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Larvicidial, Enzymology and Histological Alterations Caused by *Balanites aegyptiaca* Extract in the Larvae of *Culex pipiens* (Diptera: Culicidae)

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Article Information

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Abstract:

Culex pipiens is a mosquito responsible for the transmission of various vector-borne diseases including elephantiasis and West Nile Fever (WNF) to the humans. The aim of the current work is to find an efficient safe and low-cost mosquito control system that might be suitable for a wide range of communities affected by these disease vectors. This study is carried out by evaluating the larvicidial, biochemical and histological effect of different extract concentrations of *Balanites aegyptiaca* plant on the third instar larvae of *Culex pipiens*. The median lethal concentration (LC_{50}) was determined and then used to make a histological and biochemical studies with LC_{50} value for leaves 21.5 %, for fruits 63.26 %. An aqueous extract of *Balanites aegyptiaca* leaves and fruits was tested, serious different concentrations between 10 and 100% were prepared and applied to the larvae. The tested *Balanites aegyptiaca* induced a biochemical and histological changes on the third instar treated larvae. The biochemical changes were presented by an increase in the levels of alpha Amylase, decrease in the levels of the total protein and the acid phosphatase, alkaline phosphatase and lactate dehydrogenase activity levels were disturbed. Histological studies revealed that the treated larvae had undergone obvious changes in the muscles, cuticle and midgut. Conclusion overall, this study suggests that the use of *Balanites aegyptiaca* leaves and fruit extract can be useful for controlling *Cx. pipiens*.

Keywords: Mosquito control, *Culex pipiens*, *Balanites aegyptiaca*, Larvicidal activity, Biochemical, histology.

Introduction

The most important insect in terms of public health importance is undoubtedly the mosquito (Kovendan & Murugan, 2011**)**. Mosquitoes play a vital role in the transmission of a number of infectious diseases that are harmful to humans and other organisms (Thanigaivel *et al*., 2012**)**. According to Paixão *et al*., (2018), mosquitoes are the main cause of a number of deadly diseases like dengue, malaria and *Chikungunya*, which are more common in tropical and subtropical nations like India. In Egypt, *Culex pipiens* is widely distributed and it consider the main vector of rift valley fever virus (Darwish and Hoogastraal, 1981**)**, *Wuchereria bancrofti* Gad *et al* (1996) and western Nile virus El-Bahnasawy *et al*, (2013)**.** In addition, *Culex pipiens* was identified as the filarial vector (El-Naggar *et al*., 2017) and is unequivocally recorded by all governorates (Abdel-Shafi *et al*., 2016**).**

Mosquito control methods

Chemical pesticides such as methoprene, carbamates, pyriproxyfen, diflubenzuron, fenthion, malathion and DDT have been employed for mosquito control (Su & Mulla 1999). Although, chemical pesticides have been used to control Culex pipiens for many years, Hemingway and Ranson, (2000) pointed out that these chemicals have harmful effects on the human health and the environment, as well as induce resistance in many mosquito species. Among these strategies, focusing on mosquito breeding grounds is a simple and effective way to reduce mosquito populations. Water is necessary for all mosquito species to complete their life cycles. Consequently, any source of

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stagnant water usually serves as a mosquito breeding sites (Emidi *et al*. 2017).

On the other hand, many plants that have larvicidal effects have been suggested for reducing the larval population of mosquitoes, and *Balanites aegyptiaca*, a popular tree of the dry land areas of the Africa, the Middle East, and South Asia (Hall & Walker, 1991) is one of them.

In an earlier communication**,** Wiesman and Chapagain, (2003) have pointed out saponin-rich extracts from *Balanites aegyptiaca* as a possible candidate for natural bioactive agent against *Aedes aegypti* and *Culex pipeins* mosquito larvae. Additionally, Hall and Walker (1991) reported that *Balanites aegyptiaca* is a spiny evergreen tree about 6-10 meters in height. *B. aegyptiaca* fruits have various primary and secondary metabolites such as flavonoids, furanocoumarin, saponins, fixed oil protein, fat, carbohydrates and vitamin C. The present plant is found in the drier regions of Africa, the Arabian Peninsula, India and Myanmar. Different parts of *B. aegyptiaca* have several bioactive substances which possess miscellaneous medicinal properties (Gnoula *et al*., 2008; Montasser *et al*., 2017).

The current study will hopefully find a viable alternative to synthetic insecticides for *Culex pipiens* control as it aims to evaluate the effect of *Balanites aegyptiaca* extract in controlling *Cx. pipiens* and biochemical and histological changes in the third instar larvae of *Cx. pipiens*.

Materials and Methods

Maintenance of mosquito colony

Mosquito egg rafts were collected from small ponds in south valley university farm, Qena, Egypt. Batches of newly hatched larvae were transferred to a large plastic cups containing dechlorinated tap water, fed daily with a fish food powder (TetraMin, Germany), Stock colony of adult mosquitoes Cx. pipiens was maintained at zoology department, faculty of science under laboratory conditions (27 + 2 °C and 60-70% RH). Adult mosquitoes were kept in wooden cages. A Petri-dish containing a cotton pad soaked with 10% sugar solution as a source of carbohydrate for both males and females. The sucrose solution was replaced daily to avoid fungal contamination. Females were allowed to get a blood meal on a pigeon many times aweek for egg production. No attempt was made to control the light conditions in the laboratory which received supplementary illumination during the day from overhead fluorescent lamps according to the method described by (Adham *et al*., 2003). The 3rd instar larvae were used for toxicological studies.

Preparation of Plant extract

Plants used in the present study were obtained from farm of botany department. Two different medical plants were selected in this study to evaluate their insecticidal activity against *Cx. pipiens* mosquitoes. The taxonomic identification of the collected plant was done by department of botany, faculty of science, south valley university. Extraction of all the parts was carried out using local methods. This is to allow for easy adoption by the local population.

Preparation of the plant extracts

1-Aqoues leaf extract

Fresh leaves were collected, washed, chopped, dried, and ground to powder. 100 gram of powdered leaves was dissolved in 1 liter water letting them on stirrer overnight for 12 hour. Then, centrifugation and filtration was made for them. A serious of different concentrations was prepared and larvae exposed to them. The procedure used for testing mosquito larvae was recommended by WHO (1975).

2-Aqueous fruit extract

Fruits were collected from the farm, to obtain extract. The outer cover (Epicarp) of the fruit was removed by hand. Then, the pulp was scraped manually, dried, and pulverized. 100 gm of them were dissolved in 1000 ml water putting them in shaker for 12 hours. The mixture was filtered using a very fine muslin cloth and the final volume adjusted. A series of dilutions was prepared using this stock solution using tap water. The procedure used for testing mosquitoes larvae was recommended by WHO (1975)**.**

Larvicidal activity

The larvicidal effect was tested using the 3rd instar larvae of *Cx. pipiens* (WHO, 2005). Different concentrations were used from the extract ranges of each considered extract from 10% to 90%. The efficacy of plant- extracts, aquatic *Balanites aegyptiaca* leaves and aquatic *Balanites aegyptiaca* fruits were tested against larvae of *Cx. pipiens* under laboratory conditions. Sets of twenty five early third instar larvae of *Cx. pipiens* were transferred by a dropper into small plastic cup filled with 50 ml of the tested concentrations of leaf and fruit extract. The technique for estimating LC_{50} is illustrated by taking different range concentrations of each concerned extract between 10 % and 90% which was prepared in order to obtain full number of killed larvae of *Culex* through 24 and 48 hours from the beginning of experiment to determine the LC_{50}.

Larval mortality was recorded at 24 and 48 hrs, after exposure. The control experiments were carried out alongside treatments. Mortality data was recorded in a

probit regression line and LC_{50}, slope function was calculated **(**Finney, 1971). The control mortality was corrected by using Abbott's formula of Abbott (1925). For each treatment, the experiment was performed using four replicates. Percentage mortality was assessed using the following formula:

Percentage mortality= (Number of dead individuals / Number of treated individuals) *100.

Biochemical studies

For biochemical analysis, the third instar larvae of *Culex pipiens* were treated with a lethal concentration (LC_{50}, it kills 50% of the tested larvae). They were separately taken and washed with double distilled water and removed the water content from body surface by blotting with tissue paper, weighted and mechanically homogenized in phosphate buffer. Homogenates were centrifuged at 3500 rpm for 10 min at 2°C in a refrigerated centrifuge. The supernatant was used directly or stored at 5°C larvae of *Cx. pipiens* were prepared as described by **(**Amin, 1998**).** Double beam ultraviolet/visible spectrophotometer (Spectronic 1201, Milton Roy Co., USA) used to measure absorbance of colored substances. The activity of five enzymes was detected and measured.

Determination of total protein

Total Protein concentrations were determined according to Gornal *et al*. (1949)**.**

Determination of acid phosphatase

Acid phosphatase was determined using colorimetric method Kind**,** P.R.N. & King, E. J. (1954) J. Clin. Path. 7, 322.

Determination of alkaline phosphatase

Alkaline phosphatase were determined using colorimetric method (Belfield & Goldberg, 1971**).**

Determination of α-Amylase:

Alpha amylase was determined using colorimetric method according to (Caraway, 1959**).**

Determination of the lactate dehydrogenase (LDH)-Liquizyme

Lactate dehydrogenase was determined using Kinetic ultraviolet method. Zimmerman & Hennery (1979).

Histology

The histological techniques aim to obtain thin and colored sections of biological material, observable under an optical microscope. The biological material underwent different treatments before being analyzed under a microscope (Vebteo and Velot), incorporated with the LC_{50} of different extracts after 48 h. Only live larvae were examined. Treated larvae were prepared for ultra-structural studies (Bowen & Ryder, 1976). Then they were fixed in formalin solution for 48 hrs. After dehydration in a graded ethanol series, the materials were embedded and cut with glass knives in a rotary microtome (Leica RM2126, Wetzlar, Germany). The sections were stained with haematoxylin-eosin, analyzed, and photographed with a photomicroscope. The ventral muscle tissues, midgut digestive cells of treated larvae was observed and compared to control.

Statistical analysis

Prism software was used to analyze the results followed by a one way analysis of variance (ANOVA) to test the level of significance. The findings were expressed as (means ± SE) of untransformed data. P <0.05 was used to determine the significantly variations. The obtained toxicity data were fitted to the log-probit model according to Finney (1971) using an LDP line program, LC_{50} was determined for each material. Data were analyzed for determination of LC_{50} using Log-Probit analysis software developed (LdP line software) by Dr. Ehab Bakr, (Plant Protection Research Institute "http://www.Ehabsoft.com" (Finney, 1971).

Results

1-Larvicidal effect of Balanites aegyptiaca

1-1- Balanites aegyptiaca fruits

Effect of the various aqueous extracts of *B. aegyptiaca* on the mortality of the *Cx. pipiens* mosquito larvae are presented in table 1, figure 1 and 2. Larval mortality of *Culex pipiens* after the treatment by the extract of aquatic *Balanites* fruits extract was observed. Data presented in table, 1 and figure, 1&2 showed that the toxic effect of *B. aegyptica* increased with increasing the concentration.

At the highest concentration (100 %) mortality was LC_{50} 63%). Meanwhile, the larval mortality was 9.5% at the lowest concentration of (10g/L) compared to control.

Table 1: Effect of different concentrations of aquatic *Balanites* fruits extract on mortality (%) of the 3rd larval instar of *Culex pipiens.*

Conc. (g/L)	No. of larvae Tested	No. of dead larvae	Percent mortality (%)	Percent Corrected mortality (%) after 48h	LC_{50} (g/L)	Slope
Control	80	7	8.75	0	63.263	1.5013
10	80	14	17.5	9.589		
30	80	35	43.75	38.356		
50	80	36	45	39.726		
70	80	44	55	50.685		
90	80	50	62.5	58.904		
100	80	63	78.75	63.014		

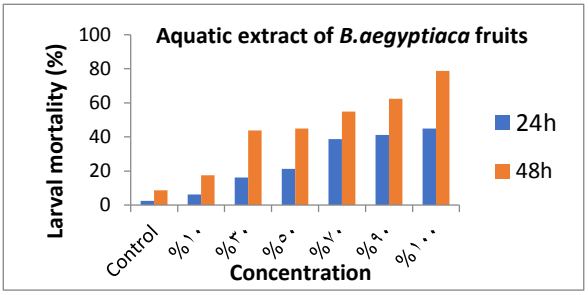

Figure 1: Effect of different concentrations of aquatic *Balanites* fruits extract on mortality (%) of the 3rd larval instar of *Culex pipiens***.**

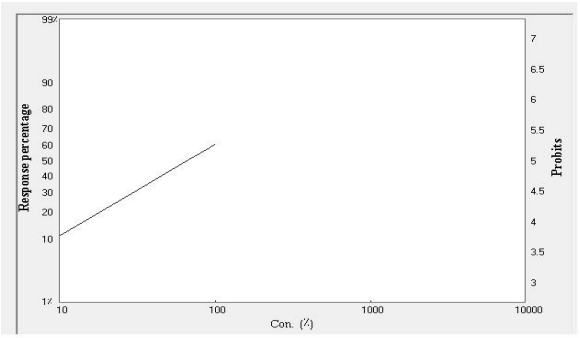

Figure 2: Probit analysis curve of toxicity of different concentrations of aquatic *Balanites* fruits extract on mortality (%) of the 3rd larval instar of *Culex pipiens.*

1-2- *Balanites aegyptiaca* leaves

Data presented in (Table 2, Figure 3,4) exhibited the larval mortality of *Cx. pipiens* after the treatment with (Aquatic *Balanites* leaves extract). The percentage of larval mortality in the 3rd instar was 95.9 % after 48 hour when the highest concentration 100 g/L was used. The lowest concentration, 25 g/L, also showed high mortality 59.5%. Controls showed 0% mortality. So, *B. aegyptiaca* leaves exhibited a potent larvicidal effect and was found to be one of the most effective aqueous extract on the 3rd larvae of *Cx. pipiens* after 48h with LC50 value of 21.5 %.

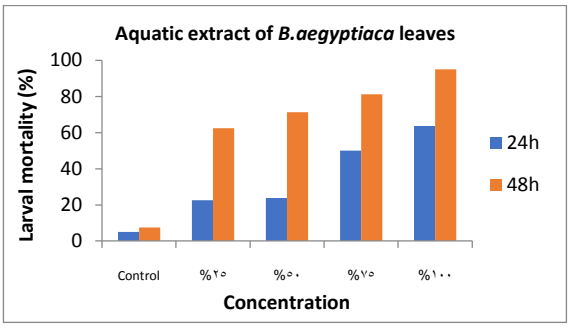

Figure 3: Effect of different concentrations of aquatic Balanites leaves extract on mortality (%) of the 3rd larval instar of Culex pipiens.

Table (3) and Figure (9) results of the two different plants aqueous extract toxicity were summarized where auatic *Balanites* leaves extract exhibited the most potent effective followed by aquatic *Balanites* fruits extract.

2-Biochemical effect

2-1-Total protein

The comparison of mean values showed that a significant decrease in the total protein amount was recorded larvae treated with aquatic. extract of *B. aegyptica* fruits exhibited (9.692 ± 0.33 mg/ml) and a low decrease for aquatic extract of *B. aegyptica* leaves (11.13 ± 0.12) which displayed the weakest inhibitory effect among the tested plant extracts compared with control mean value (13.04 ± 0.03 mg/ml).

Table 2: Effect of different concentrations of aquatic *Balanites* leaves extract on mortality (%) of the 3rd larval instar of *Culex pipiens.*

Conc. (g/L)	No. of larvae Tested	No. of dead larvae	Percent mortality (%)	Percent Corrected mortality (%) after 48h	LC_{50} (g/L)	Slope
Control	80	6	7.5	0	21.517	1.876
25	80	50	62.5	59.459		
50	80	57	71.25	68.919		
75	80	65	81.25	79.730		
100	80	76	95	95.946		

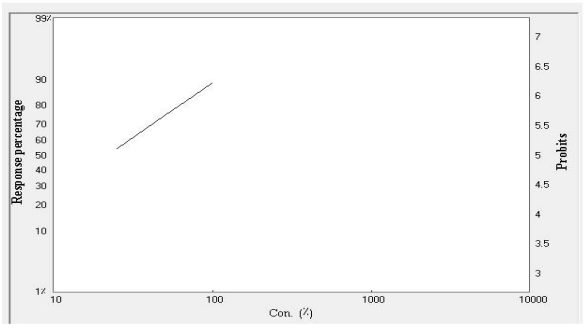

Figure 4: Probit analysis curve of toxicity concentrations of aquatic *Balanites* leaves extract on mortality (%) of the 3rd larval instar of *Culex pipiens*.

2-2-Alpha-Amylase activity

In the determination of alpha-Amylase the authors observe that in all concentrations value increase, with mean values in case of aquatic extract of *B. aegyptiaca* fruits 0.525±0.00067, in case of aquatic extract of *B. aegyptiaca* leaves 0.715±0.00208, respectively compared to control 0.493±0.00145.

2-3-Alkaline phosphatase activity:

There is a different increase in alkaline phosphate activity in the treated larvae compared with control of mean values aqueous extract get from *B. aegyptiaca* leaves 486.9 ±1.299, aqoues extract of *B. aegyptiaca* fruits 31.9 ± 1.7, respectively, compared to control 86.56 ± 0.9707.

Table 3: Effect of different concentrations of different plant extract on mortality (%) of the 3rd instar of *Cx. pipiens.*

Treatment	LC_{50} %	Confidence limits of LC_{50} (%)		LC_{90} %	Confidence limits of LC_{90} (%)		χ2	Slope± SE
		LCL	UCL		LCL	UCL		
Aquatic *balanites* fruits extract	63.26	52.80	78.02	451.66	281.20	989.88	2.86	1.5013±0.198
Aquatic balanites leaves extract	21.5	17.49	25.38	103.72	85.65	134.09	7.50	1.8762±0.349

Table 4: Effect of different types of plant extracts on total protein concentration of *Culex pipiens* 3rd instar larvae

Total Protein Concentration (Mean± SE)			
Treatments	Control	Aquatic extract of *B. aegyptiaca* fruits	Aquatic extract of *B.aegyptiaca* leaves
Total Protein Concentration (µg/µl)	13.04 ± 0.03	9.692 ± 0.33 ***	11.13 ± 0.12 **

**.The mean difference is highly significant at P value < 0.001

***. The mean difference is very highly significant at P value < 0.0001.

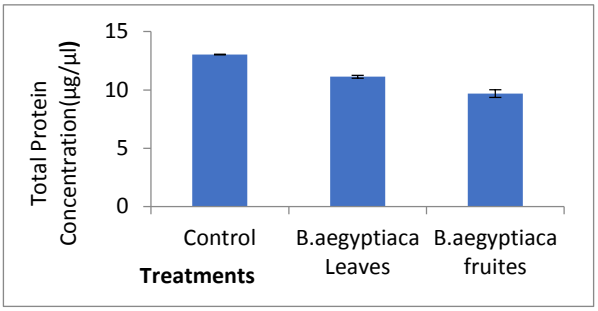

Figure 5: Effect of different types of plant extracts on total protein concentration of *Culex pipiens* 3rd instar larvae.

Table 5: Effect of different types of plant extracts on amylase activity of *Culex pipiens* 3rd instar larvae.

Alpha-Amylase Concentration (Mean± SE)			
Treatments	Control	Aquatic extract of *B. aegyptiaca* fruit	Aquatic extract of *B. aegyptiaca* leaves
Amylase Activity (U/L)	0.493± 0.00145	0.525± 0.00067	0.715± 0.00208***

The mean difference is not significant at P value >0.05.

***. The mean difference is very highly significant at P value < 0.0001.

2-4-Acid phosphatase activity

Data concerning acid phosphatase activity measurements revealed that there is a few decrease (P < 0.05) in acid phosphatase activity in the treated larvae with mean values of and, 149.5 ±0.4028 in case of aquatic extract of *B. aegyptiaca* leaves, and 144.6 ± 0.8254 in case of aquatic extract of *B. aegyptiaca* fruits respectively, compared to control 172.6 ± 0.473.

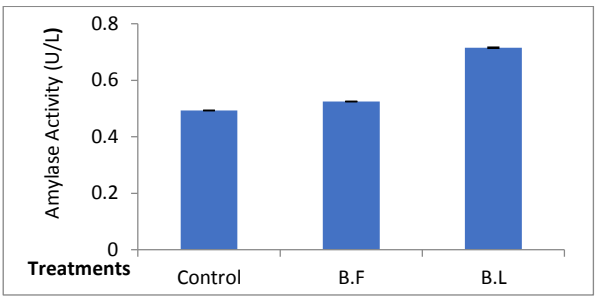

Figure 6: Effect of different types of plant extracts on amylase activity of *Culex pipiens* 3rd instar larvae.

Table 6: Effect of different types of plant extracts on alalkaline phosphatase activity of *Culex pipiens* 3rd instar larvae.

Alkaline phosphatase activity (Mean± SE)			
Treatments	Control	Aquatic extract of *B. aegyptiaca* fruits	Aquatic extract of *B. aegyptiaca* leaves
Alalkaline phosphate activity (IU/ L)	86.56± 0.971	31.9 ± 1.7*	486.9 ± 1.299***

*. The mean difference is significant at P value < 0.05.

***. The mean difference is very highly significant at P value < 0.0001.

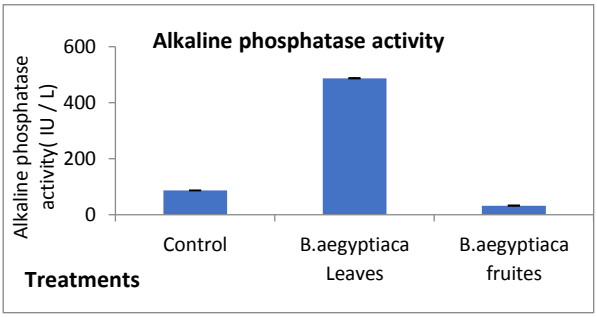

Figure 7: Effect of different types of plant extracts on alalkaline phosphatase activity of *Culex pipiens* 3rd instar larvae.

Table (7): Effect of different types of plant extracts on Acid phosphatase activity of *Culex pipiens* 3rd instar larvae.

Acid phosphatase activity			
Treatments	Control	Aquatic extract of *B. aegyptiaca* fruits	Aquatic extract of *B. aegyptiaca* leaves
Acid phosphatase activity (U / L)	172.6 ± 0.473	144.6 ± 0.8254***	149.5± 0.4028 ***

***. The mean difference is very highly significant value < 0.0001.

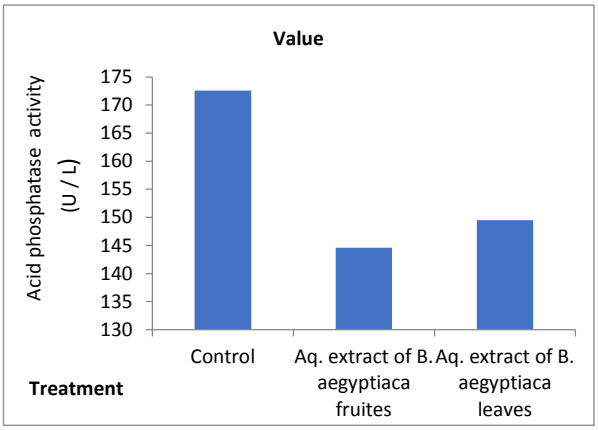

Figure 8: Effect of different types of plant extracts on Acid phosphatase activity of *Culex pipiens* 3rd instar larvae.

2- 5-Lactate dehydrogenase activity

Table 6 that shows means of lactate dehydrogenase activity levels. The highest mean of cholesterol was recorded for control 0.01233±0.007623with decrease in case of aquatic xtract of *B. aegyptiaca* fruits 0.01833 ± 0.02345, aquatic extract of *B. aegyptiaca* leaves 0.006333 ± 0.002028.

Table 8: Effect of different types of plant extracts on Lactate dehydrogenase activity of *Culex pipiens* 3rd instar larvae.

Lactate dehydrogenase activity			
Treatments	Control	Aquatic extract of *B. aegyptiaca* fruits	Aquatic extract of *B. aegyptiaca* leaves
Lactate dehydrogenase activity (U/L)	0.01233± 0.0076	0.01833± 0.0235	0.00633± 0.0021

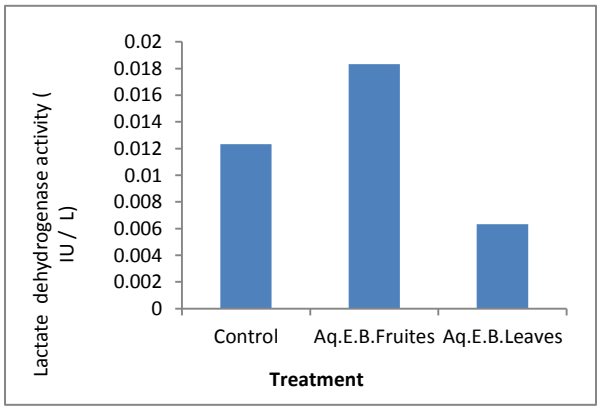

Figure 9: Effect of different types of plant extracts on Lactate dehydrogenase activity of *Culex pipiens* 3rd instar larvae.

Histology

Gut histology of mosquito larvae has been one of the most promising methods to evaluate insecticidal effects of plant extracts (**Al-Mehmadi &Al-Khalaf, 2010**). Several histopathological alterations in the midgut, including separation of the epithelial cells from the basement membrane, as well as swelling, elongation, and degredation of the epithelial cells in larvae can be occurred. All plant extract treatments showed a similar pattern of larval gut damage, such as cross-sectioned of aquatic *Balanites* fruits showing degeneration of the epithelial cells of mid gut with vacuolation, lysis of cell's gut in lumen of the gut ,(EP.), basement membrane (BM), and per trophic membrane while in (Figure 10,11) *Balanites* leaves showing thickness in circular and longitudinal muscle fibers, dilution in main muscles and the destruction of epithelial cells in gut:

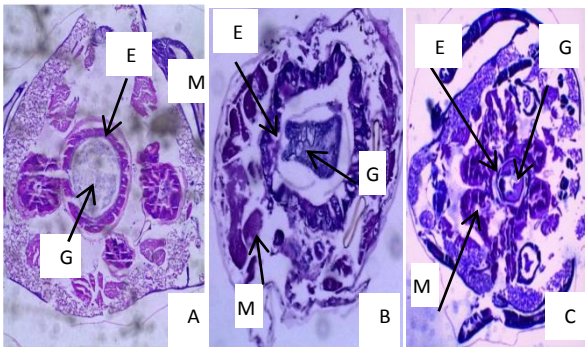

Figure 10: Cross-section of **A**: control (The untreated) larvae consists of (G = Gut; AT = Adipose Tissue, M = Muscle; C = Cuticle) and the gut contain a single layer of ciliated columnar cells resting on a basement membrane and surrounded by circular and longitudinal epithelial cell.40X. **B** = A cross-section of larvae treated with aquatic *Balanites* fruits.40X. **C** = A cross-section of larvae treated with aquatic *Balanites* leaves .*40X*.

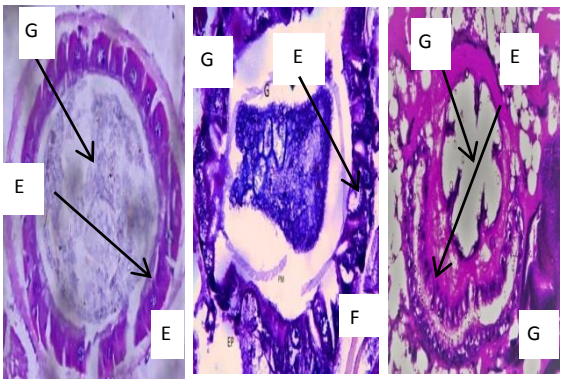

Figure 11: **E** = A cross-section of The untreated larvae consists of (G = Gut) surrounded by epithelial cells.. 400X. **F** = A cross-section of larvae treated with aquatic Balanites fruits showing degeneration of the epithelial cells of mid gut with vacuolation , lysis of cell's gut in lumen of the gut, (EP.). 400X**. G** = A cross-section of larvae treated with aquatic Balanites leaves showing destruction of epithelial cells in gut. 400X.

Discussion

It is clearly proved that plant or crude extracts are low cost-effective and more effective in controlling mosquitoes than chemicals (Jang *et al*., 2002**)**. Aqueous extracts of *B. aegyptiaca* from fruits and leaves showed a powerful larvicidal activity and were determined to be one of the effective aqueous extracts on the third larvae of *Cx. pipiens* after 48 hours. As evidenced by the obtained data. The toxicity of the saponin against the various *Cx. pipiens* larval stages was demonstrated by the mortality that increase by increasing concentration, this may be due to plant constituents according to earlier research (Farid *et al*., 2002), these tissues of *B. aegyptiaca* plants contain a significant amounts of saponins. As a result, the high mortality of different extract components may be related to the presence of saponin compounds in the *Balanites tissues*. The most likely cause of the larvae's death could be the interaction of saponin molecules with their cuticle membrane, which ultimately caused this membrane to become disorganised (Morrissey & Osbourn, 1999). It was impossible to ignore the lack of dissolved oxygen in the water caused by the active antioxidant saponin molecule. Previous researches have revealed that numerous plant extracts do have insecticidal properties (Arnason *et al*., 1989).

The present results are in agree with (Zarroug *et al*., 1990**)** which showed that Various saponin sources can be used for efficient bioactive preparation in *Aedes aegypti* and *Culex pipiens* mosquito control. Similary (Wiesma & Chapagain, 2005) reported that aqueous extracts of the *Balanites* plant can be utilized as sustainable and eco-friendly insecticides to manage mosquito where *Balanites aegyptiaca* fruit mesocarp was discovered to be sufficient in larvicidal activity of the fruit mesocarp extract of *Balanites aegyptiaca* and its saponin fractions against *Aedes aegypti* to prevent the emergence of 50% of the test larvae population.

Generally, obtained notable results of bioassays using the saponins against the larval stages of the mosquito species *Culex pipiens* showed a larvicidal activity expressed by a high larval mortality of the treated series compared to the control ones. The confirmed toxicity of the saponin was mentioned previously against other mosquito species agreed with (Pelah et al., 2002), such as *Aedes aegypti* (Bishnu et al., 2005), and *Anopheles stephensi* (Ghosh and Chandra, 2006).

Biochemical study

Biochemical studies were carried out to understand the effect of median lethal concentration (LC_{50}) of the tested plant extract on *Cx. pipiens* larvae. The overall content of protein of the treated larvae was lower than that in control samples was observed. Protein is a structural element inside the cell and plays an important role in metamorphosis step, chitin manufacturing and formation of cuticle. The significant shortage in the protein contents of the tissue samples in all treatments might be due to protein binding with foreign compounds as the tested agricultural waste extracts or may be due to mobilization of amino acids during plant extracts stress to meet the energy or might be due to the destructive effects on some of the cerebral neurosecretory cells of the brain responsible for secretion of the protein of the treated larval instars of *Cx. pipiens*. Protein is considered a key enzymes in the formation of non-essential amino acids (Mordue &Goldsworthy, 1973), which the changes in their levels could be correlated with anabolism or catabolism of protein. Maintenance of the balanced amino acid pool in insects is the result of various biochemical reactions carried out by a group of amino-transferase enzymes (Meister, 1957).

The protein levels decreased in our study and the similar findings were obtained by (Senthikumar *et al*., 2009**)** and **(**El-Sheikh *et al*., 2015**)** who found that protein levels were decreased in the *Anopheles stephensi* larvae treated with certain plant extracts as a result of interfering with the process of natural protein synthesis. This is agreed with (Nadia Bouguerra, 2018**)**.

(Khosravi & Sendi, 2010**)** have reported a reduction in the total protein, this investigation shows that after treatment, the protein levels decreased. This is in

accord with the observations of (Sharma *et al*., 2011**)** who noticed a reduction of protein levels in Anopheline and in Culicine larvae after treatment with *Artemisia annua* and *Azadirachta indica* extracts, respectively. This decline in protein content is probably due to insecticidal interference of the extract with the hormones regulating protein synthesis.

Acid phosphatase is known as a lysosomal marker enzyme that is active in the gut. (Qari *et al*., 2017**)**. So it could be used as a parameter for the determination of antifeedant activity (Abdel-Aziz, 2000**).** The detoxifying enzymes react against insecticides or compounds exhibiting insecticidal activities, they include general esterases, glutathione S-transferase, and phosphatases (Zibaee, Zibaee, & Sendi, 2011**)**. The obtained results of the present study clearly showed a significant decrease in the acid phosphatase after treatment with the plant extract may indicate the involvement of this enzyme in the detoxification process against the tested larvicides, as suggested by **(**Shekari *et al*., 2008**).** The obtained data clearly showed a highly significant inhibition in the acid phosphatase after treatment with the tested extracts as recorded by (Farag *et al*., 2021). The alkaline phosphatase enzyme increase in the present study in case of aquatic extract of *B. aegyptiaca* leaves perhaps revealed that the resistance of some insects to insecticides and that agreed with (Chang *et al*., 1990) who reported that alkaline phosphatase activity increasing might be due to the activation of ecdysone which is followed by a subsequent increase in the number of lysosomes acid and alkaline phosphatases (ALP) are the hydrolase enzymes responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids in alkaline and acidic conditions, respectively under the name of dephosphorylation (Zibaee *et al*., 2011), According to Ranson *et al*. (1997), the increased alkaline phosphatase activity was similar to increased alkaline phosphatase activity which was recorded by Wu, (1990) after the author treated the larvae of *Culex pipiens* with IGR diflubenzuron, the author attributed that increase in activity to developmental disturbance. Shekari *et al*. (2008) Also, attributed that increase to the involvement of this enzyme in the detoxification process. In case of aquatic extract of *B. aegyptiaca* fruits the alkaline phosphatase enzyme decrease this is and this is may be due to the resistance of the insect.

Results indicate that all tested materials significantly increased amylase, α-amylases catalyze the endohydrolysis of long α-1,4-glucan chains such as starch and glycogen (Terra & Ferriera, 2005), and this is similar to results of (El-Sheikh & Selem., 2015**)** that indicate increasing amylase. Significant increase effects on amylase, a hydrolytic enzyme that found in microorganisms, plants and animals, could affect catalyze of carbohydrates (Hoffmann & Franco *et al*., 2004). This is disagreed with Riseh & Ghadamyari (2012) and Darvishzadeh & Bandani (2013) who stated that plant extract reducing amylase amount, being one of the most important carbohydrates in midgut and haemolymph of the larvae and this is may be due to the resistance of the insect to the extract.

LDH is an important glycolytic enzyme involved in carbohydrate metabolism in many tissues (Shekari *et al*., 2008). (LDH) is involved in carbohydrate metabolism and has been used as an indicative criterion of exposure to chemical stress (Wu & Lam, 1997; Diamantino *et al*., 2001) and it is used as an index of anaerobic metabolism (Chamberlin & King, 1998). The obtained notable results of lactate dehydrogenase enzyme level were significantly reduced in this study in case of aquatic extract of *B. aegyptiaca* leaves and this is similar to (Arshad *et al*., 2002) who showed that the activity level of lactate dehydrogenase in *Culex* after treatment with DDT, malathion and cyfluthrin decreased respectively. Nathan *et al*. (2005) showed that feeding of *Spodoptera litura* on *Ricinus communis* treated with *azadirachtin* and nucleopolyhedrovirus decreases the amount of this enzyme in midgut that demonstrates low nutritional efficiency of the larvae. Similar results were also observed on effectiveness of *Melia azedarach* on rice leaf folder (Nathan, 2006). Enzyme level increased in case of Aquatic extract of *B. aegyptiaca fruits*, that may be due to the resistance of some insects to the plant extract.

Histology

One of the most promising ways to assess the insecticidal effects of plant extracts is through the gut histology of mosquito larvae (Al-Mehmadi & Al-Khalaf, 2010; Lija-Escaline *et al*., 2015). The midgut, which is the longest portion of the digestive tract in *Cx. pipiens* larvae and the most crucial functional component of the digestive system, is responsible for digesting, nutrient absorption, feeding, and the growth of the larvae (Taha *et al*. 2010). The midgut shows different and progressive damage to the larvae's intestinal tissue, causing the mixing of gut cells content with hemolymph, which is responsible for larval mortality (Zerroug *et al* 2017**)**.The histological studies showed many alterations and malformation in the treated instar larvae midgut. In case of *Balanites* leaves showing thickness in circular and longitudinal muscle fibers, dilution in main muscles and the destruction of epithelial cells in gut and this is similar

to **(**Assar & El-Sobky, 2003**)** observed that the water extract of *Eichhornia crassipes*, revealed drastic effect on larval midgut as the brush border and some of the epithelial cells were apically degenerated after 48 h and after 72 h, most of the epithelial cells completely degenerated and vacuolated.

In case of aquatic *Balanites* fruits midgut showing degeneration of the epithelial cells of mid gut with vacuolation, lysis of cell's gut in lumen of the gut, (EP.), basement membrane (BM), and per trophic membrane (PM). Changes were seen the midgut, included separation of the epithelial cells from the basement membrane with damage of the peritrophic membrane. The mixing of the gut contents with the haemolymph caused the larval mortality. The border of midgut showed a striated appearance due to the presence of the microvilli which line the inner edge of the epithelial cells agreed with **(Nasiruddin & Mordue, 1993)** and **(Bakkali *et al*. 2008)** whom showed the histopathological changes in treated insects with alternative insect control as a toxic action were previously investigated and with botanical insecticides were also studied**.**

In the same context, the results reported by (**Seye *et al* 2006**), on the effect of *Citrus limon* and *Allium sativum* oils against *Culex pipiens* larvae, reveal that there was a clear damage to the epithelial cells of the midgut. Intestinal tissue, muscles and cuticle were the most severely damaged by the treatment, as well as the separation of the midgut cells from their basal membrane.

Conclusion

The findings of the present study revealed that *Balanites aegyptiaca* has a larvicidal effect on *Culex pipiens* larvae. Larvae treated with *Balanites aegyptaica* exhibited alterations represented in damage in the cuticle, muscles, and midgut in comparison with the untreated one, reduced total protein LDH, acid phosphatase and disturbed alkaline phosphatase, while alpha amylase content increased. The results of this study may contribute to a reduction in the application of synthetic insecticides which in turn decrease the environmental pollution.

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