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Insecticidal Activity of Some Chemicals of Mosquitoes *Culex pipiens molestus* Forskal

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Abstract:

The chemicals materials were used to study the effect of citric acid, detergents, sodium carbonate, sodium bicarbonate on the vitality of the third stage of mosquito *Culex pipiens molestus*. The highest larvicidal efficacy was established from citric acid solution followed by detergents solution, sodium carbonate, sodium bicarbonate with (LC_{50}) values 2096, 2715, 13930, 13960 ppm respectively. The concentration of 1000 ppm of citric acid showed a fatal effect on the third stage larvae of mosquitoes which gave a mortality percent of 100% on the second day of the treatment. It was followed by the concentrations of 750 ppm and 500 ppm respectively which caused the death of all larvae on the fifth and sixth days respectively. On the other hand, the concentration of 1500 ppm for the detergents killed 100% of the larvae on the third day of treatment. Then, it was followed by the concentration of 1000 ppm, leading to the destruction of all the larvae on the fifth day of the exposure. As for the use of sodium carbonate, the mortality percentage reached to 100% at a concentration of 8000 ppm on the third day of treatment followed by the concentration of 6000 ppm, which led to a 100% killing rate on the sixth day of treatment. While the less impact of studied chemicals was sodium bicarbonate, the 8000 ppm concentration of which caused 84.21% mortality rate of larvae on the fourth day of treatment.

Key words: Citric acid, Detergents, Larvae, Mosquito, Sodium carbonate, Sodium bicarbonate.

Introduction:

Female mosquitoes attack animals to obtain the blood which is necessary to form eggs. Some diseases transmitted by mosquitoes include yellow fever, malaria, dengue, Lymphatic filariasis and zika according to the WHO report (2) . Malaria, the leading cause of mortality in Nigeria causes (1-2) million death (3) . The medical value of mosquito as vectors of serious diseases that cause mortality, morbidity, economic loss and social confusion (4). *Cx. pipiens molestus* appears to have a limited impact on the transmission of viruses in Europe. The highest form of these is West Nile Virus (WNV), which has been present in this area for at least twenty years (5). The mosquitoes are originated in Africa, but are now found in sub-tropical and tropical regions throughout the world (6).

The successful use of active components depends on its correct formulation into an equipment which can be applied for output protection with safety to those applying materials to the environment and to human, and animal life (7-9). Researchers have recently turned to the use of alternatives to pesticides that are effective against insects that remain for a short period and are safe and cheap, such as inorganic salts and antioxidants. The precise preparation of these materials leads to the eradication of insects and achieves the goal in small quantities and in a safe materials leading to the eradication of insects in a safe manner that does not harm human health (10 , 11).

Mosquito control is carried out by habitat control, use of insecticides, larvicides. In this situation, safe larvicides were needed for human. Some chemicals and natural substances combat larvae of mosquitoes, one of which is detergents. Detergents are one of cleansing agents used in households. They are available having the potential to function like larvicide for mosquitoes. Some studies showed that detergents could inhibit oviposition and kill the larvae of mosquitoes in different concentrations (12). Detergents and soaps are used for pest control,

many of which are liquids that perform better as insecticides and miticides (13). The present study was carried out in order to find the lethal concentrations of some chemicals solutions for killing of mosquitoes.

The aim of this study is to investigate some available, cheap and safe chemicals such as (citric acid, detergents, sodium carbonate, sodium bicarbonate) that are harmless towards human, animals and environment in addition to being effective in mosquitoes control.

Materials and Methods:

Preparation of mosquitoes culture

Mosquitoes eggs rafts were collected from the culture of *Cx. pipiens molestus* F. and transferred to the laboratory of entomology in University of Mosul College of Science in September of 2018. The collected egg rafts were put in a plastic container (enamel trays) containing 2 liters of dechlorinated tap water with (two egg rafts) per tray. The larvae were fed on rabbits food by mixing yellow corn %25, wheat %25, protein %40 and dry milk %10 (2.3 g per trays) and placed in screened cages (60× 30× 45)cm where the adult emerged under laboratory conditions of 70±10 relative humidity and 27±1℃ controlled temperature with 14:10 (light:dark). The adults were fed with 10% sugared solution and after 3-4 days periodically a pigeon placed in resting cages overnight for blood feeding (14).

Preparation of the solution

Citric acid solution

The stock solution of citric acid solution was prepared by dissolving (1 g) of citric acid powder in (5 ml) of distilled water and then completed to (10 ml) of distilled water to obtain a stock solution with concentration of 10%. From this stock solution different concentrations of 250, 500, 750 and 1000 ppm were prepared.

Detergent solution

The detergent solution was prepared by dissolving (1 g) of the detergent powder [(Super Topper, Turkey) Ingredients: Anionic Surfactans 16% STPP-Perfume-S. Carbonate-F.W.A., S.Silicate-S.Sulphate)] in (5 ml) of distilled water and then completed to (10 ml) of distilled water to obtain a stock solution with concentration of 10%. From this stock solution different concentrations of 250, 500, 1000 and 1500 ppm were prepared.

Sodium carbonate solution and Sodium bicarbonate solution

The sodium carbonate solution and sodium bicarbonate solution were prepared in the same way as the citric acid solution was prepared, in order to obtain the sodium carbonate solution and sodium bicarbonate solution at a concentration of 10%. From this stock solution, different concentrations of 2000, 4000, 6000 and 8000 ppm were prepared.

Stock solution of 100000 ppm was prepared by dissolving 1 g of each substance used in this study in 10 ml of distilled water.

20 healthy 3rd instar larvae of *Cx pipiens molestus* were put in a disposable plastic cup containing 50 ml of treatment solution of four concentrations of citric acid 250, 500, 750 and 1000 ppm, four concentrations of detergent solution 250, 500, 1000, and 1500 ppm and four concentrations for each of sodium carbonate and sodium bicarbonate 2000, 4000, 6000 and 8000 ppm with 0.3 grams of rabbits food, using three replicates for each concentration with a control group represented by the presence of water and food only. The effect of the treatment solution was monitored by counting the dead larvae at the end of 24, 48, hours and continuing for 7 days.

Calculation and data analysis:

The percentage of mortality was calculated. The percentage of larval mortality was corrected according to the Abbott's equation (15), utilizing LDP-line to estimate the LC_{50} slope values in addition to the test of less significant difference LSD so as to make sure of the significance of the differences between the results.

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae in control}} \times 100$$

$$\text{Correct percent of mortality} = \frac{T-C}{100-C} \times 100$$

Where, T = The percentage of mortality in the treatment

C = The percentage of mortality in the control

Results and Discussion:

In this study, after one day of treatment the 1000 ppm concentration was the most effective on larval mortality were 85% followed by the concentration of 750 ppm which killed 70% of mosquito larvae, while the concentration 250 ppm caused a lower percentage of mortality 15%. In the second day of experiment the concentration 1000 ppm gave mortality percent 100%, while the concentration 750 ppm gave mortality of a 100% in the fifth day of the experiment and the concentration 500 ppm gave mortality percent 100% in sixth day. However, the concentration 250 ppm caused a lower percentage of mortality 52.63% in the sixth day of the experiment compared to control group with a death rate of 5% (Table 1). The LC_{50} value of citric acid was obtained as 2096.

Table 1. The effect of citric acid solution on 3rd instar larvae of *Culex pipiens molestus* F.

Days	Concentrations ppm				
	250	500	750	1000	Control
	Mortality percentage %				
1	15	50	70	85	0
2	25	65	80	100	0
3	30	80	85	100	0
4	40	90	90	100	0
5	47.36	94.73	100	100	5
6	52.63	100	100	100	5
7	52.63	100	100	100	5

The concentrations 1500, 1000 ppm respectively are likely a significant reason for a higher percentage of killed larvae in the first day of experiment, which was 80, 70% respectively. As for the concentrations 500 and 250 ppm, they gave a mortality percent of 15, 0% respectively. In the third day of the experiment outweigh the concentration 1500 ppm was found to kill all the larvae, as the percentage of killing was 100%. The 1000 ppm concentration killed 100% of the larvae in the fifth day of the experiment, but the concentrations 500, 250 ppm caused percentage of mortality
40, 25% respectively in the fifth day of the experiment which remained constant until the seventh day. It is noteworthy that there was no mortality in the control group during the experiment for all concentrations (Table 2**).** The LC_{50} value of detergents was obtained as 2715.

Table 2. The effect of detergent solution on 3rd instar larvae of *Culex pipiens molestus* F.

Days	Concentrations ppm				
	250	500	1000	1500	Control
	Mortality percentage %				
1	0	15	70	80	0
2	5	25	75	90	0
3	15	30	80	100	0
4	20	35	95	100	0
5	25	40	100	100	0
6	25	40	100	100	0
7	25	40	100	100	0

After one day of treatment the 8000 ppm concentration was the most effective on larval mortality which was 50%. It was followed by the concentration of 6000 ppm that caused the killing of 30% of mosquito larvae. Interestingly, the concentrations 4000, 2000 ppm caused a lower percentage of mortality 10, 0% respectively. In the third day of the experiment outweigh the concentration 8000 ppm is killing all the larvae, where the percentage of killing was 100%, while the concentration 6000 ppm gave mortality of a 100% in the sixth day of the experiment. The 4000 ppm concentration killed 84.21% of the larvae in the sixth day of the experiment, compared to control group with a death rate of 5%. Nonetheless, the concentration 2000 ppm caused a lower percentage of mortality 5.26% only in the third day of the experiment which remained constant until the seventh day (Table 3**).** The LC_{50} value of citric acid was obtained as 2096.

Table 3. The effect of sodium carbonate solution on 3rd instar larvae of *Culex pipiens molestus* F.

Days	Concentrations ppm				
	2000	4000	6000	8000	Control
	Mortality percentage %				
1	0	10	30	50	0
2	5	25	60	90	0
3	5.26	47.36	68.42	100	5
4	5.26	57.89	78.94	100	5
5	5.26	73.68	89.47	100	5
6	5.26	84.21	100	100	5
7	5.26	84.21	100	100	5

After one day of treatment the 8000 ppm concentration was the most effective on larval mortality which was 40%, followed by the concentration of 6000 ppm which caused the killing of 10% only. In the third day of experiment the 8000 ppm concentration killed 75% of the larvae and the concentration 6000 ppm gave mortality 40%, while the concentrations 4000, 2000 ppm caused a lower percentage of mortality 10, 5% respectively. In the fourth day of the experiment outweigh the concentration 8000 ppm where the percentage of killing was 84.21%, while the concentration 6000 ppm gave mortality of a 42.1% which remained constant until the seventh day compared to control group with a death rate of 5% (Table 4**).** The LC_{50} value of sodium bicarbonate solution was obtained as 13960.

Table 4. The effect of sodium bicarbonate solution on 3rd instar larvae of *Culex pipiens molestus* F.

Days	Concentrations ppm				
	2000	4000	6000	8000	Control
	Mortality percentage %				
1	0	0	10	40	0
2	0	5	25	50	0
3	5	10	40	75	0
4	0	5.26	42.1	84.21	5
5	0	5.26	42.1	84.21	5
6	0	5.26	42.1	84.21	5
7	0	5.26	42.1	84.21	5

Table 5. Less significant difference test (LSD)

Chemicals	Days							Mean of mortality
Citric acid	1	2	3	4	5	6	7	2.057
Detergent	8.800	2.000	1.000	1.000	1.200	0.400	0.000	1.514
Sodium carbonate	6.600	1.200	1.200	1.000	0.600	0.000	0.000	1.714
Sodium Bicarbonate	3.600	3.600	2.200	0.800	1.000	0.800	0.000	0.857
Mean of days	2.000	1.200	2.000	0.800	0.000	0.000	0.000	
LSD					0.2541			0.1921
LSD for days and chemicals					0.5083			

According to the value of the LSD (Table 5) for the studied substances, it was found that the citric acid surpassed the rest of substances due to a significant difference between it and the rest of the substances used in the experiment. Likewise, sodium carbonate and detergents are superior to sodium bicarbonate due to the significant differences between them.

The results of the current study show that percentage of mortality increased proportionally with the increase of exposure period and concentration of solutions. The mechanism of action of detergents is to remove fat. The detergent molecules have a hydrophilic head and a hydrophobic tails, so the molecules are attracted to water from one end repelled by the other end. Hydrophobic attacks the fat molecule and surrounds it, pulling the molecules and their fats out . This is attributed to the fact that fat surrounds the body of the insect where the fat body composed of many big cells, called trophocytes. In the larval phases, the fat body occupies most of the body cavity which helps accelerate its destruction (16). The results of the current study are expected to be of significance when it comes to mortality rates after 24 hours up to 7 days of treatment by chemical solution. (16) reported that Lice sp., *Melanoplus mexicanus, Periplaneta americana, Musca domestica* and Ants were placed in a petri dish filled with washing powder solution whose ingredients are: sodium silicate, carboxymethylcellulose sodium, sodium sulphate, fluorescent materials, sodium telions sulphate. In different concentrations, the Lice sp. were killed by detergent solution in (17-22) seconds, *Melanoplus mexicanus* in (30-35) seconds, *Periplaneta americana* in (25-30) seconds and (15-20) seconds for both *Musca domestica* and Ants. This study is consistent with the finding of (17) as dish washing liquids were toxic against the adult of *Periplaneta americana* after 24 hours of exposure. The knockdown effect of the detergents was found to be 70-78% in contact with detergents. However, when dipping 100% of the cockroaches, they are found to be knocked down and ultimately died at 24-houre exposure. (18) reported that the effect of detergent solutions with concentrations 23.3, 11.6 and 7.7 on two species of the hard ticks: *Hyalomma ana. anatolicum* and *Ripicephlus turanicus* takes several hours to cause mortality. It was found that the highest concentration of solution has the greatest activity. The highest concentration of detergent stopped the embryonic development of eggs, and hatching percentages was zero. The period of death was less in females full with eggs due to shield area is elongated and permeable. The results of this study are in agreement with (12) who reported that the detergent with the concentrations of 0.18, 0.20, 0.22, 0.24, g/l caused the killing of *Aedes aegypti* larvae 85.6, 89.6, 95.2 and 100% respectively. (19) found a significant effect of dishes washing detergent on common *Pistachio psylla*, *Agonoscena pistaciae*, soil and plant with five treatments and four replicates. The results showed that the percentage of mortality was higher in 14^{th} and 21^{st} days after spraying. Furthermore, (20) reported that few makeup products like citric acid were applied to the skin of rats, mosquitoes were attracted towards, the results showed that *Anopheles gambae* was less attracted than *Aedes aegypti* towards the makeup products. The current study is similar to those conducted by (8), in which the citric acid was found to be effective on all tested stages of *Aphis craccivora*. This effectiveness was barely noticed after 2 hours, yet it increased after 12 hours. (10), noticed that the insecticidal activity of citric acid against the striped meal bug increased gradually to give the highest effectiveness after 18 days when the reduction percentage is 93.3 at 125 ppm. (21) reported that the mortality responses of *Epiphyas postvittana* larvae to immersion in solution of 0.5-5% sodium bicarbonate at 20-45 ℃ resulted in a maximum of 60% up to 100% mortality. (22), demonstrated that the soup solutions were prepared in 4 concentrations 10%, 20%, 30%, 40% then sprayed into 15 cockroaches. The soup solution has a lethal effect on the cockroaches with LC_{50} at 32% after 24 hours of treatment.

Conclusions:

The results of this study showed the acute toxicity and repellency of chemicals solution,

particularly citric acid and detergents solution, against larvae of *Cx pipiens molestus* F. at varied concentrations. Citric acid and detergents solution showed strong toxicity and repellent activities against *Cx pipiens molestus* larvae. Since these chemical solutions are safe, cheap, abundant and biodegradable, they can be used in the control of *Cx pipiens molestus.* The sodium carbonate and sodium bicarbonate were found to kill larvae of *Cx. pipiens molestus* yet at high concentrations.

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Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in University of Mosul.

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الفعالية السمية لبعض المواد الكيمياوية على يرقات البعوض *Culex pipiens molestus* Forskal

الفت تحسين ياسين **خالدة عبد الله سليمان**

قسم علوم الحياة، كلية العلوم، جامعة الموصل، الموصل، العراق.

الخلاصة:

تضمنت الدراسة الحالية إجراء سلسلة من التجارب للتعرف على تأثير اربع مواد كيمياوية وهي حامض الليمون ، مسحوق الغسيل، كاربونات الصوديوم ، بيكاربونات الصوديوم على حيوية الطور الثالث للبعوض *Culex pipiens molestus* وقد اظهر التركيز 1000 ج ف م لمادة حامض الليمون تأثيرا مميتا ليرقات العمر الثالث للبعوض وبنسبة 100% في اليوم الثاني من المعاملة يليه التركيزان 750 و 500 ج ف م على التوالي واللذان تسببا في هلاك جميع اليرقات في اليوم الخامس والسادس على التوالي بينما اعطى التركيز 1500 ج ف م لمادة مسحوق الغسيل نسبة قتل 100% في اليوم الثالث من المعاملة يليه التركيز 1000 ج ف م والذي تسبب في هلاك جميع اليرقات في اليوم الخامس من المعاملة . اما عند استخدام مادة كاربونات الصوديوم فقد تسبب التركيز 8000 ج ف م في هلاك جميع اليرقات في اليوم الثالث من المعاملة يليه التركيز 6000 ج ف م والذي اعطى نسبة قتل 100% في اليوم السادس من المعاملة وكانت اقل المواد تأثيرا هي مادة بيكاربونات الصوديوم والتي اظهر فيها التركيز 8000 ج ف م اعلى نسبة قتل والتي بلغت 85% في اليوم الرابع من المعاملة .

الكلمات المفتاحية: حامض الليمون، مسحوق الغسيل، كاربونات الصوديوم، بيكاربونات الصوديوم، يرقات البعوض