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Biological and Morphological Changes in *Culex Pipiens* after Rose Water Exposure

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ABSTRACT Rose water is a well-known culinary supply; available in many homes but its insecticidal effect has not been reviled. This study tests the potentiality of rose water insecticidal effect on *Culex pipiens* adults and larvae. Chromatographic characterization of tested rose oil was mainly nerol (20.88%), Citronellol (18.05%) and Geraniol (10.42%). The performed insecticidal bioassay on *Culex pipiens* adults and larvae showed a dose-dependent relationship with LC_{50} of 0.732 percent after 24h and 0.119 percent after 48h consequently. Prolongation of both larval and pupal duration in addition to the decrease in both pupation and adult emergence percentages were reported. Spots of pigmentation of the malformed stages of development and pharate pupae were recorded and photographed. Rose water showed a potential insecticidal property that deserves to be highlighted.

INTRODUCTION

Culex is long known for its ability to transmit many vector-borne diseases. The profuse usage of synthetic insecticides facilitated the development of resistance (Vézilier et al. 2013). Although persistent trials of control, still numbers of mosquitoes are increasing. This may be attributed to meteorological, hydrological changes and global warming, aside from expansion in condominiums and gardening allowing new breeding areas for mosquitoes (Patz et al. 2003; Shaman et al. 2011).

Rosa damascena (family *Rosaceae*) is one of the most ancient flowers known to mankind as indicated by fossils dating back 3.5 billion years (Baser et al. 2012). It has long been praised as beauty and passion symbol. The fragrance, the fresh taste and the smooth clear appearance of its water tempted many to apply it in old and new remedies including culinary, perfumery, cosmetics, medical (Yang et al. 2014), aside from the floral offerings as a part of many religious ceremonies (Elango and Govindasamy 2018). It grows around the world especially in the Middle East (Yilmaz 2015). Hydrodistillation is the method used for ages to produce rose water. With the uprising economic value of rose cultivation, its contribution to the economy is devastating (Hummer and Janick 2009). With the successful development of iron nanoparticles from Rosa leaves extract; a new prospect of green chemistry is established (Ebrahiminezhad et al. 2017). Studying the insecticidal effect of rose products is not fulfilled in text and is limited to repellency effect of rose oil (Salman and Erbas 2014). The lack substitutes, the low yield of rose oil necessitate the use of a huge number of petals and the narrow harvesting season makes it one of the most expensive essential oils in the global markets (Baydar and Baydar 2005; Yilmaz 2015). Also, rose water contains hydro-soluble components that still holds a generous amount of rose oil but with easier and cheaper cost of preparation (Nunes and Miguel 2017) so this work aimed at evaluating the insecticidal activities of rose water on *Culex pipiens* adults and larvae by monitoring biological and morphological effects.

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METHODOLOGY

All the research study was done after the agreement of the ethical committee Faculty of Medicine, Ain Shams University.

Rose Water Preparation and Essential Oil Isolation

Rose petals (*Rosa damascena*) were purchased from local market, washed with distilled

water to remove dust particles and taxonomically identified by Department of Botany, Faculty of Science, Ain-Shams University staff members. To remove moisture from the flowers, they were shade-dried at room temperature. Sixty grams of shade-dried rose petals were hydro-distilled with one liter of water for 4 h to prepare 800 ml of rose water by clevenger apparatus at the Experiments and Advanced Research Unit, Faculty of Pharmacy-Ain Shams University using hexane as extracting agent.

The essential oil layer was separated and dried over anhydrous sodium sulphate to remove residual moisture. The solvent was evaporated under reduced pressure (35°C) to obtain a concentrated volatile fraction (Verma et al. 2011). Moisture removal was done by shade drying the flowers at room temperature. Sixty grams of shade-dried rose petals were hydro-distilled with one liter of water for 4 h to prepare 800 ml of rose water by clevenger apparatus using hexane as extracting agent. The essential oil layer was separated and dried over anhydrous sodium sulphate to remove residual moisture. The solvent was evaporated under reduced pressure (35°C) to obtain a concentrated volatile fraction (Verma et al. 2011).

Gas Chromatography/Mass Spectrometry (GS-MS) Analysis of Essential Oils

The GC-MS analysis was done using a Shimadzu 2010 Plus GC-MS (Germany) equipped with a Quadrupole (QP-5050) detector. The following conditions were applied throughout the analysis; a capillary column, CP-Wax 52 CB (50 m x 0.32 mm, film thickness 0.25 ìm), with injector temperature at 240 °C and detector temperature at 250 °C, oven temperature program ranged from 60 °C (10 min. hold) reaching 220 °C rising at 2 °C/min. and increasing to 220 °C (11.5 min. hold) rising at 20 °C/min with a flow speed of 10 pounds per square inch (psi) and ionization type detector of 70 electron volt (eV) using helium as carrier gas (20ml/min.), injecting 1 ìl of sample. To identify the constituents; comparison of the retention times of standard substances was done with corresponding data from the WILEY, NIST, and TUTOR libraries.

Mosquitoes Rearing

Laboratory- reared larvae provided by Research Institute of Medical Entomology, Mosquitoes Research Department, Dokki, Giza. These larvae were reared in plastic cups measuring 30cm x 15cm, containing dechlorinated water. With temperature of 25±2 °C and 70 ± 5 percent R.H in 12hour light: dark cycle. Larvae were fed dried bread and yeast mixture. Adults were reared in 0.5×0.5×0.5m cages and fed ten percent sugar solution. Guinea pigs were provided for females feeding three times per week for a blood meal (Alouani et al. 2009).

Biological Assays

Adults Bioassay

The bioassay was performed by fumigation method according to Palacios et al. (2009) and Rossi and Palacios (2013) using a screw-capped glass jar with a suspended 7cm length cotton yarn. Serial dilutions of the rose water (0.05, 0.1, 0.5, 1, 2, and 3%) were prepared using absolute ethanol (El Gomhoureya, Egypt) according to Emtithal and Thanaa (2012) and incorporated in the yarn using cotton yarn soaked in water as a control. Mortality was assessed by softly stimulating each insect with a pen tip. The positive control was carried out by using deltamethrin with different doses (0.0125, 0.025, and 0.05%). Mortality was recorded after 1h, 6h, and 24h of treatment.

Larvae Bioassay

The larvicidal bioassay was done on third instar larvae held using 1ml of 0.005, 0.01, 0.5, 1, and 2 percent rose water in 249ml water in exposure plastic chambers. Mortality was recorded up to 48h after exposure (Kumar et al. 2011) by the loss of movement. Control group using 1ml absolute ethanol was applied. Living larvae were exposed for further daily examination to estimate the effect on the larval duration after treatment, the percentage of pupation and the successfully emerged adults according to Jimenez Peydro et al. (1995) and Sripongpun (2008).

In addition, morphological abnormalities were recorded and photographed at all developmental stages. Each test was replicated three times with concurrent controls. The positive control was carried out by using temephos with different doses (0.00001- 0.00005- 0.0001- 0.0005- 0.0025- 0.0125%). Mortality was recorded up to 48h after treatment. Percent mortality was calculated and LC_{50}, LC_{90} with their ninety-five per-

cent confidence limits were determined using probit analysis.

Statistical Analysis

IBM SPSS statistics (V. 23.0, IBM Corp., USA, 2015) was used for data analysis. Log dose response probit analysis was used to calculate LC_{50} and LC_{90}. Chi-square, Z score and paired *t*-tests were used with a p value of d"0.05 as significant, <0.001 as highly significant, and >0.05 as non-significant.

RESULTS

Evaluation of insecticidal properties was done using different concentrations of rose water on *Culex pipiens* adults and larvae. Results of chemical compositions of essential oils by GC-MS are shown in Table 1 with nerol, citronellol, geraniol and linalool being the major constituents. Results of *Culex pipiens* adulticidal activity of rose water and their positive controls by fumigation technique after 1h, 6h, 24h are shown in Tables 2 and 3 respectively. Results of *Culex pipiens* larvicidal activity of tested botanical materials and their positive controls after 24h and 48h are shown in Tables 4 and 5 respectively. Increase in the mortality rate with increasing the tested extract concentrations was detected in both adults and larval bioassays. A non-significant (p>0.05) difference between the observed values of mortality and expected ones indicating the efficacy of the tested models. Both slope and intercept of the tested extract showed a highly significant linear prediction of the model tested (p<0.05). Rose water had LC_{50} of 0.732 percent after 24h. For the positive control, deltamethrin showed LC_{50} of 0.013 percent after 24h (Table 3). For *Culex pipiens* larvae, the biological assay was performed and showed after 24h and 48h that rose water LC_{50} was 3.717 after 24h and 0.119 percent after 48h (Table 4). For the positive control, temephos showed LC_{50} of 2.18×10-5 ppm after 48 h (Table 5).

Effect of rose water on some biological aspects after treatment of 3rd larval instars of *Culex pipiens* are shown in Table 6 showing prolongation of development time as the concentration increased. Figure 1 shows; spots of pigmentation observed after treatment of 3rd larval instars of *Culex pipiens* with rose water in larvae and the emerging pupae with the development of pharate pupae. Also, spots of erosion and thinning of the wings observed after treatment of adults of *Culex pipiens*. Ultrastructural superficial changes in emerging *Culex pipiens* pupae showed cracks in abdominal segments and head induced by rose water seen after 72h as shown in Figure 2.

Table 1: Essential oil components and rates (%) of rose oil by GC-MS

Peak	*Retention time*	*Name*	*Percentage (%)*
1	23.55	Eicosane	0.71
2	24.24	Linalool	2.45
3	24.81	Heptadecane	3.28
4	26.02	Myrceen	0.46
5	27.07	Limonene	0.43
6	27.75	B-Ionone	3.91
7	28.17	Benzyl Acetate	0.52
8	28.61	Alpha pinene	0.36
9	29.09	Citronellyl Propionate	4.54
10	30.28	Tricosane	0.56
11	30.87	Citranellyl acetate	0.55
12	32.58	Ethyl acetate	0.31
13	32.99	Dodecanoic acid	0.81
14	35.71	Methyleugenol	0.71
15	36.02	Docosan	0.41
16	36.36	1,22-Docosanediol	0.33
17	38.31	Tetradecanol	0.62
18	40.82	Heptadecen	1.02
19	40.99	Cis-Nerolidol	1.98
20	41.58	Farnesol	1.76
21	42.06	Geranyl Acetate	1
22	43.36	Hexadecanoic acid	0.81
23	43.44	Rhodinol	0.94
24	44	Geranyl Iso Butyrate	1.31
25	45.69	Geranyl acetate	0.61
26	46.07	Trans-Caryophyllene	0.52
27	46.46	nerol	20.88
28	48.54	E-Citral	1.4
29	49.08	Alpha humulene	0.44
30	50.49	Viridiflorol	0.52
31	50.81	Citronellol	18.05
32	52.72	Rhodinyl acetate	1.42
33	53.32	n-Pentacosane	0.45
34	54.55	2,2-dideutero octadecanal	1.52
35	54.78	Geraniol	10.42
36	56.58	Phenyl Ethyl Alcohol	6.85
37	58.31	Ecosane	0.76
38	58.47	Methyl Eugenol	5.45
39	61.89	Hexacosane	0.47
40	67.27	Docacosane	0.48

DISCUSSION

In the present study, according to the GC-MS analysis of the hydrodistilled rose oil, a total of 40 volatile compounds were identified. A high percentage of identified compounds were nerol (20.88%), citronellol (18.05%), geraniol

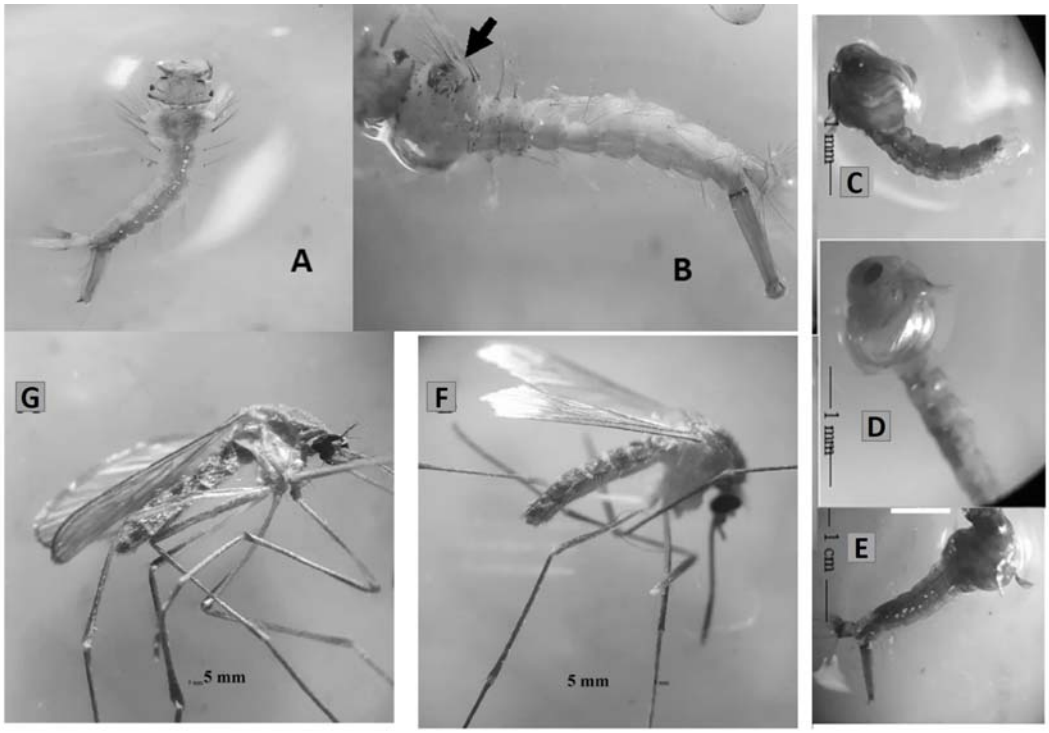

Fig. 1. Morphological changes in *Culex pipiens* larvae whole body and head, induced by rose water after 48h (A and B). (A) shows *Culex pipiens* control larva with normal appearance of whole body and head (B) shows spots of pigmentation in the thorax of the rose water exposed larva. Pupal morphological abnormalities (C, D and E) observed after treatment of 3rd larval instars of *Culex pipiens* (C) shows control *Culex pipiens* pupa with normal appearance of whole body and head (D) shows spots of pigmentation in the abdomen of the pupa exposed to rose water. (E) show larval-pupal intermediates (pharate pupae) after exposure to rose water. Adults abnormalities after exposure to rose water for 24 h (G) shows *Culex pipiens* control adult with normal appearance of the whole body and head (F) shows abnormalities in wings as spots of erosion and thinning of the wings of the adult exposed to rose water

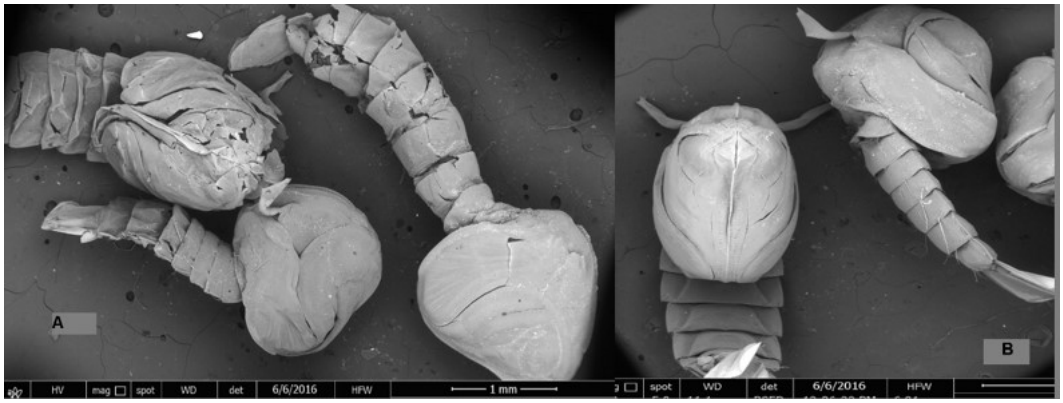

Fig. 2. Ultra structural superficial changes in emerging *Culex pipiens* pupae whole body and head induced by rose water after 72h.(A) Scanning Electron Microscope (SEM) micrograph of *Culex pipiens* pupa represents the control showing the normal appearance of the pupa. (B) SEM micrograph of treated *Culex pipiens* pupa showing cracks in abdominal segments and head

Table 2: *Culex pipiens* adulticidal activity of rose water by fumigation technique after 1h, 6h and 24h

Duration	*Responses*	*Concentrations %*							LC_{50} *and 95% confidence limits*	LC_{90} *and 95% confidence limits*	*Z-score**	
		0.05	*0.1*	*0.5*	*1*	*2*	*3*				*Slope*	*Intercept*
1h	Observed	0	1	2	5	8	10		3.261.86-10.08	61.7312.76-4924.5	4.08	-4.61
	Expected	0.16	0.452	2.835	5.017	7.864	9.709					
	Residual	-0.16	0.548	-0.84	-0.02	0.136	0.291					
6h	Observed	1	2	3	7	12	14		1.560.97- 3.11	16.3096.47-115.9	5.06	-1.80
	Expected	0.61	1.341	5.353	8.09	11.09	12.8					
	Residual	0.39	0.659	-2.35	-1.09	0.914	1.205					
24h	Observed	2	3	4	11	15	18		0.7320.47- 1.17	6.183.16-20.57	6.02	1.36
	Expected	1.07	2.318	8.19	11.49	14.54	16.03					
	Residual	0.93	0.682	-4.19	-0.49	0.459	1.967					

Dose response relationship of the tested materials showed increase in the mortality rate with increasing the tested materials concentrations with no significant ($p>0.05$) difference between the observed values of mortality and expected ones;Chi-square test was used to calculate p-value;
*Both slope and intercept of the tested materials showe

(10.42%) and linalool (2.45%) (Table 1). This goes with results obtained by Babu et al. (2002) with citronellol, geraniol, nerol and nonadecane as major constituents of rose oil with different extraction temperature and pressure. Also, Erbas and Baydar (2016), who found that over 100 components have been identified in rose oil by GC-MS analysis. Geraniol (34.9%); and citronellol (23.4%) were the major constituents of the oil reported by Salman and Erbas (2014). The concentrations of citronellol, geraniol, nerol and linalool constitute approximately sixty percent of the rose oil although their poor contribution to odour (Nunes and Miguel 2017). The yield of essential oil and the content of 1, 8-eucalyptol was within the values reported in the literature (Song et al. 2009) and the contents of the main constituents are similar to those given in the literature (Daroui-Mokaddem et al. 2010; Tadtong et al. 2015). Other studies from Egypt reported the same results as Makhlouf et al. (2015) indicated that 1, 8-eucalyptol was 55.6 percent. Said et al. (2016) indicated that the oil contains 1, 8-eucalyptol (19.8%) which was different from researchers' results. Genotype difference, seasonal variation, soil properties, the timing of rose picking, fermentation of roses, distillation timing and solvent in use were mentioned by many other studies as factors attributed to results variation (Pal et al. 2016; Ucar et al. 2017). This variation in constituents gives every oil its characteristic and authentic signature that can also define the land of rose origin (Krupèík et al. 2015).

The rose essential oil and rose water compositions of fresh flowers of *Rosa damascena* were concerned by Verma et al. (2011) and Baydar et al. (2008 and 2013) who found citronellol, geraniol, nerol to be the major components of the rose essential oil and citronellol and geraniol were the major components of the rose water. Therefore, rose water was selected as an alternative to rose oil in the present study to overcome the cost and high pricing of the oil and getting the benefit of the easy preparation and high yield of rose water extraction. To the best of researchers' knowledge, no reports have been mentioned regarding the insecticidal activity of rose water. In the researchers' study, rose water was found to have both adulticidal and larvicidal effect on *Culex pipiens.*

In the current study, the adulticidal activity of rose water was evaluated at 0.05, 0.01, 0.5, 1, 2 and 3 percent while larvicidal activity was eval-

Table 3: *Culex pipiens* adulticidal activity of deltamethrin by fumigation technique after 1h, 6h and 24h

Duration	*Responses*	*Concentrations %*			LC_{50} *and 95% confidence limits*	LC_{90} *and 95% confidence limits*	*Z-score**	
							Slope	*Intercept*
		0.0125	*0.025*	*0.05*				
1h	Observed	3	6	10	0.05 0.031-1.189	0.278 0.092-25163.7	2.36	1.94
	Expected	2.99	6.03	9.99				
	Residual	0.012	-0.028	0.014				
6h	Observed	6	10	15	0.024 0.013-0.039	0.104 0.054-2.91	2.827	2.829
	Expected	5.817	10.381	14.82				
	Residual	0.183	-0.381	0.18				
24h	Observed	10	16	20	0.013 0.007-0.017	0.029 0.022-0.063	3.384	3.642
	Expected	19.658	16.946	9.46				
	Residual	0.54	-0.947	0.342				

Dose response relationship of the tested materials showed increase in the mortality rate with increasing the tested materials concentrations with no significant ($p>0.05$) difference between the observed values of mortality and expected ones; Chi-square test was used to calculate p value;
*Both slope and intercept of the tested materials showed a highly significant linear prediction of model tested ($p<0.05$)

uated at 0.05, 0.01, 0.5, 1 and 2 percent on third instar larvae of *Culex pipiens.* Dose-response relationship of the tested materials showed an increase in the mortality rate with increasing the tested materials concentrations with no significant difference ($P>0.05$) between the observed values of mortality and the predicted ones. The residual indicates the appropriateness of the used models. All results of the present study showed significant linear predictions ($P<0.05$) from the resultant probit models as shown by z-scores (Tables 2 and 4).

In the current study, pupation rates and adults' emergences decreased as the concentration increased (Table 6). Comparable results were recorded against *Culex pipiens* following treatment with fenugreek oil (Halawa 2001) and onion oil (Khater 2003). The prolongation of developmental periods reported with low concentrations may be explained as rose water may possess an insect growth regulating activity, which may inhibit insect development (Mohsen et al. 2000; Khater 2003; Sivagnaname and Kalyanasundaram 2004). Comparable prolongation of pupal developmental periods was also recorded (Abdel-Kadder 2005; Khater and Shalaby 2008).

A similar reduction in the pupation rates and adults' emergences, following exposure to higher concentrations of rose water, were recorded after treatment of *Culex pipiens* with different plant extracts, such as neem seed kernel extract (Desoky 1995), fenugreek (Halawa 2001), sesame, onion and nigella oils (Khater 2003).

Morphological malformations resulted from treatment of larvae with rose water as larvae and pupae were pigmented and larval-pupal intermediates (pharate pupae) were reported and morphological abnormalities observed in *Culex pipiens* adults after exposure to rose water were spots of erosion and thinning of the wings after exposure to rose water (Fig. 1). With cracks in abdominal segments and head of pupae seen with scanning electron microscopy (Fig. 2). Several authors recorded similar anomalies when applied different plant extracts at low concentrations. Pigmented larvae and larval-pupal intermediates (pharate pupae) were reported after treatment with sesame oil on *Culex pipiens* (Khater and Shalaby 2008). The present results indicated a metamorphosis inhibiting the effect of the rose water which is possibly based on the disturbance of hormonal control (AL-Shorook et al. 2001). Metamorphosis abnormalities suggest a type of insect growth regulating activity that is believed to be a constituent of botanical extracts apparently the major cause of the mortalities. Likewise, such abnormalities were noted following treatment of mosquitos' larvae with juvenile hormone (JH) analogues and chitin synthesis inhibitors (Khater 2003; Sivagnanam and Kalyanasundaram 2004; Tehri and Singh 2015).

Table 4: *Culex pipiens* larvicidal activity of rose water after 24h and 48h

Duration	*Responses*	*Concentrations %*						*LC_{50} and 95% confidence limits*	*LC_{90} and 95% confidence limits*	*Z-score**	
		0.005	*0.01*	*0.1*	*0.5*	*1*	*2*			*Slope*	*Intercept*
24h	Observed	3	6	12	17	24	44	3.717 1.08-104.299	394.441 27.7-2465.8	7.6	-4.5
	Expected	2.772	4.158	12.821	23.26	28.73	34.591				
	Residual	0.228	1.842	-0.821	-6.26	-4.731	9.409				
48h	Observed	8	20	36	50	62	72	0.119 0.083-0.17	4.235 2.367-9.171	11.8	8.7
	Expected	10.19	14.937	37.979	55.72	62.19	67.542				
	Residual	-2.19	5.063	-1.979	-5.723	0.189	4.458				

Dose response relationship of the tested substances showed increase in the mortality rate with increasing the tested substances concentrations with no significant difference ($p>0.05$) between the observed values of mortality and expected ones;
Chi-square test was used to calculate p value;
*Both slope and intercept of the tested materials showed a highly significant linear prediction of model tested ($p<0.05$)

Table 5: *Culex pipiens* larvicidal activity of temephos after 24h and 48h

Duration	*Responses*	*Concentrations %*						*LC_{50} and 95% confidence limits*	*LC_{90} and 95% confidence limits*	*Z-score**	
		0.0001	*0.0005*	*0.001*	*0.005*	*0.025*	*0.125*			*Slope*	*Intercept*
24h	Observed	16	28	40	48	64	76	0.00014 6×10^{-5}-3×10^{-4}	0.008 0.002-0.077	5.401	5.461
	Expected	15.871	28.286	39.014	49.064	64.382	75.473				
	Residual	0.129	-0.286	0.986	-1.064	-0.382	0.527				
48h	Observed	32	48	60	64	76	80	2.2×10^{-5} 4.8×10^{-6}-5×10^{-5}	8.9×10^{-5} 3.4×10^{-5}-6.1×10^{-3}	4.62	5.27
	Expected	31.883	48.253	59.005	65.197	75.978	79.714				
	Residual	0.117	-0.253	0.995	-1.197	0.022	0.286				

Dose response relationship of the tested materials showed increase in the mortality rate with increasing the tested materials concentrations with no significant ($p>0.05$) difference between the observed values of mortality and expected ones; Chi-square test was used to calculate p value;
*Both slope and intercept of the tested materials showed a highly significant linear prediction of model tested ($p<0.05$)

Table 6: Effect of rose water on some biological aspects after treatment of 3rd larval instars of *Culex pipiens*

Rose water concentration percent	*Larval duration (Days)*	*Pupal duration (Days)*	*Pupation percent*	*Emergence of adult percent*
0.005	10.75*	4.72	75.47*	74.5*
0.01	8.88*	4.2	71.11*	60.55*
0.1	6.46*	3.22	69.64*	55.32*
0.5	4.68	2.89	60*	46.8*
1	3.86	2.54	49.65*	44.7*
2	3.22	1.5*	45.34*	37.08*
Control	4.5	3.45	95.5	93.4

Results were presented as the mean; P>0.05 non-significant; *P<0.05 significant;
Results were analysed by student's t-test; Statistical comparisons were done between control and exposure data

CONCLUSION

Rose water is long been used in cosmetic, culinary and medical remedies although this domestic familiarity; studies on its potentiality as insecticide are unexplainable scares. So, this study spotlights on a fine scented, edible, safe, domestic and easily prepared rose water as a potential mosquito insecticide.

RECOMMENDATIONS

Further studies, especially regarding the mechanism of rose water effect on mosquitos' growth regulation, is advised. Development of rose water derived products in combating mosquitoes is recommended.

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