, 46.67, 66.67and 96.67% for the 2nd concentration 4% while for the 3rd concentration 6% were 36.67, 43.33, 56.67, 86.67and

100% compared with control which were0.00, 0.00, 0.00, 0.00, 3.33 and 0.00% after the same exposure periods respectively. From the same table the means of treatments for the three plant essential oils and the untreated control, it was clear that concentration 6% recorded the highest mean mortality percentage 100% comparing with the other two concentrations and control.

1.3. The plant essential oil Jojoba.

Results in Table (2) and Figure (1) showed that after 1, 2, 3, 5, and 7 days the mortality percentage were 6.67, 16.67, 33.33, 43.33and 53.33% for concentration 2% of The plant essential oil Jojoba, and 16.67, 23.33, 33.33, 46.67and 70.00% for the 2nd concentration 4% while for the 3rd concentration 6% were 23.33, 33.33, 53.33, 73.33and 90.00% compared with control which approximately did not have fatal effect after the same exposure periods. From the same table the means of treatments for the three plant essential oils and the untreated one control, it was clear that increased the concentration increases the mortality rate.

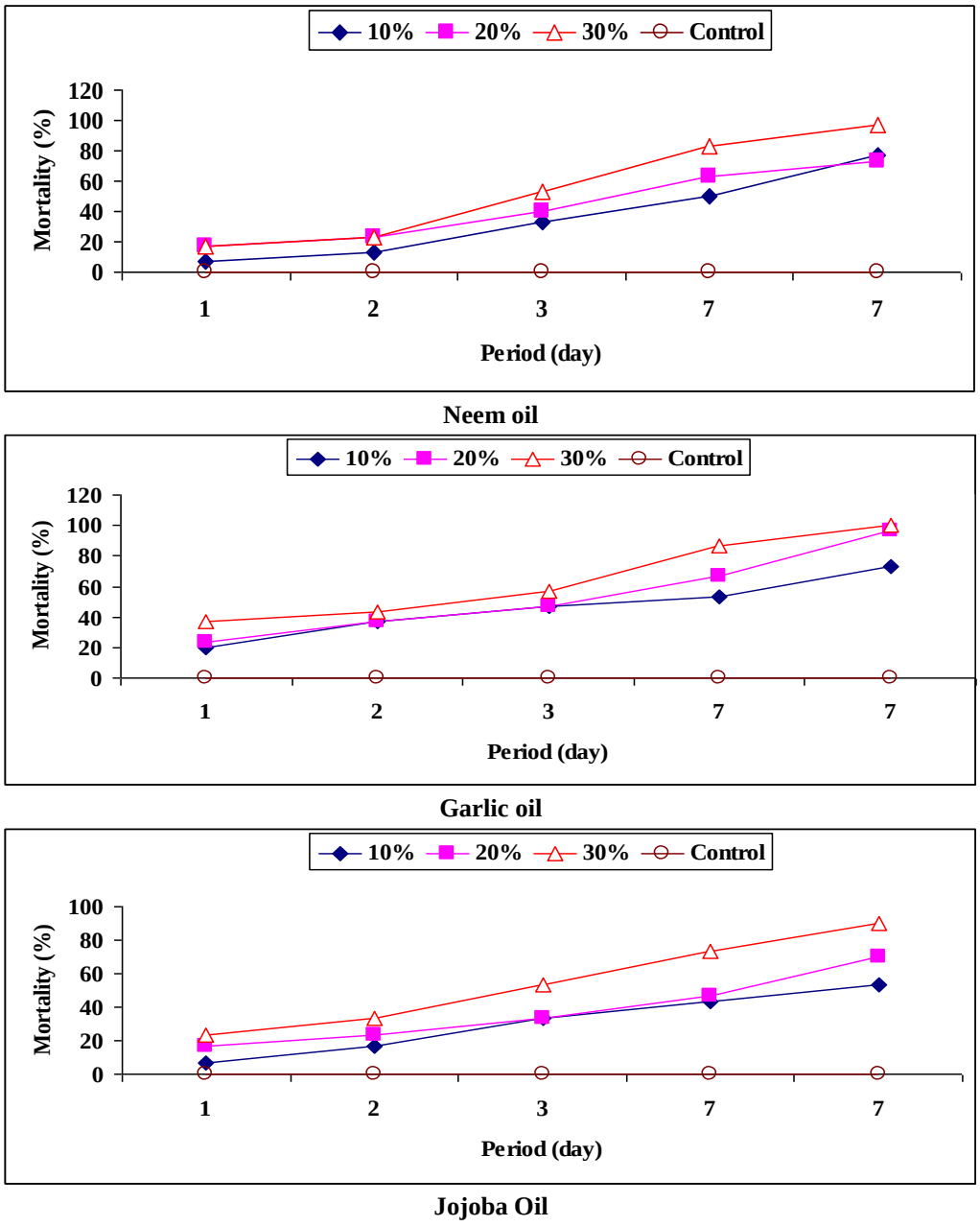


Fig. (1) Mortality percentages of the larvae of *G. mellonella* treated with three dosages of the tested plant essential oils in the laboratory.

2. The results of probit analysis obtained by the exposure of 4th instar larvae of The Greater waxworm, *G. mellonella* to different concentration of the three tested materials.

Data in Table (3) indicated that the plant essential oil, Neem recorded the value of LC_{50} (2.842%). The Garlic essential oil LC_{50} value reached to (1.983%), while the plant essential oil of jojoba recorded the value of LC_{50} (3.958%). There were significant positive correlation coefficient between concentration and mortality percentage. The LC_{50} ratio indicated that the increase of concentration increases the mortality percentage, in summery data indicated that the plant essential oil Garlic was the most effective one comparing with the other two essential oils.

Table 3. Lethal concentration of the plant essential oils against the 4th larvae of the Greater wax worm, *G. mellonella* after treatment.

Oil	LC_{50}	Slope	SE
Neem oil	2.842	1.636	0.143
Garlic oil	1.983	0.534	0.431
Jojoba oil	3.958	2.856	0.085

3. Impact of the evaluated essential oils on several biochemical parameters of fourth instar larvae of *G. mellonella* in a laboratory:

In these tests, only 4th instar larvae of *G. mellonella* were exposed to the LC_{50} concentration for each plant essential oils of Neem, Garlic, and jojoba to evaluate the activities of LDH, GST, AchE, ATPase and Alpha esterase enzymes.

3.1. Acetylcholin esterase activity (AChE):

Results in Table (5) and fig. (2) revealed that AchE level was 54.33, 116.67, and 84.33 µg AchBr / min/ g.b.wt after treatment with the 3 essential oils Jojoba, Neem, and Garlic respectively, comparing with control 165.33µg AchBr / min/ g.b.wt . The results indicated a decrease in AchE activity of the treated 4th instar larvae of *G. mellonella* after treatment with the three essential oils comparing with the untreated one (control) which recorded (165.33).

Gaaboub *et al.* (2012) demonstrated that the studied chemicals protecto, lannate, coumarin, and azadirachtin reduced the level of AChE in the treated larvae of *S. littoralis* comparing with control.

3.2. Lactate Dehydrogenase activity (LDH).

Data in Table (5) and fig. (2) showed that LDH activities were 1842.67, 1718.67, and 1324.67*mU/g.b.wt)* after treatment with the three tested material Jojoba, Neem, and Garlic respectively, comparing with control 1319.67. *mU/g.b.wt)*. Results indicated an increase in LDH levels of the treated caterpillars of this insect after exposure to the 3 plant essential oils Jojoba, Neem and Garlic comparing with the untreated one (control) which recorded 1319.67 *mU/g.b.wt*.

3.3. Glutathione S-Transferase activity (GST).

Data in Table (5) and fig. (2) showed that GST activities were 80.67, 37.00 and 58.67*mmol sub. Conjugated/ min/ g.b.wt* after treatment with the 3 plant essential oils Jojoba, Neem, and Garlic respectively, comparing with control 53.00 *mmol sub. Conjugated/ min/ g.b.wt*. Results indicated decrease in the level of GST level of the treated larvae of *G. mellonella* after treatment with the plant essential oil Neem and increase after treatment with the two essential oils Jojoba and Garlic comparing with the untreated one (control).

3.4. ATPase activity.

Data in Table (5) and fig.(2) indicated that ATPase levels were 45.33, 41.33, and 86.67 *µmol Pi liberated / min/g.b.wt* after treatment with the 3 essential oils Jojoba, Neem, and Garlic respectively, comparing with control 81.67*µmol Pi liberated / min/g.b.wt*. Results indicated a decrease in ATPase activity of the treated larvae of waxworm after treating with two essential oils Jojoba and Neem and increase after treatment with the plant essential oil Garlic comparing with the untreated one (control).

3.5. Alpha esterase's activity.

Data in Table (4) and fig. (2) stated that Alpha esterase's level were 3395.67, 1342.33, and 3084.00 b*µg α-naphyhol / min/g.* after treatment with the 3 essential oils Jojoba, Neem, and Garlic respectively, comparing with control 1548.67. *(µg α-naphyhol / min/g.*). Results showed decrease of the level of Alpha esterase's of the treated larvae after treatment with the essential oil Neem and increasing on it after treatment with the two plant Essential oils Jojoba, and Garlic comparing with the untreated one (control) which recorded (1548.67).

Gaaboub *et.al.* (2012) showed that the α-esterase activity of the treated larvae of *S. littoralis* was significantly higher than that of the control due to the studied substances coumarin and azadirachtin. When larvae were exposed to the LC50 concentrations of the tested substances, Protecto, Lannate, Coumarin, and Azadirachtin, the average levels of α-esterase enzyme activity in the supernatant of the homogenized caterpillars were observed to reach 1.643, 1.800, 3.047, and 2.655 mg/g .w, respectively, compared with 2.410 mg/ b.w. in the untreated control.

Table 4. Impact of the tested essential oils on biochemical parameters of *Galleria mellonella* larvae exposed to their LC50 concentrations. (mean±SD)

Enzymes / Essential oils	AchE (µg AchBr / min/ g.b.wt	LDH (mU/g.b.wt)	GSH (mmol sub. Conjugated/ min/ g.b.wt)	ATPase (µmol Pi liberated / min/g.b.wt)	Alpha (µg α-naphyhol / min/g.b.wt)
Jojoba	54.33 ±4.04^{d}	1842.67 ±8.74^{a}	80.67 ±2.08^{a}	45.33 ±3.06^{b}	3395.67 ±178.54^{a}
Neem	116.67 ±8.08^{b}	1718.67 ±15.57^{b}	37.00 ±2.65^{c}	41.33 ±2.08^{b}	1342.33 ±43.02^{d}
Garlic	84.33 ±5.86^{c}	1324.67 ±17.47^{c}	58.67 ±3.21^{b}	86.67 ±3.51^{a}	3084.00 ±54.56^{b}
Control	165.33 ±12.86^{a}	1319.67 ±37.23^{c}	53.00 ±4.36^{b}	81.67 ±3.79^{a}	1548.67 ±53.53^{c}
LSD	15.79	42.21	6.00	5.98	187.27

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

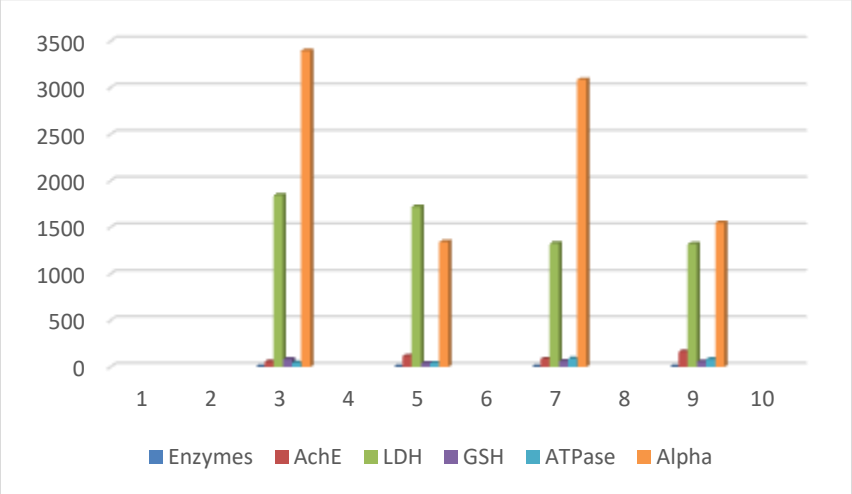


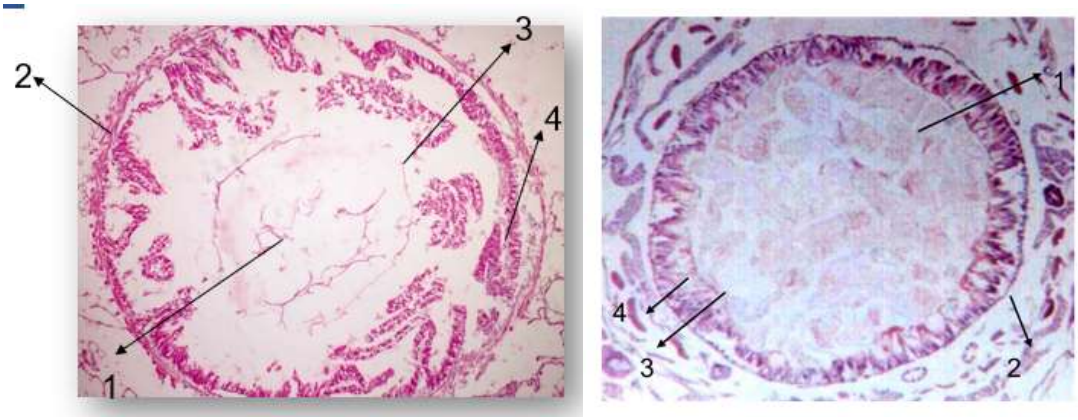
Fig. (2) Effect of tested essential oils on some biochemical aspects of *G. mellonella* larvae treated with LC_{50} of these essential oils.

4. Histopathological Alterations in the Midgut of Fourth Instar Larvae of *G. mellonella* Induced by the three essential Oils:

The digestive tract is one of the most important tissues in the body of insects. and is essential to their existence. Because Digestion, purification, and transportation all take place there, and semi-chemical production all crucial processes in the insect life cycle research on the alimentary canal is crucial. **(Nardi *et al.* 2002).** There are three areas in the insect gut, each with a distinct purpose. The midgut's main job is to absorb nutrients, while the foregut and hindgut, which originate from the ectodermal layer of the embryo, are mostly in charge of food ingestion, water absorption, and osmoregulation. **(Corley and Lavine, 2006).** Additionally, luminal enzyme production and secretion as well as microvillar enzyme final digestion depend on midgut tissue. In lepidopteran insects, the midgut epithelium is composed of goblet cells that transport ions and tiny spherical stem cells at the base of the epithelium, and columnar absorptive cells with apical microvilli. **(Smagghe *et al.* 2005).**

4.1. Effect of the plant Essential oil, Neem:

The present histological study on the impact of plant Essential oil Neem on the mid gut of treated larvae of *G. mellonella* revealed specific alterations in Fig. (3) which revealed an increased thickness of the epithelial layer within the midgut of the larvae, the separation of epithelial cells at various places from the basement membrane, and a few damaged cells that discharged their cytoplasm in the gap between the epithelial cell and peritrophic membranes.

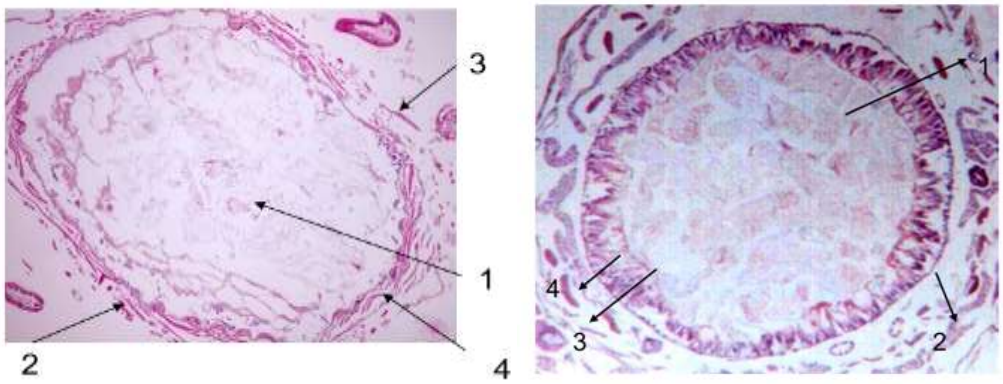


Untreated (control)

Fig (3): Cross section on the mid gut of Galleria mellonella after treatment with the plant essential oil Neem

4.2. Effect of the plant Essential oil, Jojoba:

The current histological investigation of the impact of the plant essential oil jojoba on the midgut of treated *G. mellonella* larvae revealed specific alterations in Fig. (4) that demonstrated the epithelial cells' separation from the basement membrane and their destruction in some locations. Additionally, the peri-trophic membrane is damaged.

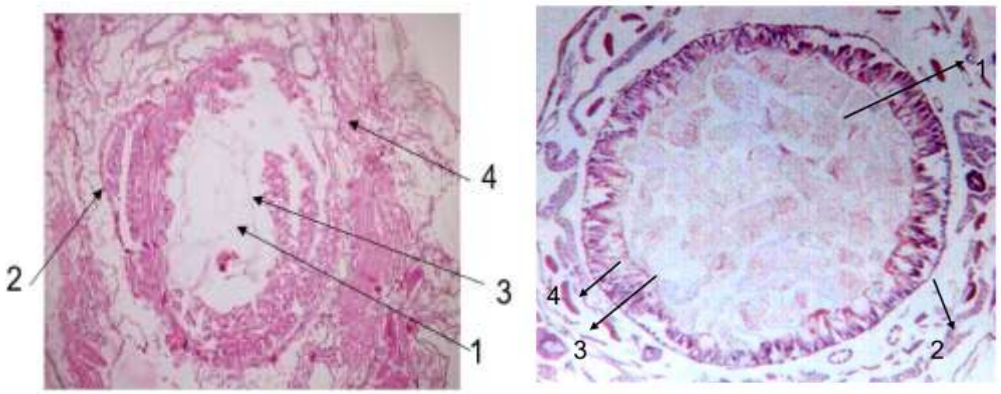


Untreated (control)

Fig (4): Cross section on the mid gut of *G. mellonella* after treatment with the Jojoba essential oil.

4.3. Effect of the plant essential oil Garlic.

The present histological study on the effect of plant essential oil Garlic on the mid gut of treated larvae of *G. mellonella* revealed certain changes appeared within Fig (5) that showed epithelium cells detached from the basement membrane in many areas and it has become thick in some areas and caused disappearance in the epithelium layer in some points .The mid gut of larvae and some cells were broken and emptied their cytoplasmic contents in the space between the epithelial and peritrophic membrane .



Untreated (control)

Fig (5): Cross section on the mid gut of *G. mellonella* after treatment with the plant essential oil Garlic.

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