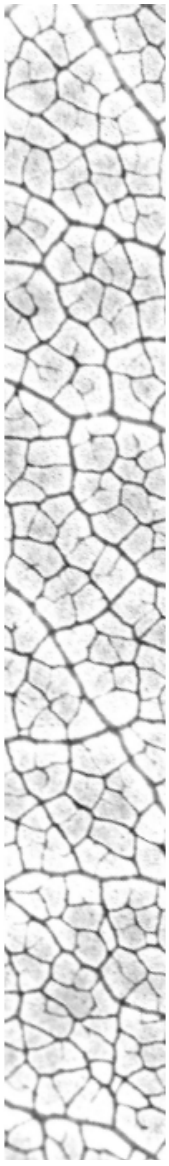

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Effect of crude extracts of the peels of *Musa acuminate* L. Banana plant in some biological aspects of *Culex Molestus* Forskal (Diptera: Culicidae) with an estimation of the enzymatic effectiveness of Tyrosinase

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Abstract
The effectiveness of the crude extract of organic solvents, hexane, ethyl acetate, and ethanol was studied for peels of *Musa acuminate* L. banana that contains the Tyrosinase and determination of the enzymatic efficacy and protein, The concentrations of (0.5, 1 and 2) mg / ml were used in the treatment of the fourth larval instar of the insect and its effect on some biological aspects of *Culex molestus* mosquitoes in Karbala governorate in February 2018 for mosquitoes control. The results showed an increase in the rate of the fourth larval instar and pupae resulting from the fourth larval instar treatment and the percentage of cumulative mortality of immature stages and the increase in the percent of inhibition of emergence of adult. The use of these extracts led to a decrease in the rate of ages of the immature stages of male and female insect and the appearance of phenotypic deformation in the stages of different insects. The study also evaluated the enzymatic efficacy of Tyrosinase at 0.5 mg / ml and protein concentration of 1.25 mg / ml and the specific efficacy was 0.4 unit/ mg protein.

Keywords:

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INTRODUCTION

Culex molestus mosquitoes belong to order: Diptera to the Culicidae family, a medically and biologically important blood-sucking insect that transmits many diseases to humans, causing an epidemic. This species is the most demosticity and present in the human environment (Alahmed et al. 2010, Fodeal 2014, Ilango et al. 2016, Kassim et al. 2012). The use of chemical pesticides pull faces many criticisms and warnings (Al-Jinabi and Al-Essa 2013), so researchers began to use other methods of control, including control by using plant extracts as insecticides (Al-Kahfagi et al. 2017). The larvae and pupae mosquitoes are generally affected by the toxic effects of these extracts (Al-Raheemy and Al-Essa 2017). Musa acuminate L. belongs to the Musaceae family, a plant that contains Tyrosinase (monophenol, o-diphenol: oxygen oxidoreductase, EC 1.14.18.1). It is due to a large group of proteins called copper proteins of the third type, which include catechol oxidases enzymes in plants (Velichkova et al. 2015). Amino acid Tyrosine is responsible for the hardening of the cuticle, by which the transfer of the pale color and soft cuticle after the moulting directly to the cuticle rigid after a few hours and given the special pigments of the insect (Arakane et al. 2016, Sterkel and Oliveira 2017, Sugumaran and Barek 2016, Vavricka et al. 2010). In most insects, there was a significant increase in the concentration of tyrosine before the process of moulting directly and decreased at or after the process of moulting significantly decreased and the rise of Tyrosinase concentrations represents the first start of the process of moulting and melanozation (Kim et al. 2002, Kubo et al. 2003, Sugumaran and Barek 2016). The function of this enzyme was detected in the process melanozation in mosquitoes Called dopachrome conversion enzyme (DCE) (Li et al. 1994, 2007). Therefore, Tyrosinase was used as a means of control insect pests by inhibiting its effectiveness (Kubo et al. 2003), and to control the adult stage of mosquitoes. So, the research was aimed at control the fourth larval instar of the insect by studying the effect of the crude extract of banana plant peels in

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some biological aspects of the insect's by treatment of Fourth-instar larvae and calculation of the percentage of mortality of the treated larvae and their resulting pupae. Also, studying the percentage of phenotypic deformities of the immature stages resulting from treatment with these extracts, the average age of immature stages and the adult stage of the insect result of treatment with this extracts.As well as the investigation of Tyrosinase in the banana peels plant and assesses its effectiveness.

MATERIALS AND METHODS

Collecting and Diagnosing Plant Samples

The samples under study were collected from the household waste of the banana plant *Musa acuminate* L. in Karbala Governorate/ Iraq, in February 2018. It was dried and grinded and placed in glass bottles and the name of the plant was recorded and stored in the fridge until it was used, the plant was diagnosed at the University of Karbala - Faculty of Science - Department of Life Sciences - by Dr. Khansaa Abdul Alee Shaheed.

Preparation of Crude Extracts for Organic Solvents of Plants

Crude extracts were prepared for organic solvents for banana peels by method (Ladd et al. 1978). Three organic solvents different polar were selected, ethyl alcohol as the polar solvent and Ethyl acetate as a medium-polar solvent, and hexane (n-Hexane) as a non-polar solvent (Harborne 1984). For the purpose of estimating the effectiveness of organic solvent crude extract for banana plant peels and its effect on *Cx. molestus* mosquitoes, a (2) gm of dry matter for each organic extract was dissolved in 2 ml of each organic solvent extracted from the sample. The volume was supplemented to 100 ml with distilled water, the concentration of the stock solution became 2% equivalent 2 mg / ml, the solution was about 20 mg / ml, i.e., from this solution concentrations was prepared (0.5, 1 and 2) mg/ml. The control treatment was performed using 1 mL of each solvent and 1 ml of ethyl alcohol with 98 mL distilled water.

Collecting and Diagnosing of Mosquito Samples

Boats of eggs samples of *Cx. molestus* were collected in the Karbala Governorate from the (3X3) m exposed **wastewater dumps** that contain Typha sp (Cane plant) at their edges in February of 2018. The boats were placed in laboratory conditions for the purpose of hatching and were reared until they reached the fourth larval instar and samples were taken for diagnostic purposes using the diagnostic key (Abul-hab and Kassal 1989). In order to confirm the diagnosis, it was based on a vital phenomenon, namely Autogeny, the ability of females to put the first egg boat without the need for a blood meal (Hayes 1973).

Mosquito Breeding

The colony was purified for three generations before the experiments were started. The egges' boats collected from the study site were transferred to plastic containers space 500 ml containing water, each egg boat was transferred to a 100 ml plastic container. After the eggs were hatched and turned into larvae, the breadcrumbs were added to feed them. When they were turned into pupae, they were transferred to the breeding cages with dimensions 40X40X40 cm, closed by the first cloth on one side and the metal wire from the four sides, In addition to a wooden base, the adults were not fed to the blood but were fed on a 10% glucose solution. This method of breeding was used for the purpose of obtaining adults and the fourth larval instar of diagnosis and conducting the necessary experiments.

Study of the Effect of Crude Extracts of Organic Solvents for Banana Peels in Biological Aspects of the Insect

10 larvae were taken from the fourth larval instar with three replicates per concentration and transferred to 100 mL plastic containers containing the above extracts with concentrations (0.5, 1 and 2 mg/ml), with the addition of bread crumbs to feed them, and in the control treatment, distilled water was used with the organic solvent to follow up on its life. The mortality ratios for the fourth instar larvae and for the pupae resulting from the treatment of fourth instar larvae were recorded until they reached the adult stage and the growth rate was calculated for fourth instar larvae, pupae and adults and the percentage of phenotypic deformation was calculated and the rate of percentage mortality were corrected for according to Abbott Forula equation (Abbott 1925).

$$\text{The Percentage of Corrected mortality} = \frac{\%\text{mortality at treatment} - \%\text{mortality at control}}{100 - \text{mortality at control}} * 100\%$$

The percent Inhibition of Emergence (IE%) was calculated according to the Mulla equation and others (Mulla et al. 1974).

$$IE\% = 100\,(T\,X\,100\,/C)$$

T = the percent of emergence in the treatment

C = the percent of emergence in the control treatment

Investigation about Tyrosinase in Banana Peels Extract

The enzyme was investigated according to the method (Frary et al. 2010):

1- banana peels were cut and crushed 0.1 g of them and dissolved with 0.2 g of the regulated solution Tris_HCl, pH = 7 during crushing in a ceramic mortar and under refrigerated conditions.

2- The extract was then filtered as the plant parts were removed.

3- Centrifugal of extract under refrigerated conditions (4) ℃ at 8000 cycles / min for 10 minutes to obtain a pure enzymatic extract.

Determination of the Effectiveness of Tyrosinase

The efficacy of Tyrosinase was determined according to the method described by (Kubo and Kinst-Hori 1998) using 1 Mm of L-Tyrosine as an interaction substance, as follows:

1- Pumping pure oxygen for (5-3) minutes to the interaction substance.
2- The reaction mixture consisting of water that removed from it the ions and the regulated solution (50 Mm Potassium Phosphate Buffer, pH = 6.5) and solution of the reaction substance.
3- Add 0.1 mL of the enzymatic extract and record the increase in absorption at a wavelength of 280 nm.

Enzyme efficiency is calculated according to the following equation:

$$Units\ /\ ml\ enzyme\ =\ (\Delta A280nm/min\ Test - \Delta A280nm/min\ Blank)\ (df)\ /(0.001)\ (0.1)$$

The enzyme activity unit is defined as the amount of the enzyme that causes an increase in absorption at a wavelength of 280 nm at 0.001 per minute and underestimation conditions.

Determination of the Protein

Determination of the protein in the enzymatic extract using the method (Lowry et al. 1951) using the bovine serum (BSA) is a standard protein.

The specific efficacy of Tyrosinase was calculated according to the following equation:

$$specific\ efficacy = Efficacy\ (unit)\ /\ protein\ (mg) = Unit\ /\ mg\ of\ protein$$

Statistical Analysis

The results of the study were analyzed according to the model of the factorial experiments with completely randomized design and the Least Significant Difference (L.S.D.) was used below a significant level of 0.05 to indicate the significance of the differences between the different treatment (SAS 2012). The mortality ratios were adjusted according to the equation listed in (Abbott 1925, AlRawi and Khalf Allah 2000) and the corrected values were converted to angled values for their insertion in the statistical design.

RESULTS AND DISCUSSION

Effect on the Percent of Corrected Mortality of the Fourth –Instar Larvae and Pupae Resulting from the Treatment of the Fourth-instar Larval of *Cx. molestus*

The results indicated in **Table 1** existence a relationship between the concentrations used and the percent of mortality, This relationship differed according to the type of solvent used in the extraction, It was found that when using the solvent of hexane, the percent of mortality was (12.5)% at the concentration (0.5 mg / ml)and increased to 62.5% at the concentration of 1 mg / ml, but when the concentration increased to 2 mg / ml we note a decrease in the percent of mortality to 12.5%,In principle, this result was similar to that obtained using the ethyl acetate extract. We note that the effect of the concentration increased from 0.5 to 1 mg / ml but when increased to 2 mg / mL, note the low effect, but the results were different when using the ethanol extract, The correlation between the concentrations used and the mortality rate was 0% at the concentration of 0.5 mg / ml and raise with the concentration increased to 25% at the concentration of 2 mg / ml compared to the control treatment of 4.8%, pupae resulted of these larvae had a patent effect on the rate of their mortality, We can note in the hexane extract, the mortality rate of 30% in the concentration was 0.5 mg/ml and decreased to 10% in the concentration of 1 mg/ml and this percent increased with a concentration of 70% in the concentration of 2 mg/ml. When using the extract Ethyl acetate was found to have no effect on pupae in the concentrations of 0.5 and 1 mg/ml, as it reached a mortality rate of 0% respectively, but in concentration 2%, the mortality rate was 10%.

When the ethanol extract was used, it was observed that there was no effect on the pupae in the concentration of 0.5 mg/ml as it reached mortality rate was 0%. In the concentration of 1 mg/ml, the mortality rate increased to 70% and then decreased to (60%) in concentration (2mg/ml) compared with 0% in the control treatment.

The results of **Table 1** showed that the effect for the hexane extract of the banana peel was more effective than the ethyl acetate and ethanol extract in the percent of the mortality of the fourth–instar larval of the insect and the effect of the hexane and ethanol extract on the plant was found to be more effective than the ethyl acetate in the percent of mortality of pupae resulting from fourth-instar larvae treatment and the effect varied with different concentrations of the extract and the results of the statistical analysis showed the significance of the differences in the results obtained.

A study (Abdul Rahuman 2011) confirmed in India conducted three types of mosquitoes: *Anopheles vagus*, *Argemeres subalbatus*, and *Culex vishnui*. The crude extracts of organic solvents (chloroform, methanol, hexane, ethyl acetate, and acetone) were used for leaves, flowers, flower buds, and seeds of several plants including banana flowers *Musa paradisiaca*. With a mortality rate of 84 and 75%, respectively, in the concentration of 1000µg / ml in the hexane and ethyl acetate extract when treated with *Culex vishnui* fourth-instar larvae.

Table 1. Effect of Interaction of crude Extract Concentrates of Organic Solvents to *Musa acuminate* peels in Percent of corrected mortality of the fourth-instar larval and the pupae resulting from the treatment of the fourth-instar larval of *Cx. molestus*

Concentrations mg / ml	Hexane Extract		Ethyl acetate extract		Ethanol extract	
	% Mortality of the fourth-instar larval	% Mortality of the resulting pupae	% Mortality of the fourth-instar larval	% Mortality of the resulting pupae	% Mortality of the fourth-instar larval	% Mortality of the resulting pupae
Control	4.8	0	4.8	0	4.8	0
0.5	12.5	30	0	0	0	0
1	62.5	10	25	0	12.5	70
2	12.5	70	12.5	10	25	60
L.S.D	18.828	13.347	15.583	0.941	16.306	10.525

L.S.D. The interaction between the three extracts and the concentrations was 16.143 in the percent of mortality in fourth-instar larval decay.
L.S.D. Interaction between the three extracts and concentrations was 9,753 in the percent of mortality inpupae of resulting from the treatment of the fourth – instar larvae.

Table 2. Effect of the intraction of the crude extract concentrations of the organic solvents of the *Musa acuminate* peels in the cumulative percent of cumulative mortality of the immature stages of the *Cx. molestus*

	Banana Plant		
Concentrations mg / ml	Hexane Extract	Ethyl Acetate Extract	Ethanol Extract
Control	4.8	4.8	4.8
0.5	0.5	0	0
1	75	25	100
2	100	25	100
L.S.D	14.121	6.656	21.051

L.S.D. value of the interaction between the three extracts and the concentrations was 13,543 in the cumulative mortality of immature stages of the insect

The results of the (Narayanan et al. 2014) study showed that the concentration of 500ppm for the chloroform extract of *Terminalia retz* plant, It was the most effective and gave a percent of mortality 56% of the *Cx quinquefasciatus*, and that the percent mortality of pupae in the concentration of 250 ppm reached 39.20%.

The high percent of mortality of larvae and pupae resulting from the treatment of the fourth-instar larvae of the insect with crude extracts of organic solvents for banana peel may be caused by the tyrosinase that is responsible for the stiffening of the body wall after a few hours of moulting, this enzyme is the first start of the moulting process, then the insect gave the dark colors to protect them from predators.

As the enzyme rate of increase before the process of moulting and it decreases during or after the process (Kubo et al. 2003), and the mortality of insects treated with these extracts is the survival of the percent or concentration enzyme is high that indicate to the incomplete of cuticle hardening and non-coloration, which led to the emergence of cases of partial moulting in insects treatment with the emergence of larvae and pupae Albino.

The efficacy of the organic solvent extract of hexane to banana peel to contain the above plant may be due to a high amount of toxic compounds present in the plant extract that affect the target tissues and cause larval tissue poisoning (Kuusik et al. 2001, Sukumar et al. 1991).

Effect of Interaction of Crude Extract Concentrations of Organic Solvents for *Musa acuminate* Peels in the Percentage of Cumulative Mortality of Immature Stages (Fourth-instar Larval and the Pupae Stage) of *Cx. molestus*

Table 2 shows the effect of the crude extract of organic solvents of banana plant peels in the percent of cumulative mortality of immature stages of the insect. The highest rate of cumulative mortality of immature stages of the insect in the extract of hexane, ethyl acetate, and ethanol (100, 25 and 100%) in concentration (2) mg / ml respectively compared with (4.8%) in the control treatment.

It is noted from **Table 2** that the extract of hexane and ethanol for *Musa acuminate* peels were more effective than the ethyl acetate plant extract in the percent of cumulative mortality of immature stages, the results indicate of the statistical analysis showed the differences in the results obtained.

Notes from the current study that the greater the concentration and the time period, the higher the percent of cumulative mortality of immature stages, the reason may be due to the effect of these extracts in the insect stager because they contain toxic substances or other effective compounds acting as feeding deterrents the insect to die. The killing reason of insect stages of their nervous and digestive system may be effect when touching these extracts to the body surface of the insect or entering these substances through the respiratory openings (Halawa et al. 1998). Or may cause the reason for mortality of the fourth-instar larvae to starvation because the active compounds of the plant extract works as feeding deterrents and may combine with the fatty substances in the digestive system and cause the mortality of larvae, where the fatty substances removed

Table 3. Effect of the Interaction of the Crude Extract of Organic Solvents to *Musa acuminate* peels in the Percent Inhibition of Emergence of *Cx. molestus*

Concentrations mg / ml	Banana Plant		
	Hexane Extract	Ethyl Acetates Extract	Ethanol Extract
	% To percent Inhibition of emergence	% To percent Inhibition of emergence	% To percent Inhibition of emergence
Control	20	20	20
0.5	96	90	0
1	97.5	92.5	100
2	100	92.5	100
L.S.D	23.742	16.972	32.06

L.S.D. Value For the interaction between the concentrations of the extract and the percent inhibition of emergence 19.22

Table 4. The Effect of the Interaction of the Crude Extract of Organic Solvents on *Musa acuminate* peels in the Rate of Age of the Fourth-instar Larval ,pupae and Adult stages of *Cx. molestus*

Concentrations mg / ml	Crude extract of organic solvents for *Musa acuminate* peels plant											
	Hexane Extract				Ethyl acetate extract				Ethanol extract			
	Age of larva	Age of pupae	Age of male	The age of female	Age of larva	Age of pupae	Age of male	The age of female	Age of larva	Age of pupae	Age of male	The age of female
Control	5	7	6	12	5	7	6	12	5	7	6	12
0.5	4	4.42	5	9	2.25	3	5	-	11	-	-	-
1	4.25	3	3	-	7.28	4.33	5.66	7	1.66	2.14	-	-
2	1.25	-	-	-	2	3	-	-	1.5	2	-	-
L.S.D	1.882	1.630	2.306	1.331	2.977	1.882	2.306	2.105	3.646	1.630	0.941	2.824

D for interaction between the three extracts and 1,685 concentrations in larval age
L.S.D for the interaction between the three extracts and 1.583 concentrations in the age of the pupae
L.S.D for the interaction between the three extracts and concentrations 1.287 at the age of male
L.S.D for interaction between the three extracts and 1.087 concentrations in the age of the female
*** - Indicates the Mortality of the insect**

without benefit, or may affect the Epithelial cells of the gastrointestinal tract and cause poisoning of the insect (Metspalu et al. 2001, Pederson et al. 1976).

A study (Al-Khafagi 2010) confirmed when continuous exposure to the immature stages of *Cx. pipiens* of the extract of the terpene compounds of the leaves of the *Ricinus communis* plant caused the most cumulative mortality of 100% for all concentrations.

Results in (Al-fetlawy 2014) showed a cumulative mortality of immature stages of *Cx. pipiens* 100% when treated with terpene compounds extract to *Tamarix ramosissma* for concentrations (2.5, 5, 10 and 20) mg / ml respectively.

Effect of the Interaction of Crude Extract of Organic Solvents of *Musa acuminate* peels in the Percent Inhibition of Emergence of Adult of *Cx. molestus*

The effect of the crude extract of organic solvents of *Musa acuminate* peels was significantly observed in the percent Inhibition of Emergence of *Cx. molestus* and the different concentrations used (**Table 3**), with the highest percent Inhibition of Emergence in the extract of hexane, ethyl acetate and methanol (100, 92.5 and 100%) in concentration (2) mg / ml respectively compared with 20% in the control treatment.

It was observed in the results in **Table 3** that the extract of hexane and ethanol were more effective than the ethyl acetate extract of the plant in the percent inhibition of emergence of adult insects. The results of the statistical analysis showed the significance of the differences in the obtained results.

A study (Al-Rahimy 2017) confirmed the use of chloroform extracts for the roots, stems, and leaves of boht the *Mentha spicata* L. and *Eucalyptusmicrotheca* F.Muell. plants, when treating the fourth larval instar of *Cx. molestus*. The value of Percent Inhibition of Emergence (IE%) reached to (100%, 39%, and 78%) in the concentration of 0.5 mg/ml and (100, 81.82 and 100%) respectively in the concentration of 1 mg/ml for the roots, stems and leaves for the mint plant. Where in the roots, stems and leaves of the Eucalyptus plant reached (100, 61.04 and 61.04%) in the concentration of 0.5 mg / ml and (100, 100 and 100%) respectively in the concentration of 1 mg / ml.

Effect of the Interaction of the Crude Extract of Organic Solvents on the *Musa acuminate* peels in the Rate of Age of the Fourth-instar Larval, Pupae and Adult Stages and Complete Role of *Cx. molestus*

Table 4 shows the effect of the crude extract of organic solvents on the *Musa acuminate* peels in the period of growth of the fourth-instar larval, pupae and adults. The average age of the immature and adults of the insect (1.25, 0, 0 and 0) days respectively in the hexane extract, (2, 3, 0, and 0) days respectively in the ethyl acetate extract. In the ethanol extract, the average age (1.5, 2, 0 and 0) days respectively in the concentrations of 2 mg / ml compared to (5, 7, 6 and 12) days in the control treatment.

Table 4 shows a decrease in the average age of the larval instar, pupae and adult stage of the insect in all the crude extract concentrations of organic solvents for *Musa acuminate* peels plant. The results of the statistical analysis showed the differences in the results obtained.

The low rate of age of the insect in the current study may be because of the contains of these extracts on

substances that inhibited the effectiveness of Tyrosinase in the peels of banana plant and mosquito larvae, and this enzyme is directly responsible for the hardening of the cuticle, which resulted in mortality of adult stages and immature stages by age less than the average of the control treatment (Evans and Kaleysa 1992).

A study (Mahdi 2010) confirmed the effect of the organic extract(alcohol) of the plants *Peganum harmala* and *Ricinus communis* in the period of growth of different stages of *Cx. pipiens molestus*, as it reached the duration of the larval and pupae and adult stage of the *Peganum harmala* plant was 2mg / ml (12.2, 3.7 and 18.3) compared with (9.5, 3.0 and 14.1) in the control treatment, For the *Ricinus communis* plant, the duration of the larval ,pupae and adult stage of the same concentration above (10.0, 3.3, and 15.3) compared to (9.7, 3.2 and 14.7), respectively, in the control treatment.

A study (Al-Rahimy 2017) confirmed the use of chloroform extracts of *Mentha spicata* and *Eucalyptus microtheca* when treating the fourth-instar larval of *Cx. molestus* , The mean age of the fourth-instar larval and the male and female were: (2, -, -, -) days, and (1.6, 2.5, 2, 5.5) days, and (1.5, 3, 3, 5.3) days respectively in the concentration of 0.5 mg/ml, and (1, -, -, -) days, and (1.6, 2, 2, 4) days, and (1, -, -, -) days respectively in the concentration of 1 mg/ml for the roots, stems and leaves of *Menthaspicata*. While (2, 3.5, -, -) days, and (1, -, -, -) days, and (1, 3.5, 4, 6) days respectively in the concentration of 0.5 mg/ml, in the concentration of 1 mg/ml the mean age of the fourth-instar larval, pupae, male and female was reached (1, 2.54, -, -) days, and (1, -, -, -) days, and (1.14, -, -, -) days respectively for roots, stems and leaves of *Eucalyptus microtheca*plant in comare to (13, 8, 2, 2) days in the control treatment.

The Effect of the Interaction of the Crude Extract of Organic Solvents on Musa acuminate Peels in the Mortality and the Appearance of the Phenotypic Deformation of the Stages of *Cx. molestus*

The results of the present study showed the appearance of somephenotypic deformation in the shape of the fourth-instar larval, pupae and adult stage of the insect. As a result of the use of the crude extract of the organic hexane solvent, it was observed that the legs were adhesion with the body of the insect and atrophy in the abdominal region by 33.33% respectively in the concentration of 0.5 mg/ml, in the concentration of 1 mg / ml the appearance of the Albino larvae with atrophy at the beginning of the abdomen was observed at 33.33%, In the concentration of 2 mg/ml, the larvae appeared with a black head and a less color by 100%. In the ethyl acetate extract, Albino larvae were shown with 100% partial moulting, In the 1 mg / ml concentration, Albino larval, and atrophy in the adult in abdomen showed a 50% respectively. The Concentration of 2 mg / ml showed partial moulting of pupae 100%. And the extract of ethanol was the effect of this extract on the larvae by remaining in the fourth-instar larval without completing its life cycle as it reached 100%. In the concentration of 1 mg/ml, the combination of the head and thorax regions of the larvae and the partial emergence of adult appeared at 10%, respectively, compared with (20, 0, 0) % respectively of larval, pupae and adult deformation.

It is clear from the results in **Table 5** that the crude extract of organic solvents for *Musa acuminate* peels plant had an effect on the appearance of the morphological abnormalities of the treated insect. The results of the statistical analysis showed the significance of the differences in the results obtained.

The emergence of a number of Albino larvae and pupae when treated with crude extracts of organic solvents for the peels of banana plant, may be caused by the tyrosinase found in the peels of the plant under study, Where the study conducted by (Kubo et al. 2003) had confirmed that this enzyme represents the first start of the process of moulting and melanozation of insects and the appearance of white or transparent to insects treated with these extracts evidence of high concentration, which led to the moulting and non-decline led to non-hardening of the wall body of the body, which leads to non-discoloration of the insect in dark colors, which caused their mortality (Kubo et al. 2003, Vavricka et al. 2010).

The appearance of these abnormalities of the stages of the insect may be due to the inhibitory action of the plant studied on the growth of the fourth-instar larvae, pupae or adult stage, as the work may be similar to the work of these growth regulations, orimmature stages are unable to build the cuticle, which causes the mortality of the insect because the contains of these extracts with inhibitors of chitin formation in immature stages (Kader et al. 2010).

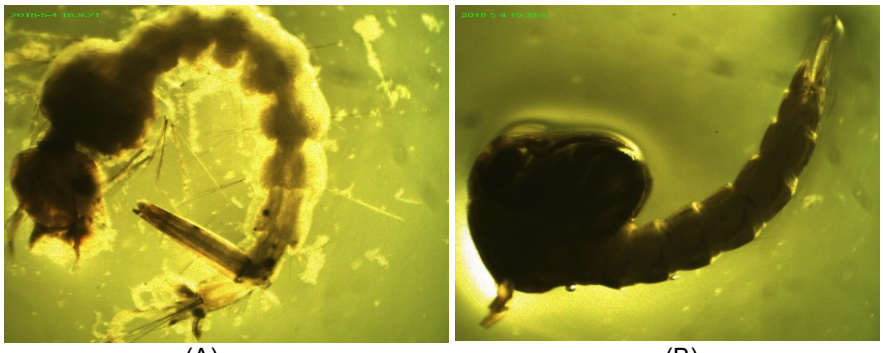
The results of the study (Kader et al. 2010) confirmed the use of organic extracts of *Datura innoxia* and *Nerium oleander* leaves, as the petroleum extract had an effect on appearance of deformities of the larvae *Cx. quinquefascitus* that treated with these extracts.

A study (Al-Rahimy 2017) showed the appearance of a number of phenotypic deformities of the fourth-instar larval and adults of *Cx. molestus* when treating the fourth-instar larval of the mosquito with extracts of chloroform of the *Mentha spicata* L. and *Eucalyptus microtheca* F.Muell. Using concentrations (0.25, 0.5 and 1) mg / ml.

Table 5. Effect of the organic solvent extract of *Musa acuminate*peels plant on the mortality and the appearance of the deformities of the insect stages*Cx. molestus*

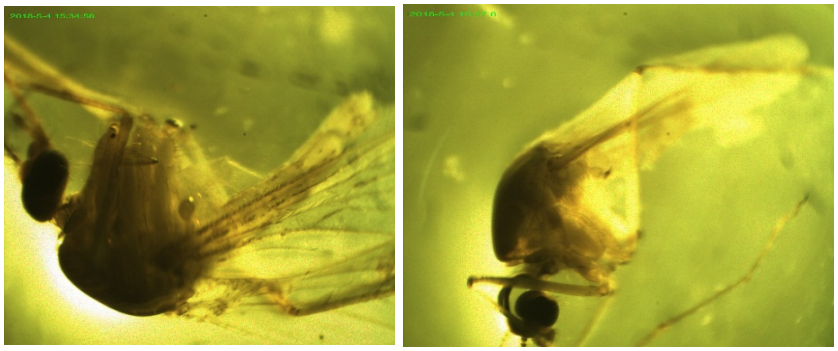
Banana Plant												
Concentration	Hexane extract				Ethyl acetate extract				Ethanol extract			
mg/ml	Larva	Pupae	Adult	Deformation	Larva	pupae	Adult	Deformation	Larva	pupae	Adult	Deformation
Control	20	0	0	0	20	0	0	0	20	0	0	0
.05	-	1 pupae with black colour	2 sticking of adult legs to body	16.66	2 Albino larvae and partial moulting		-	100	10 Survival of the larvae in the larval stage			100
			2 Atrophy of the adult abdomen	33.33		1 Inflate the head area of the pupae		50	without dissociation			
1	1 larva with a curved end			16.66	2 Albino larvae			50	1 larva with yellow colour			10
	2 pallid larvae with atrophy at the beginning of the abdomen	1 The appearance of a transparent area in the fourth and fifth rings of the belly of the pupa		33.33	2 Blackness of the larval body		3 sticking the wings with the body	50	1 larva with green colour			10
				16.66				50	2albino larva			20
							3 Atrophy of the adult abdomen	50	1 albino larva with combination in the head and thorax regions	1 pupa with partial moultingn		10
											1 adult with partial emergence	10
2	8 larva with black head and pale body	-	-	100		4 pupae with partial moulting		100	4 larva with curved end			40
										6 pupae with partial moulting 2 albino pupae		60
						1 elongation with inflated in the head of the pupae		25				20
							3 with sticked wings and body	50				
								50				
							3 inflated in adult abdomen					
L.S.D	1.882			9.507	1.882			16.306	1.630			13.347

L.S.D. Interference between extracts and concentrations was 11.946% in deformation
L.S.D. For the interaction between the extracts and concentrations in the mortality 1.613



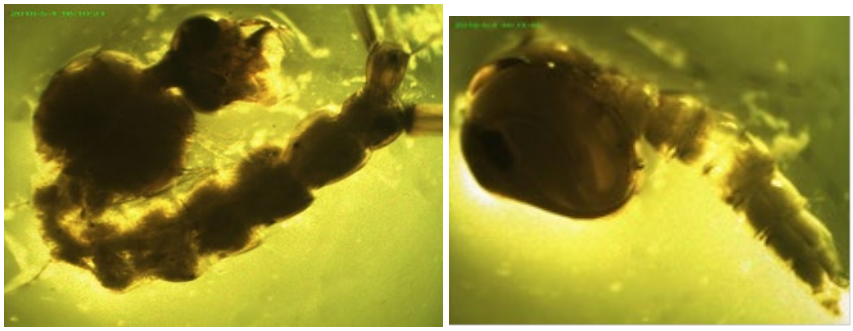
(A) (B)

Fig. 1. (A) A pupa with a black color at a concentration of 0.5 mg/mL, (B) larva with a curved end at a concentration of 1 mg / ml for *Cx. molestus* which were treated with a hexane extract for *Musa acuminate* peels Power zoom 4x



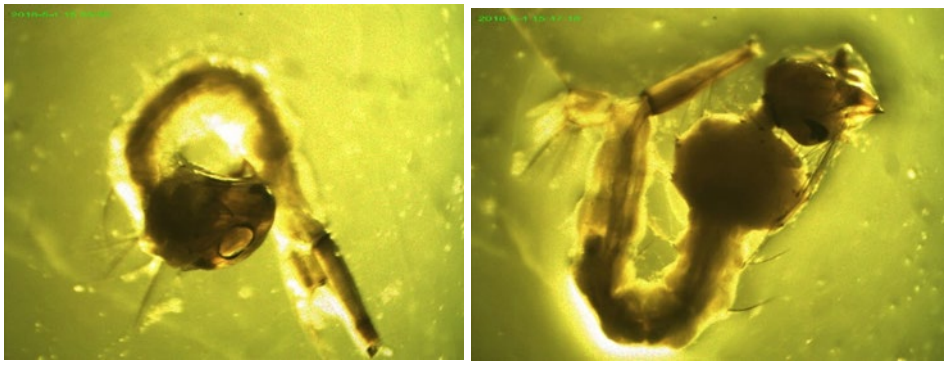
(A) (B)

Fig. 2. (A) Atrophy in adult abdomen, (B) shows that insect legs are sticked to the body of adult of *Cx. molestus* Treatment with a hexane extract for the *Musa acuminate* peels with a concentration of 0.5 mg / m. 4x magnification



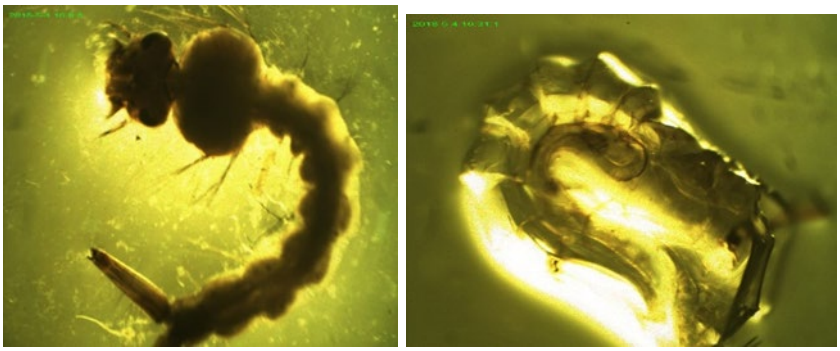
(A) (B)

Fig. 3. (A) White larva body with atrophy at the beginning of the abdominal region, (B) pupa colorless in the fourth and fifth abdominal rings of the *Cx. molestus* insect treated with a hexane extract of *Musa acuminate* peels with a concentration of 1 mg/ml. Power zoom 4x



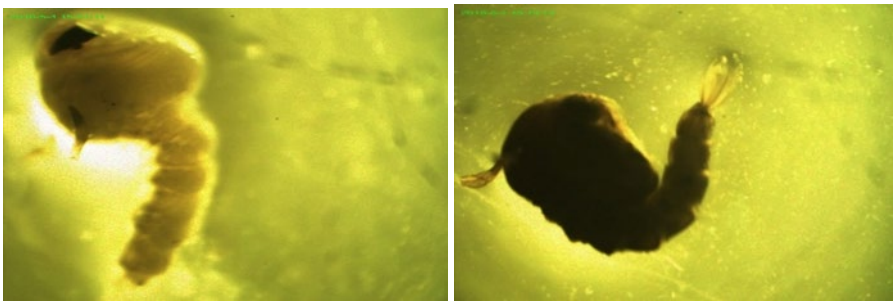
(A) (B)

Fig. 4. (A) Albino larvaeand partial moulting in 0.5 mg / ml, (B) Albino larvae at 1 mg / mL of the *Cx. molestus* treatment with Ethyl acetate extract for *Musa acuminate* peels The power zoom 4x



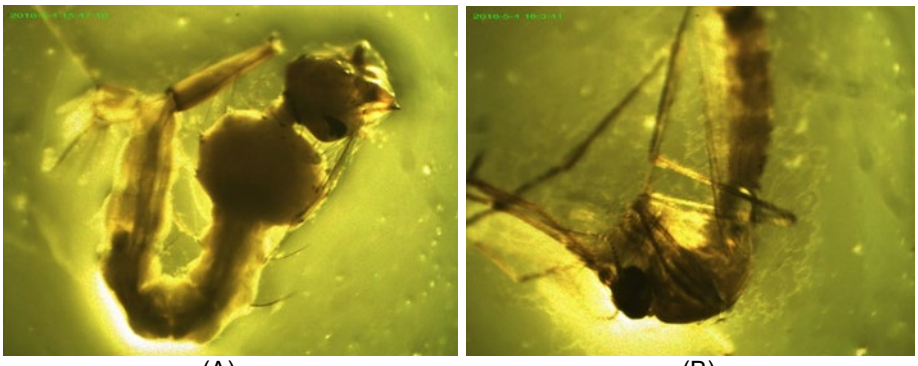
(A) (B)

Fig. 5. (A) The Black body of larvae at a concentration of 1 mg/ml, (B) showed a partial moulting of a pupa with a concentration of 2 mg/ml for *Cx. molestus* treatment with Ethyl acetate extract for *Musa acuminate* peels Power zoom 4x



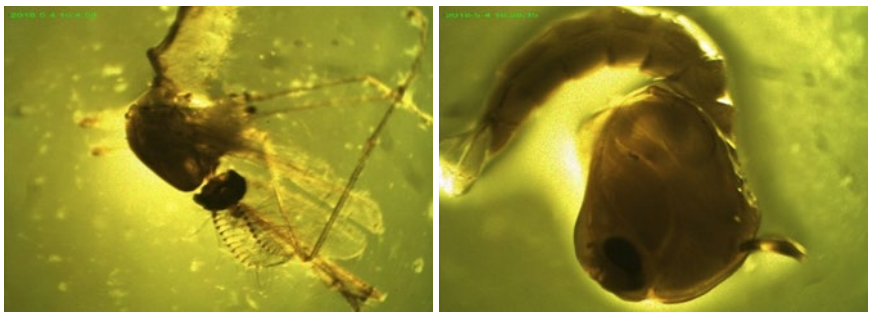
(A) (B)

Fig. 6. (A) Albino pupa, (B) Inflated pupa's head of *Cx. molestus* treated with Ethyl acetate extract for *Musa acuminate* peels in concentration of 0.5 mg/ml. The power of zoom 4x



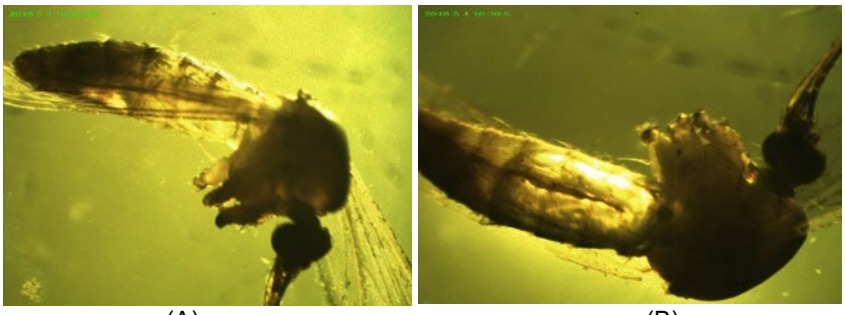
(A) (B)

Fig. 7. (A) Albino larva, (B) The wings attach to the adult body of the *Cx. molestus* treated with Ethyl acetate extract for *Musa acuminate* peels at 1 mg/ml. 4x magnification



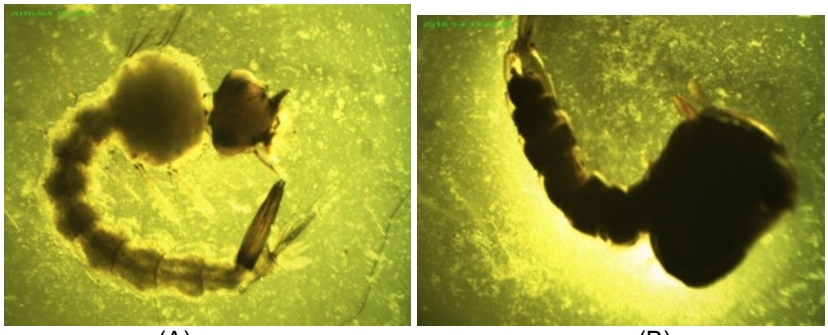
(A) (B)

Fig. 8. (A) Atrophy in the adult abdomen region of the concentration of 1 mg/ml, (B) showed elongation and enlargement of the pupa head at a concentration of 2 mg/ml for the *Cx. molestus* treatment with Ethyl acetate extract for *Musa acuminate* peels The power zoom 4x



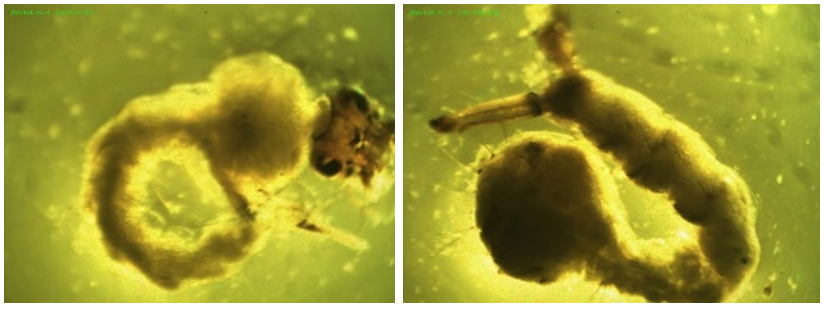
(A) (B)

Fig. 9. (A) the wing are attached to the adult body, (B) Atrophy in the adult region of the concentration of 2 mg/ml for *Cx. molestus* treatment with Ethyl acetate extract for banana peels *Musa acuminate* The power zoom 4x.



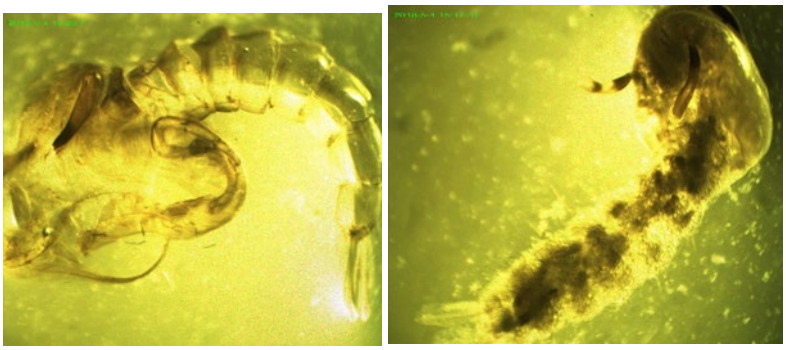
(A) (B)

Fig. 10. (A) The larvae do not moulting were detected with a concentration of 0.5 mg/ml, (B) partial moulting of the pupa at a concentration of 1 mg / mL for *Cx. molestus* treated with ethanol extract for banana peel *Musa acuminate*. the power zoom 4x



(A) (B)

Fig. 11. (A) Albino larvae, (B) Albino larvae showing the combination of the head and thorax region of the *Cx. molestus* treated with a methanol extract of banana peels *Musa acuminate* with a concentration of 1 mg/ml. 4x magnification



(A) (B)

Fig. 12. (A) Albino pupa, (B) Albino pupa with partial moulting of *Cx. molestus* treated with ethanol extract for banana peels *Musa acuminate* at 2 mg/ml concentration. with 4 x magnification

Fig. 13. Showing an adult of Cx. molestus treated with distilled water only

Investigation of Tyrosinase in Bananas Peel Extract

The current study, which investigated about the enzymatic efficacy of banana peels, showed an enzyme efficacy of 0.5 units/ml also, the results showed that the concentration of the protein was 1.25 mg/ml, so the specific efficacy was 0.4 mg/mg.

The results of (Silva et al. 2013) study the crude extract of banana plant (Musa sp.) The enzyme efficacy, quality and protein estimation were study of the polyphenol oxidase (PPO), it reached the enzyme activity2558AU/ml, the total protein concentration of the enzyme in the crude extract was 18.66mg/ml and the specific efficacy was 137 UN/mg.

A study (Farrokh et al. 2018) confirmed the use of banana peels as a source of tyrosinase in pharmaceutical, cosmetic, food and commercial research instead of mushroom. The specific efficacy was 0.005 µmol/min mg, the total efficacy was 0.148 µmol/min and the protein concentration was 30.5 mg/ml.

REFERENCES

Abbott WS (1925) A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267. https://doi.org/10.1093/jee/18.2.265a

Abdul Rahuman B (2011) Evaluation of larvicidal activity of medicinal plant extracts against three mosquito vectors. Asian Pacific J. Trop. Medi.: 29-34.

Abul-hab J, Kassal S (1989) Lecture notes; Regional training center. Advanced course in mosquito control.

Alahmed AM, AlKuriji MA, Kheir SM, AlHaqawi HM, Sultan EAA (2010) Mosquitoes (Diptera: Culicidae) and their seasonal activity in Jazan region, Saudi Arabia. J. Saudi Soc. Agr. 9(2a).

Al-fetlawy GAA (2014) Effect of crude secondary compound extracts for Tamarix ramosissma on some biological aspects of Culex pipiens L. (Diptera: Culicidae) and study their role in transmission of hepatitis C virus. thesis. college of Science for Women, University of Babylon.102 p.

Al-Jinabi AA, Al-Essa RA (2013) Effect of water and alcohol extract from Nerium oleander L. s Meloidogyne incognita and some in control nematode larvae non-target organisms. J. Univer. Kerb. 11(3).

Al-Kahfagi HS, Al-Essa RA, Al-Rubiei THT (2017) Study of repellent and attractant effect of aqueous and ethanolic of same plant extracts against adults of house flies Musa domistica (Diptera: Muscidae). J. Univer. Kerb. 15(1).

Al-Khafagi HAA (2010) Effect of plant leaves extracts Ricinus communis in some aspects of mosquito life Culex pipiens (Diptera: Culicidae. Thesis. College of Science/University of Qadisiyah. 86 p.

Al-Myah ARAA, Al-Mansour N, Al-Dhahir AHS (2011) Effect of some plants extracts on the mortality of the larval mosquitoes Culex pipiens molestus Forskal (in Arabic). J. Basra Sci. (B) 29(1): 47-61.

Al-Raheemy SK, Al-Essa RA (2017) Effect of aqueous and alcohol extracts of plants for Mentha spicata L. and Eucalyptus microtheca F. Meull. on some biological aspects in Culex molestus Forskal. Acceptable for publication in Al-Bahir Journal at 3/12/2017.

Al-Rahimy SKI (2017) Effect of plant extracts for Mentha spicata L. and Eucalyptus microtheca F. Muell. on some biological aspects of Culex molestus Forskal (1775) (Diptera: Culicidae). thesis. college of Education for Pure Science, University of Karbala.105 p.

AlRawi KM, Khalf Allah AM (2000) Design and Analysis of Experiments Agricultural. Ministry of Higher Education and Scientific Reserarch. Dar AlKutub for Printing and Publishing. University of Mosul. Edi. 2nd. 488 p.

Arakane Y, Noh MY, Asano T, Kramer KJ (2016) Tyrosine metabolism for insect cuticle pigmentation and sclerotization. From book (Extracellular composite matrices in arthropods (pp.165-220). https://doi.org/10.1007/978-3-319-40740-1_6

Arizo MAM (2018) Ripe banana peel wastes for a mosquito-free environment. Int. J. Waste Resour. 8: 90.

Evans DA, Kaleysa RR (1992) Effects of quassin on the metabolism of catechol amines in different life cycle stages of Culex quinquefasciatus. Indain. J. Biochem. Biophys., 29(4): 360-363.

Farrokh A, Sariri R, Nasoohi N (2018) Extraction and characterization of tyrosinase from Banana Wastes. App. Sci. Report. 23(1): 31-38.

Fodeal NK (2014) Bioeffect of two strains of Bacillus thuringensis and growth regulator Applaud and pesticide Abte to control of mosquito insect Culex molestus Forskal (Diptera: Culicidae) in the province of Karbala. Thesis. Science in Agriculture - plant protection. 75 p.

Frary A, Gol D, Keles D, Okmen B, Pinar H, Osigva H, Yemenicioglu A, Doganlar S (2010) Salt tolerance in Solanum pennellii: antioxidant response and related QTL. BMC PlantBiology, 10: 58. https://doi.org/10.1186/1471-2229-10-58

Halawa ZA, Mohamed RA, El-Kashlan II (1998) Labaratory evaluation plant and insecticides against beetle Callobruchus maculates infesting stored product. Eygpt. J. Agr. Res. 79(1): 85-93.

Harborne JB (1984) Phytochemical methods. Chapman and Hall. New York. 2nd ed. 288 p. https://doi.org/10.1007/978-94-009-5570-7

Hayes J (1973) A study of Culex pipiens Linnaeus. In the Ohio Mississippi river basin in relation to St. Louis encephalitis (SLE). Mosq. News. 33(2): 447-454.

Ilango S, Athirstalaxmi D, Manonmani P, Malarvizhi M (2016) Repellency of aromatic plants based ingredients against mosquitoes. Int. J. Appl. Biol. Pharm. Tech. 7(2): 55-61,

Kader TA, Abid TM, Thaker AA (2010) Accumulative and non-accumulative effects of Nerium oleander extracts on the larvae of Culex quinquefasciatus. Anbar J. Agri. Sci. 8(4): 321-333.

Kassim NFA, Webb CE, Russell RC (2012) The importance of males: larval diet and adult suger feeding influences reproduction in Culex molestus. J. Amir. Mosq. Contr. Associ. 28(4): 312-316. https://doi.org/10.2987/12-6274R.1

Kim YM, Yun J, Lee C, Lee H, Min KR, Kim Y (2002) Oxyresveratrol and hydroxystilbene compounds inhibitory effect on tyrosinase and mechanism of action. J. Biol. Chem. 277: 16340-16344. https://doi.org/10.1074/jbc.M200678200

Kubo I, Kinst-Hori I (1998) Tyrosinase inhibitors from cumin. J. Agric. Food Chem. 46: 5338-5341. https://doi.org/10.1021/jf9708958

Kubo I, Kinst-Hori I, Nihei K, Soria F, Takasaki M, Calderon JS, Cespedes CL (2003) Tyrosinase inhibitors from galls of Rhus javanica leaves and their effects on insects. Z. Naturforsch. 58c: 719-725. https://doi.org/10.1515/znc-2003-9-1022

Kuusik A, Tartes U, Vanatoa A, Metspalu L, Hiiesaar K (2001) Body stereotypic movements and their possible role as triggers of heart activity in pupae of Colorado potato beetle Leptinotarsa decemlineata (Say). Physiological Entomology, 26(2): 152-158. https://doi.org/10.1046/j.1365-3032.2001.00229.x

Ladd JL, Jacobson M, Buriff CR (1978) Japaan beetle extracts from neem tree seeds as feeding deterrent. J. Econ. Entomol. 71: 810-813. https://doi.org/10.1093/jee/71.5.810

Li J, Zhao X, Christensen BM (1994) Dopachrome conversion activity in Aedes aegypti: significance during melanotic encapsulation of parasites and cuticular tanning. Insect Biochem. Mol. Biol. 24: 1043-1049. https://doi.org/10.1016/0965-1748(94)90142-2

Li JS, Vavricka CJ, Christensen BM, Li J (2007) Proteomic analysis of N-glycosylationin mosquito dopachrome conversion enzyme. Proteomics 7: 2557-2569. https://doi.org/10.1002/pmic.200601053

Lowry OH, Rosebrough NS, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol. J. Biol. Chem. 193: 256-257.

Mahdi NKh (2010) The effects of aquous extracts and organic solvents of Peganum harmala and Ricinus communison the different instars of mosquito Culex pipiens molestus (in Arabic). J. Tik. Univer. Agri. Sci. 10(2): 119-128.

Metspalu L, Hiiesaar K, Joudu J, Kuusik A (2001) The effects of certain toxic plant extracts on the larvae of Colorado potato beetle Leptinotrasa decemlineala (Say). Institute of plant protection, Estonian Agriculture University: 93-100.

Mulla MS, Darwazeh HA, Norland RL (1974) Insect growth regulation: evaluation procedures and activity against mosquitoes. J. Ecom. Entomol. 67(3): 329-332. https://doi.org/10.1093/jee/67.3.329

Narayanan PCS, Devi RK, Pushpalatha M, Raveen R, Lingathurai S (2014) Bio-Efficacy of Terminalia chebula Retz. (Combretaceae) against Culex quinquefasciatus Say (Diptera: Culicidae). Biosci. Plant Biol. 1(3): 41-49.

Pederson MW, Barner DK, Sorensen EI, Griffin GP, Nickon M, Howath E (1976) Effect of low and high saponin selection in alfa on organomic and pest Resistance traits and the interrelationship of these traits. Crop. Sci., 15: 254-256.

SAS (2012) Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Institute Incorporated Cary. N.C. USA.

Silva SCI, Wisniewski C, Luccas PO (2013) Enzyme from Banana (Musa sp.) extraction procedures for sensitive adrenaline biosensor construction. Amir. J. Analytic. Chem. 4: 293-300.

Sterkel M, Oliveira PL (2017) Developmental roles of tyrosine metabolism enzymes in the blood-sucking insect Rhodnius prolixus. Proc. R. Soc. B 284: 20162607. https://doi.org/10.1098/rspb.2016.2607

Sugumaran M, Barek H (2016) Critical analysis of the melanogenic pathway in insects and higher animals. Int. J. Mol. Sci. 17: 1753. https://doi.org/10.3390/ijms17101753

Sukumar K, Perich MJ, Boobar LR (1991) Botanical derivatives in mosquito control: a review. J. Amir. Mosquito Control Ass. 7(2): 210-211.

Vavricka CJ, Christensen BM, Li J (2010) Melanization in living organisms: a perspective of species evolution. Protein Cell, 1(9): 830-841. https://doi.org/10.1007/s13238-010-0109-8

Velichkova P, Marinkova D, Yaneva S, Yotova L (2015) Isolation and purification of tyrosinase from different plant sources. First Nat. Conference Biotech. Sofia, 100(4): 70-75.