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Effect of Some Plant Extracts on the Greater Waxworm *Galleria mellonella*

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Abstract

This study was conducted to evaluate the effect of three concentrations (10, 20 and 30%) of three Plant extracts: Thevetia, (*Thevetia peruviana)*, Neem *(Azadirachta indica)* and Common Oleander *(Nerium oleander)* against the fourth instar larvae of greater wax moth, *G. mellonella* and the mortality percentages were calculated. In comparison to the control, mortality rates were higher at Neem treatments, followed by Common Oleander and Thevetia. In addition, the values of LC_{50} were 12.656, 20.268 and 23.416% respectively. In the fourth instar larvae treated with the three studied plant extracts, the activity of the enzymes Lactate Dehydrogenase (LDH), Glutathione S-Transferase (GST), ATPase-acetylcholinesterase, and Alpha esterases was assessed. The results showed that, in comparison to the control, LDH activity increased following treatment with neem and common oleander. The levels of Glutathione S-transferase (GST) and alpha-esterases increased following treatment with Common Oleander and Thevetia extracts. In contrast, ATPase activity showed a marked decrease across all treatments, while acetylcholinesterase (AChE) activity declined after exposure to Common Oleander and Thevetia. Overall, Neem and Common Oleander extracts exhibited stronger effects compared to Thevetia, and all three plant extracts were more active than the untreated larvae.

Keywords*:* Waxworm, *Galleria menonella*, plant extracts, enzymes, histological, biochemical Studies.

Introduction

The greater wax moth one of the most destructive pests of honeybee colonies, as its larvae severely damage the wax combs within beehives. The larvae make the damage by feeding on the wax, bee brood, honey remnants, and pollen kept inside the wax frames. In cases of severe infestation, the combs deteriorate into a brittle, blackened mass. Prior to pupation, the larvae bore into the wooden structures of the hive and secrete silk threads that obstruct the movement of bees within the colony. These threads damage the hexagonal cells, leading to the collapse of weak colonies and the weakening of strong ones. Additionally, the silk produced during feeding serves as a protective barrier against attacks by worker bees. Despite the widespread prevalence of the greater wax worm in all areas of beekeeping, it is more active in warm regions **(Charriere and Imadorf, 1997**, **Abddel Jabbar, 2001).** Various methods have been employed to control this pest, including chemical insecticides and fumigation with phostoxin and methyl bromide tablets. However, ongoing research focuses on alternative control strategies aimed at reducing environmental pollution and mitigating the development of insect resistance. These approaches include biological control, as well as the using the natural materials, which are considered environmentally friendly and have been successfully applied against numerous insect species **(Szabo and Heikel, 1997 & Hood *et al*., 2003 & AL-Khazraji *et al*. 2016).** Previous studies showed that the most successful approach for controlling of wax moth Larvae were stored under deep-freezing conditions at −17 °C. Temperatures slightly Below the freezing point, such as those typically employed in household refrigerator units, also demonstrated effectiveness following application for 10–20 days. Numerous synthetic chemicals have been tested for controlling this pest the storage; however, these chemicals present significant drawbacks. They can contaminate honey and other hive products and exert harmful side

effects on bees. The use of synthetic pesticides such as sulfur and para-dichlorobenzene is particularly detrimental to bee populations, which has prompted interest in safer alternatives such as plant extracts. **(Whitecomb, 1967).**

Although plant extracts and essential oils have been tested against various lepidopteran insects, no studies have specifically investigated their potential to induce mortality in wax worm larvae. Identifying a Materials of plant origin with high efficacy against wax moths and minimal toxicity to honeybees is crucial. Such as plant extracts could be integrated into IPM programs to control wax moth infestations in honeybee colonies and storage areas without contaminating hive products such as wax, pollen, and honey with pesticide residues. Therefore, this study was conducted to evaluate the efficacy of various plant extracts against *G. mellonella* larvae under control conditions.

Histological examination of the midgut in treated larvae is essential for understanding and explaining the structural changes induced by different tested compounds. Additionally, assessing variations in enzyme activities particularly transaminases such as GOT and GPT, as well as trehalase, invertase, amylase, and soluble protein content is crucial for clarifying their roles in the biological and physiological processes of insects. (**Mead-Hala, 2000 and Khedr, 2002)**.

The current study's objective was to evaluate the histological effects of the tested materials in the *G. mellonella* midgut of larvae in their fourth instar. Additionally, a biochemical analysis of the treated larvae was carried out, including enzyme activities, non-specific esterases, Glutathione S-transferase (GST) transaminase enzymes, Lactated hydrogenase (LDH), ATPase enzyme, and Acetyl cholinesterase enzymes.

Materials and methods

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

English name	Scientific name	Family	Used part
Common Oleander	***Nerium oleander***	**Apocynaceae**	Leaves
Neem	***Azadirachta indica***	**Meliaceae**	Leaves
Thevetia	***Thevetia peruviana***	**Apocynaceae**	Leaves

2. Insect Rearing Technique*:*

Laboratory established colony of this insect(*G.mellonella*), was originally sourced from the apiary of theFac. of Agri. Benha Univ., and maintained under standardized rearing conditions in accordance with **Hussein (2004).** This pest was reared on artificial diet (corn & wheat flours, bran, milk powder, honey and glycerin), under laboratory conditions according to the formulation of **Wiesner (1993).** Field-collected larvae were introduced into clear plastic rearing chambers provisioned with 150 g of the prepared diet. Rearing units were secured with breathable muslin covers to ensure adequate ventilation and kept at 27 ± 2 °C with 65 ± 5% relative humidity. After completion of larval development and adult emergence, a folded sterile tissue paper was placed inside each chamber to promote egg laying. Oviposited eggs adhering to the tissue paper and chamber surfaces were collected and move into fresh rearing containers containing 150 g of diet. These containers were sealed with a double-layer muslin barrier to prevent neonate larval escape and supplemented with fresh diet at 1–2-day intervals.

3. Method for Preparing Plant Extracts:

Freshly collected leaves of common oleander, neem, and Thevetia. were shade-dried at room temperature for a period of three weeks. The dried material was mechanically ground to produce the powder using an electric milling device. For extraction, 200 g of the powdered plant material were immersed in solvent within flask container and allowed to extract by maceration for 72 h, following earlier established protocols (**Su, 1985 & Abo-ElGhar and El-Sheikh, 1987 & Sharaf El-Din, 1998 & Barakat, 2012).** The extracts were agitated mechanically for 30 min, then clarified by filtration. Solvent removal was carried out under vacuum at 60 °C using a rotary evaporator, yielding crude extracts that were weighed and subsequently reconstituted in the same solvent to obtain a 20% (w/v) stock solution. The concentrations of 10, 20, and 30% (w/w) through serial dilution of the stock solution were made.

For bioassay evaluation, five grams of the diet were homogenized with 1 ml of each plant extract concentration. Control treatments consisted of diet mixed with solvent only. The treated diets were transferred into glass jars and maintained under

controlled conditions (30 °C and 65% relative humidity). Each treatment level and control was conducted in triplicate, with ten fourth-instar larvae of *G. mellonella* introduced per replicate. Mortality and survival data were collected daily over a 14-day observation period.

4. Toxicological studies:

Larval mortality and LC_{50} determination for the tested plant extracts were evaluated at three concentrations (10%, 20%, and 30%). Each extract was dissolved in ethanol, homogenized by shaking, and incorporated into the artificial diet at a ratio of 1 cm^3 per 5 g of diet per container, while control diets contained ethanol only. Three replicates were prepared for each treatment, with ten fourth-instar larvae per replicate. Mortality data were recorded, and corrected mortality percentages were calculated. LC_{50} values were estimated according to **Finney (1971)**

5. Biochemical studies:

5.1. Preparing of Biological Samples for Biochemical analysis:

Fourth instar of *G. mellonella* larvae were collected after 48 hours from applying the LC_{50} concentration of each tested compound while untreated larvae were considered as controls. During each trial, larvae were housed in disinfected jars, then homogenized in distilled water using a Teflon homogenizer. The homogenized material was centrifuged at 600 rpm for 10 minutes at 5 °C. The resulting supernatants were promptly analyzed to measure levels of acetylcholinesterase (AChE), lactate dehydrogenase (LDH), glutathione S-transferase (GST), ATPase, and alpha-esterases.

5.1.1. ATPase activity

The ATPase's total enzyme level was calculated by **(Amaral *et al*., 2001).**

5.1.2. LDH determination

The method used for this assay was based on **(Dgkc, 1972).**

5.1.3.Nonspecific esterases

According to **Vanasperen (1962),** alpha esterases (α-esterases) and beta esterases (β-esterases) were detemined.

5.1.4.Acetylcholinesterase (AChE) activity

The method used for this assay was based on **(Simpson *et.al*., 1964)**.

5.1.5.Glutathione S-transferase(GST)

That enzyme was determined using the technique of **Habig *et al*. (I974).**

6. Histological studies:

Caterpillars were sampled after 48 h of treatment with the LC_{50} concentrations and placed in Bouin's solutionfor fixation, followed by dehydration of the larvae through a graded ethanol series to eliminate the yellow coloration. The larvae were then kept in 60% ethyl alcohol for 2 h, they were left for a whole day. After that, for two hours at room temperature, the larvae were exposed to a graded sequence of ethanol 80%, 90%, 96%, and 100%. Following dehydration, the larvae were kept at 50°C for a whole day in a solution of amylacetate and soft paraffin wax. Three times, at intervals of 24 hours, the larvae were immersed in soft paraffin wax at 50°C. Larvae were embedded in hard paraffin wax. The final step was embedding it in the wax mixture. A microtome was used to create serial slices at 6 microns, and Mayer's albumin was used to mount the sections on sterile slides. Sections were prepared for inspection and photo microscopy after mounting them on glass slides, staining them with hematoxylene.

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 extract against 4th instar larvae of the Greater wax worm *Galleria mellonella* in laboratory:

1.1. Thevetia leaves extract.

Results in Table (2) cleared that after 1,3,5,7,10,12 and 14 days post treatment the mortality percentage were 13.33 ,16.67 ,20.00 ,23.33 ,40.00 and 50.00%, respectively for concentration 10%, and reached 13.33 ,26.67 ,43.33, 43.33 ,56.67 and 76.67% with 20%, While at 30% the precent mortality were 20.00 ,40.00 ,60.00,60.00 ,70.00 and 93.33% after the same mentioned period of exposure respectively, Compared with control which were 0.00, 0.00, 0.00, 0.00, 3.33and 0.00% after 1,3,5,7,10,12 and14 days, respectively. It was clear that concentration 30% had the highest mean mortality percentage (93.33%) compared with the other two concentrations and control. These results conclusively demonstrated that increasing the concentration of the plant extract and

the exposure period increases the insect mortality rate.

1.2. Neem leaves extract.

As shown in Table 2 it was observed that, at 1, 3, 5, 7, 10, 12, and 14 days post-treatment, the mortality rates were 13.33, 20.00, 33.33, 43.33, 63.33 and 80.00% in the concentration 10% of the Neem extract and it were 23.33, 33.3, 40.00 ,53.33, 66.67 and 100% in the 2nd concentration (20%). While in the 3rd concentration (30%) the mortality rates were 43.33, 70.00, 83.00, 100.00 and 100.00 %, respectively. By contrast, the control group exhibited no mortality (0.00%) on all trial days. Data analysis presented in the same table indicates that, among the three tested concentrations of plant extracts, the 30% concentration resulted in the highest mean larval mortality, reaching 100%, compared to the other two concentrations and to the control group.

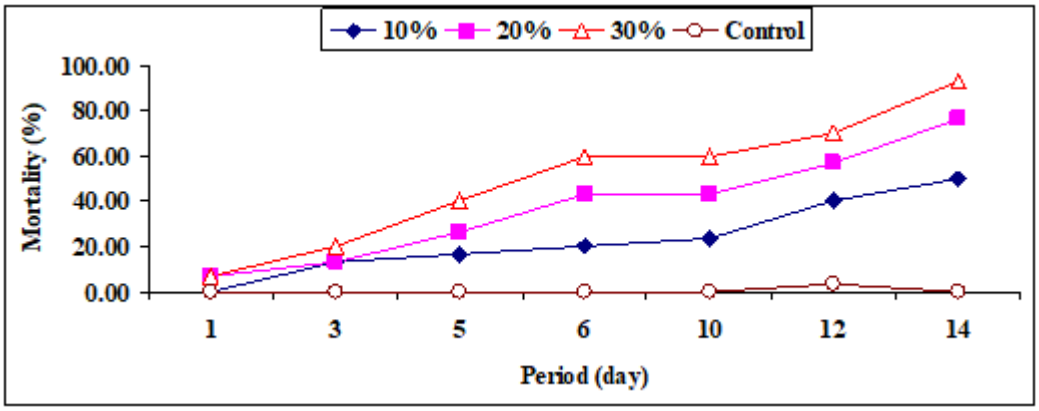
1.3. The plant extracts Common Oleander.

Data in table (2) showed that after 1,3,5,7,10,12 and 14 days the mortality percentage were 3.33 ,13,33 ,23.33 ,36.67 ,40.00, 60.00 and 73.33% for concentration 10% of the plant extract Common oleander, and 3.33, 20.00, 30.00 ,46.67, 53.33,66.67 and 80% for the 2nd concentration 20% while for the 3rd concentration 30% were 10.00 ,23.33 ,36.67,60.00,73.33,90.00 and 100.00 %. Compared with control which were 0.00, 0.00, 0.00, 0.00, 0.00and 0.00% after 1,3,5,7,10,12 and 14 days respectively. The 30% concentration clearly resulted in the highest mean mortality rate of 100% when compared to the other two concentrations and the control.

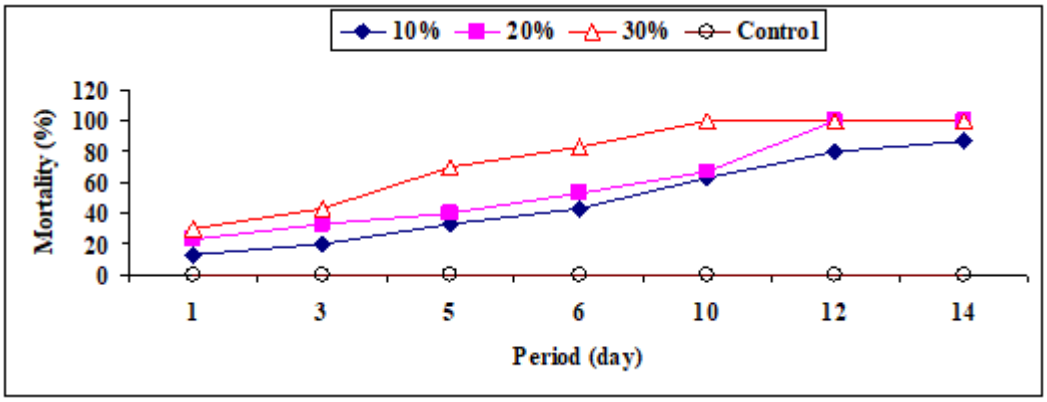
Table 2. Effect of the three different concentrations of the tested plant extracts on the mortality rates of the 4th instar larvae of *Galleria mellonella*

Concentration (%)	Accumulative larval mortality (%) after indicated days.						
	1	3	5	7	10	12	14
				Thevetia			
10	0±0^{Bf}	13.33±3.33 Be	16.67±3.33 Cde	20.00±0.00^{c} CD	23.33±3.33 cC	40.00±0.00 cB	50.00±0.00 cA
20	6.67±3.33^{a} F	13.33±3.33 bE	26.67±3.33 bD	43.33±3.33^{b} C	43.33±3.33 bC	56.67±3.33 bB	76.67±3.33 bA
30	6.67±3.33^{a} F	20.00±0.00 aE	40.00±0.00 aD	60.00±5.77^{a} C	60.00±5.77 aC	70.00±0.00 aB	93.33±3.33 aA
Control	0±0b^{A}	0±0^{cA}	0±0^{dA}	0±0^{dA}	0±0^{dA}	3.33±3.33^{dA}	0±0^{dA}
				Neem			
10	13.33±3.33 cG	20.00±0.00 cF	33.33±6.67^{c} E	43.33±3.33^{c} D	63.33±6.67 bC	80.00±5.77 bB	86.67±6.67 bA
20	23.33±3.33 bF	33.33±3.33 bE	40.00±0.00 bD	53.33±3.33^{b} C	66.67±8.82 bB	100±0.00^{a} A	100±0.00^{aA}
30	30.00±0.00 aE	43.33±6.67 aD	70.00±0.00 aC	83.33±3.33^{a} B	100±0.00^{aA}	100±0.00^{a} A	100±0.00^{aA}
Control	0±0^{dA}	0±0^{dA}	0±0^{dA}	0±0^{dA}	0±0^{cA}	0±0^{cA}	0±0^{cA}
				Common oleander			
10	3.33±3.33^{b} F	13.33±3.33 cE	23.33±3.33^{c} D	36.67±3.33^{c} C	40.00±0.00 cC	60.00±0.00 cB	73.33±3.33 cA
20	3.33±3.33^{b} G	20.00±0.00 bF	30.00±0.00 bE	46.67±3.33^{b} D	53.33±3.33 bC	66.67±3.33 bB	80.00±5.77 bA
30	10.00±0.00 aG	23.33±3.33 aF	36.67±3.33 aE	60.00±0.00^{a} D	73.33±3.33 aC	90.00±5.77 aB	100±0.00^{aA}
Control	0±0^{cA}	0±0^{dA}	0±0^{dA}	0±0^{dA}	0±0^{dA}	0±0^{dA}	0±0^{dA}

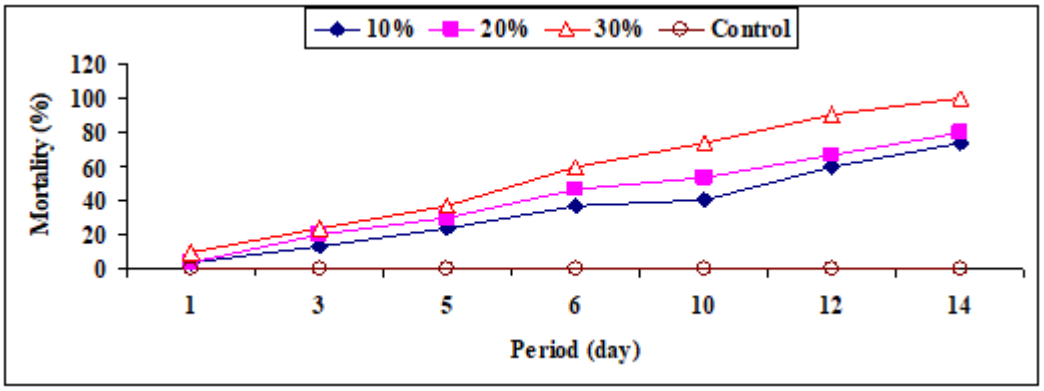
There is no significant difference (P>0.05) between any two means within the same column that share the same superscript letter.



Thevetia



Neem



Common oleander

Fig. (1): Effect of the three different concentrations of the tested plant extracts on the mortality rates of the 4th instar larvae of *Galleria mellonella*.

2. Probit analysis for the effect of the tested plant extracts on the 4th instar of greater wax moth.

Data in Table 3 showed that the LC_{50} values were 23.416 and 12.656 for thevetia and neem extracts, respectively. While in common oleander extract it was 20.268 after 7 days of treatment. Statistically significant positive relationship was observed between the tested concentrations and the corresponding mortality percentages. Analysis of the LC_{50} values further indicated that increasing concentration levels were associated with elevated mortality level. A similar pattern was observed with LC_{95} and LC_{90}. In conclusion, the data cleared that the Neem plant extract was the most effective, followed by Common Oleander, while Thevetia extract proved to be the least effective.

Table 3. Lethal concentrations of the tested plant extracts against the 4th larvae of the Greater wax worm, *Galleria mellonella* after 7 days after treatment

Plant extract	LC_{50}	LC_{90}	LC_{95}	Slope	SE
Neem	**12.656**	**55.896**	**85.163**	**2.026**	**0.118**
Common oleander	**20.268**	**235.963**	**473.207**	**1.203**	**0.192**
Thevetia	**23.416**	**95.002**	**122.507**	**2.289**	**0.105**

3. Effect of some plant extracts on the activity of some enzymes of 4th instar larvae of *G. mellonella* in the laboratory:

In these experiment, only 4th instar larvae of *G. mellonella* were treated with the LC_{50} concentration for each plant extract (Thevetia, Neem and Common oleander) to evaluate the activities of LDH, GST, AChE, ATPase and Alpha esterase enzymes.

3.1. Acetylcholine esterase activity (AChE):

Data in Table (3) revealed that AChE activity were 214.67, 129.33 and 158.33 after treatment with Neem, Thevetia and Common Oleander respectively, comparing with control 165.33µg AchBr / min/ g.b.wt. The results indicated a decrease in the AchE level of the larvae of *G. mellonella* treated with the plant extracts Thevetia and Common Oleander. In contrast, an increase in AchE level was observed after treatment with the Neem plant extract, In comparison to the untreated group, which exhibited a value of 165.33.

3.2. Lactate Dehydrogenase activity (LDH).

Data in fig. (2) showed that LDH levels were 1410.00, 1176.33, and 2160.67*mU/g.b.wt* after treatment with Neem, Thevetia, and Common oleander respectively, comparing with control 1319.67. *mU/g.b.wt)*. The extract of Common Oleander was the only treatment that induced a statistically significant elevation in lactate dehydrogenase (LDH) activity in fourth-instar larvae of wax moth, compared to both control and those treated with Neem and Thevetia extracts.

3.3. Glutathione S-Transferase activity (GST)

Results in fig. (2) indicated that GST rate were 30.67, 112.67, and 62.33*mmol sub. Conjugated/ min/ g.b.wt* after treatment with Neem, Thevetia, and Common oleander respectively, comparing with control (53.00. *mmol sub. Conjugated/ min/ g.b.wt*). Treatment with Thevetia and Common Oleander extracts resulted in a marked increase in glutathione S-transferase (GST) level in fourth-instar larvae of *G. mellonella* compared to both the untreated control and larvae exposed to Neem extract. Glutathione S-transferase (GST) plays a important role in detoxification and catalyzing the conjugation to provide cellular antioxidant defense of reduced glutathione (GSH) to electrophilic centers of both natural and synthetic compounds, whether exogenous or endogenous. **(Ortelli *et al.*, 2003; Enayati and Ranson, 2005 and Lumjuan *et al.*, 2005).** The degree of changes in GST activity can differ according to the type of insect that is being targeted and the concentration of the compounds applied. GST activity showed an increase during the early stages of treatment, up to 96 hours after fungal application, after which enzyme activity was reduced, resulting in metabolic disruptions and mortality in *Spodoptera littoralis*. **(Wu and Yi, 2016).**

3.4. ATPase activity.

Data in fig. (2) revealed that ATPase rate were 56.00, 80.33, and 35.67*µmol Pi liberated / min/g.b.wt* after treating with Neem, Thevetia, and Common oleander respectively, comparing with control (81.67 *µmol Pi liberated / min/g.b.wt* .The results demonstrated a decrease in ATPase activity in the treated fourth instar larvae of waxworms following exposure to the three tested materials, comparing with the control group.

3.5. Alpha esterases activity.

Data in fig. (2) increasing on it after treated with the two plant extracts thevetia, and Common oleander comparing with the untreated one (control). Esterases are crucial in insect defense mechanisms as they break down esters of long-chain fatty acids, which affect flight functionality, and aid in the breakdown of metabolic esters that are not active. **(Terriere, 1984 and Roslavtseva *et al.*, 1993).** GST activity increased in *Drosophila melanogaster* due to increased resistance to oxidative stress and longer lifespans for both males and females. **(Aslan *et al.*, 2019).** The most significant glutathione S-transferase (GST) level suggested that the GST enzyme facilitated the detoxification of the antifungal agent (terbinafine) in insect species. **(Kastamonuluoglu *et al.*, 2020).**

Table 4. Impact of tested Plant extracts on some biochemical aspects of the Greater waxmoth larvae treated with LC_{50} of these plant extracts.

Enzymes Plant extracts	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)*
Neem	**214.67 ±9.07^{a}**	**1410.00 ±123.66^{b}**	**30.67 ±2.08^{d}**	**56.00 ±3.61^{b}**	**857.33 ±29.69^{d}**
Thevetia	**129.33 ±7.64^{c}**	**1176.33 ±20.65^{c}**	**112.67 ±7.64^{a}**	**80.33 ±3.06^{a}**	**2279.00 ±68.46^{a}**
Common oleander	**158.33 ±4.93^{b}**	**2160.67 ±64.84^{a}**	**62.33 ±4.04^{b}**	**35.67 ±2.08^{c}**	**1857.33 ±81.25^{b}**
Control	**165.33 ±12.86^{b}**	**1319.67 ±37.23^{b}**	**53.00 ±4.36^{c}**	**81.67 ±3.79^{a}**	**1548.67 ±53.53^{c}**
LSD	**17.11**	**137.42**	**9.32**	**6.03**	**115.43**

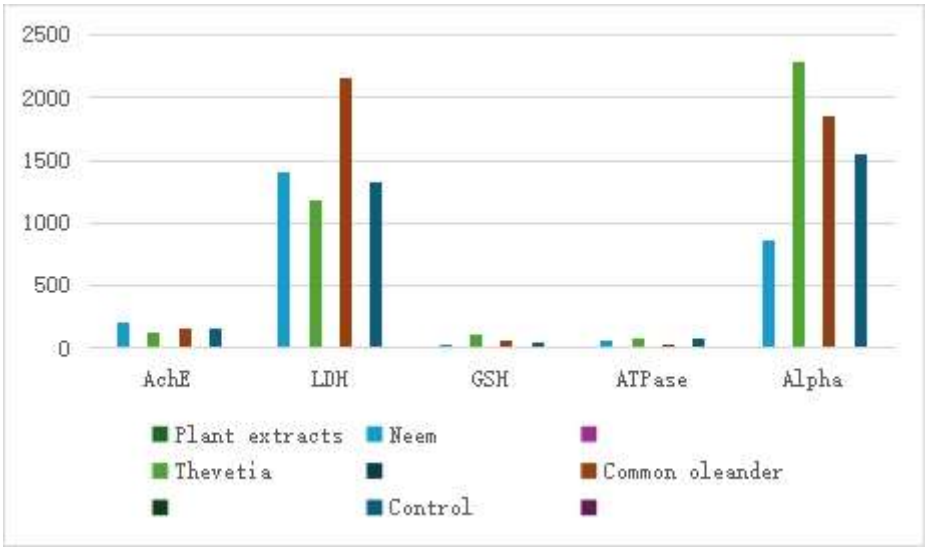


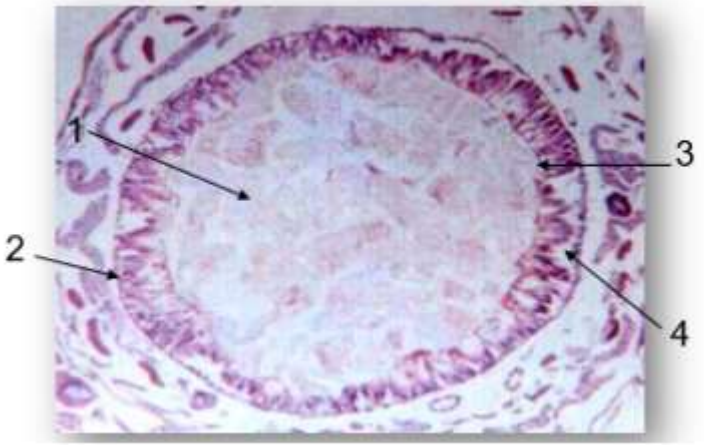
Fig. (2) Impact of tested plant essential oils on some biochemical aspects of *Galleria mellonella* larvae treated with LC_{50} of these plant extracts.

4. The effects of tested plant extracts on the midgut histological alterations of the Greater Wax moth fourth instar larvae.

Among the various bodily tissues of insects, the digestive system constitutes one of the most vital components, assuming a fundamental role in the life processes of these organisms. Investigating the insect digestive system is imperative, as it serves as the locus of digestion, detoxification, transport, and the synthesis of of semi chemicals, all of which are critical processes in the lifecycle of insects. **(Nardi *and Lavine* 2006).** The gastrointestinal tract of insects can be categorized into three distinct regions, each exhibiting unique functional roles. The foregut and hindgut originate from the embryonic ectodermal layer and primarily facilitate the processes of food ingestion (foregut),whereas the principal function of the midgut pertains to the absorption of nutrients. **(Corley and Lavine, 2006).** The midgut plays a critical role in producing and secreting enzymes and facilitating digestion through microvillar enzymes. Its epithelium is composed of columnar absorptive cells bearing apical microvilli, goblet cells are in charge of ion transport, and the basal region of the epithelium contains tiny, spherical stem cells In lepidopteran insects. **(Smagghe *et al.*, 2005).**

Data in fig (3) revealed a cross section of the mid-gut of normal *G. mellonella* larvae. The mid-gut

epithelium was encircled by the basement membrane, and it had oral, prominent nuclei that were almost centrally located. Small goblet cells with spherical nuclei and reduced granular cytoplasm were dispersed among them. The brush border of the epithelium is produced by the microvilli of columnar cells that protrude from their free ends. The gut wall contains two distinct layers of muscle fibers. Circular muscle fibers on the inside and longitudinal muscle fibers on the outside. Connective tissue nearly fills the gaps between the various layers of the gut wall.

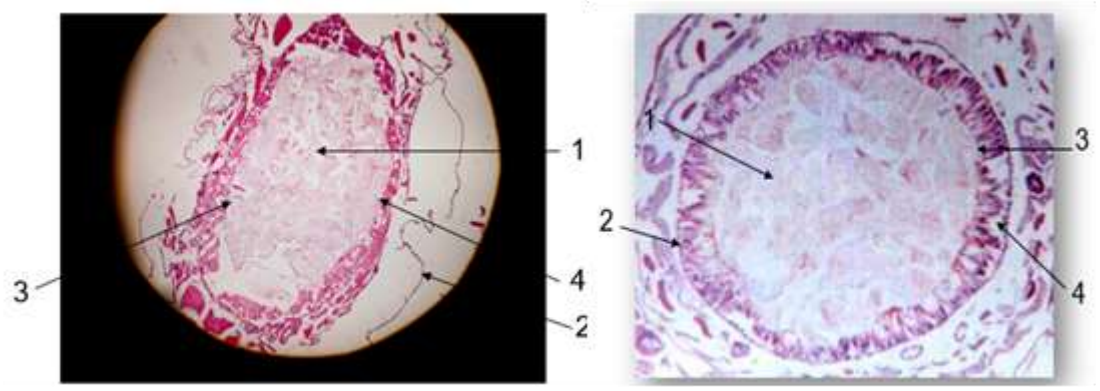


1) Lumen, (2) Basement membrane, (3) Peritrophic membrane, (4) Epithelium layer
Fig (3): mid gut of control

4.1. Effect of plant extract, (Neem)

The current histological study on the effect of plant extract Neem on the mid gut of treated larvae of *G. mellonella* showed that some changes were present in fig. (4) revealed In many places, the epithelial cells in the larvae's midgut separated from the basement membrane, and some of the cells were ruptured and released their cytoplasm in the area between the peritrophic and epithelial membranes. **Mohamed-Sondos (2002)** reported that extracts of plant such as sunflower, soybean, castor, and cotton induced structural changes in the midgut tissues of pink bollworm caterpillar. **Gamil (2004)** revealed that histological changes were obtained in *S. littoralis* mid-gut and cause destroy 1st day post treatment ,3rd day post treatment ,5th day post treatment in mid-gut tissue by tested material(neem) was comparatively similar, **Ahmed *et. al.* (2007)** investigated the impact of various neem based products on the midgut tissues of *Agrotis ipsilon*.

Gaaboub *et.al.* (2012) found that azadirachtin caused Biochemical and histological effect disintegration of peritrophic membrane compared with control. While after 5 days of treatment the epithelial cells become more apparent and the microvilli of some columnar cells are still intact, also peritrophic membrane and basement membrane not affected.

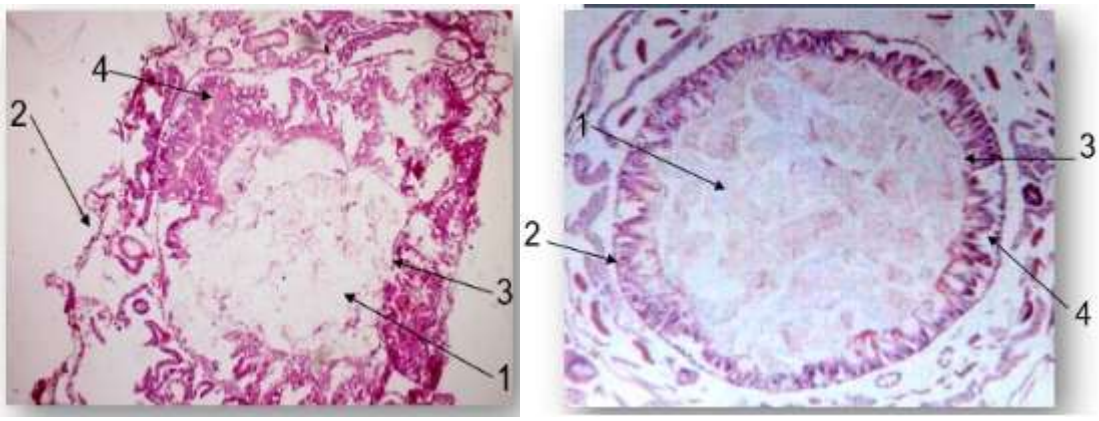


Untreated (control)

Figure (4): Cross section on mid gut of 4th instar larvae of *G.mellonella* after treatment with plant extract Neem

4.2. Effect of plant extract Common oleander on the mid gut of 4th instar larvae of *G.mellonella*:

Fig (5) indicated that, Several structural changes were showed in the midgut compared to the control. The cells of epithelium layer become distorted and partially ruined in certain regions, and in some areas, the epithelium was elongated relative to the control. There were multiple locations where epithelial cells were clearly detached from the basement membrane. Furthermore, treatment with Common Oleander extract caused noticeable disintegration of the peritrophic membrane compared to the control.



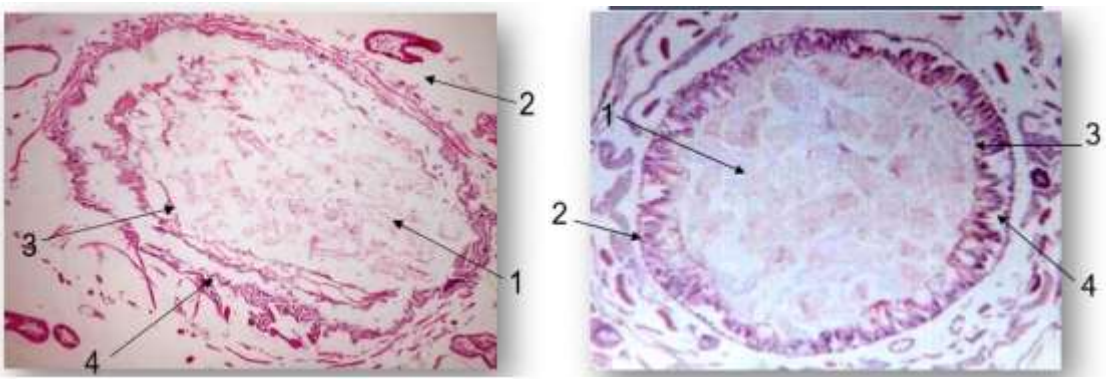
Untreated (control)

Fig (5): Cross section on mid gut of the larvae of *G.mellonella* after treatment with plant extract common oleander.

4.3. Effect of plant extract, (Thevetia)

Fig (6) showed that the epithelial cells lost their strong connection to the basement membrane, were eventually killed, lost their columnar structure, and caused the peritrophic membrane to become disorganized and, in certain circumstances, disappeared. Also, the cytoplasm lost its granular appearance, cracking highly vacuolated and showed a fibrous network, the columnar cells appeared highly vacuolated, liquefied and most of it seemed to be necrotic.

This result is agree with (**Khalil *et.al,* 2021**), It was reported that treatment of fourth-instar larvae of *Galleria mellonella* with the LD_{50} of ethanolic neem seed (*Azadirachta indica*) extract caused complete destruction of fat tissue, body wall, colon, Malpighian tubules, and muscles just prior to death. These damages were reduced in larvae examined after 12 and 24 hours of treatment. Mild muscular damage was observed when larvae were treated with lethal doses of leaf extracts from C. antiqurum and V. rosea. Similarly, lethal doses of leaf extracts from A. sativa resulted in severe damage to muscles, gonads, and the hindgut, whereas extracts from M. azedarach caused cracks in the gonads. These findings confirm the potential of using these compounds for environmentally safe control of this pest without causing pollution.



Untreated (control)

Fig (6): Cross section on mid gut of4th instar larvae of *G.mellonella* after treatment with plant extract Thevetia.

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