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## Toxicity of Nanomulsion of Castor Oil on the Fourth larval stage of *Culex quinquefasciatus* under Laboratory Conditions

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### Abstract:

Mosquitoes like *Culex quinquefasciatus* are the primary vector that transmits many causes of diseases such as filariasis, Japanese encephalitis, and West Nile virus, in many countries around the world. The development in the scientific fields, such as nanotechnology, leads to use this technique in control programs of insects including mosquitoes through the use of green synthesis of nanoemulsions based on plant products such as castor oil. Castor oil nanoemulsion was formulated in various ratios comprising of castor oil, ethanol, tween 80, and deionized water by ultrasonication. Thermodynamic assay improved that the formula of (10 ml) of castor oil, ethanol (5ml), tween 80 (14 ml) and deionized water (71ml) was more stable than other formulas. The formulated castor oil nanoemulsion was characterized by transmitting electron microscopy and dynamic light scattering. Nanoemulsion droplets were spherical in shape and found to have a Z-average diameter of 93 nm. A concentration of castor oil nanoemulsion (250, 350, 450, and 550 ppm) was tested as larvicidal agents and bulk emulsion (1000, 1500, 2000, and 2500 ppm) was tested also and compared against the fourth instar larvae. Our nanoemulsion showed higher activity when compared to bulk emulsion. LC<sub>50</sub> for castor oil nanoemulsion and castor bulk emulsion were found to be 268.21 and 409.37ppm after 72 h, respectively. The biochemical assays were carried out to examine the effect of castor oil nanoemulsion on biochemical characteristics of larvae. The treated larval homogenate showed inhibition in the activity of acetylcholinesterase.

**Keywords:** Castor Oil, *Culex quinquefasciatus*, Nanoemulsio, Plant Extract, Insect control.

### Introduction:

Human health faces serious threats like dangerous diseases, especially those transmitted by some arthropods such as the two winged ectoparasites insects that belong to the order Diptera, which are called mosquitoes. Mosquitoes are adaptive to living in many types of habitats, so they spread all over the world<sup>1</sup>, and their spread has increased in the last decade because of migration and population expansion, international treading, international travel, climate change, and unplanned urbanization of tropical regions<sup>2,3</sup>. *Culex quinquefasciatus* Say. is a species that transmits many diseases such as filariasis, Japanese encephalitis, West Nile virus<sup>4</sup>, JEV, Zika virus, and ST. Louis disease<sup>5</sup>.

The threats of such diseases are confronted by either discovering treatments or vaccines against pathogens that are transmitted by mosquitoes, or by hindering the process of transmission by finding the best and most efficient ways of controlling the vector of such pathogens such as *Culex quinquefasciatus* with no impact on the environment<sup>6</sup>. The methods of control were and are still in constant development with all modern means that are safer, more efficient, less costly, and less harmful. The use of chemical insecticides with many synthetic agents in this domain has been appointed and employed with considerable success, but there are non-selective and harmful effects on

human health and non-target organisms<sup>7</sup> even the use of alternative chemicals such as citric acid and bicarbonate which have an effects on mosquitoes larvae but there safty on environment are uncertain<sup>8</sup>. The appearance of the harm of chemical pesticides and the resistance of the pests to these pesticides leads to the use of alternative methods, such as the use of plants with bio-potential products and secondary metabolites, which have a significant role in mosquito management. Seed extracts of *Millettia pinnata*, which contain four flavonoids and two fatty acids, proved a potent toxicity against the third instar larvae of three species of mosquitoes through the ability to inhibit the activity of acetylcholinesterase<sup>9</sup>, *Dalbergia oliveri* and *Heracleum rigens*, whose leaf/seed extracts were investigated as larvicidal, ovicidal, and oviposition deterrent against *Culex quinquefasciatus* to improve as bioactive insecticides that control this species<sup>10</sup>. The aqueous and alcoholic extracts of *Piper nigrum* were tested on the third instar larvae of *Culex pipiens* which achieve high mortality rates<sup>11</sup>, castor plants (*Ricinus communis* L.) which are considered medicinal plants that belonged to the Euphorbiaceae family and grown all over the world because its tolerance to various weather conditions and for its oil that has huge use in industrial, pharmaceutical, cosmetic and engine sector. castor oil consists of many fatty acids like Ricinoleic acid, which comprises more than 75% of the total fatty acids<sup>12</sup>. The reason for this fatty acid and the protein Ricin is the use of castor plants to control many insects, such as *Plutella xylostella* L., *Tribolium confusum*, *Trogonella granarium*, *Aedes aegypti*, *Anopheles culicifacies*, and *Spodoptera littoralis*<sup>13-16</sup>.

The chemical instability of plant essential and fix oils in the presence of light, air, high temperatures, and moisture can lead to quick evaporation and degradation of some bioactive components. Nanotechnology could solve this problem by protecting active components from degradation and losses by evaporation, thereby boosting their effectiveness. Therefore, nanoformulation, such as nanoemulsion, represents a class of emulsions with a diameter of less than one micrometer (50-200 nm), low viscosity droplets, transparent or translucent appearance with intense bluish reflection or milky, depending on the size of the droplets<sup>17</sup>, which have a vast use in mosquito management programs. They are considered as delivery systems, thus the essential and fixed oils are encapsulated in nanoemulsions to achieve high stability and efficiency. Therefore, they are considered as a promising strategy to deliver essential and fixed oils in mosquito control. On this

basis, the use of toxic organic solvents can be excluded. Nanoemulsion is usually produced either by low-energy emulsification or high-energy emulsification methods. In the low energy technique, surfactant and co-surfactant physiochemical properties have a great role in emulsification, while in the high energy technique, mechanical apparatuses are used to reduce droplet size by outputting intensive disruptive forces<sup>18,19</sup>. This study was chosen to prepare eco-friendly alternatives to chemical pesticides in mosquito control based on nanotechnology in the form of nanoemulsions of castor oil and to determine some of its properties and toxicity against mosquitoes, as well as its side effects on one of the non-target organisms.

## Materials and Methods:

### Chemicals

Ethanol (Thomas Baker, India) as a solvent and as co-surfactant, tween 80 (Thomas Baker, India) as surfactant, and deionized water. Acetylcholine chloride (C7H16ClNO2) were used, Fast blue B Salt (C14H12Cl4O2Zn), Disodium phosphate (Na2HPO4,7H2O), Monosodium phosphate (NaH2PO4,H2O).

### Mosquito collection and rearing

Mosquito larvae were collected from Al Hawizeh marsh in the Abu Khasaf region in Missan governorate by using small containers. The larvae were collected from the water and then placed in plastic containers that were covered with tulle and then sent to the Natural History Museum of Iraq for identification. They were diagnosed as *Culex quinquefasciatus*. The experiments were done in the laboratory of the Agricultural Research Center in Al Twaitha.

In the laboratory, the larvae were placed in plastic containers of 500 ml which were filled with 400 ml of distilled water. The containers were then placed in the rearing wooden cages with a dimension of (30×20×20) cm. The cages' sides were covered with a metal mesh, but one of the sides was covered with tulle. The rearing cages were placed in the incubator under controlled conditions (27± 2) °C, 65± 5 RH and 10: 14 D/L photoperiod. The larvae were fed a mixture of biscuits and yeast in a ratio of (3: 1). The water in the rearing cages was exchanged every four days. When the larvae reach pupae, the pupae are collected and placed in plastic containers containing 400 mL of distilled water till the adults emerge, fed on a piece of cotton saturated with 10% sugar solution in a petri dish inside the cages. This feeding is important for mosquitoes to get the energy needed for flight and other life activities. For the purpose of getting eggs, the

females feed on the blood of pigeons by placing the pigeon on the top of the cage overnight. After removing the breast feathers of the pigeon and tying their wings and feet, the females will suck the blood and, after 2-3 days, they will produce egg rafts which they transmit with a plastic tea spoon to clean plastic containers of 500ml that contain 400ml of distilled water.

#### Plant collection and extraction

Dried castor fruits were collected from castor trees in the center of agricultural research (EPA) in Abo Guraib, in February 2020. The seeds were manually removed from the fruit capsules which were split open. The seeds were then cleaned with a clean cloth, and then crushed by mortar and pestle to obtain the seed paste. 250 g of castor seed paste was put in thimbles, and the thimbles were placed in the siphoning tube in the extractor part of the soxhlet. 1 L of the solvent ethanol was put in the round flask of the soxhlet which was exposed to heat with a heater at 65 °C, then the condenser was connected with water supply from the chiller. This process takes 8 hours. After that, the obtained material was put in the vacuum rotary evaporator to evaporate the solvent and concentrate the oil, which was kept in the refrigerator at 4 °C to be used in the preparation of the bulk and nanoemulsion of castor oil. To prepare the stock solution of the bulk emulsion, 10ml of castor oil was taken, mixed with 10ml of tween 80 (as surfactant) and 5 ml of ethanol (as co- surfactant), then 74 ml of deionized water was added to the mixture in a glass bottle and mixed in a screw vial very well to obtain the bulk emulsion with a 10% concentration that equals 100000 ppm.

#### Nanoemulsion preparation using a high-energy method (ultrasonication)

The mixture of the oil, co-surfactant, surfactant, and deionized water were mixed with a magnetic stirrer hot plate for 30 min and then exposed to an ultrasonic device with a high energy of 50kHz, a power output of 400 W (OMNI International, US) for 30 min, and input energy given by a sonotrod containing a piezoelectric crystal with a probe diameter of 13 mm. The disruptive forces generated by the emulsification probe reduced the particle size, converting the bulk emulsion to a nanoemulsion. These nanoemulsions are then characterized by many tests to select the most stable formula. The formulas as in Table 1.

**Table 1. Combinations used to prepare castor oil nanoemulsions**

Composition	F1	F2	F3
Oil phase (Castor oil)	10 ml	10 ml	10 ml
Co – surfactant (ethanol)	5 ml	5 ml	5 ml
Surfactant (tween 80)	11 ml	14 ml	18 ml
aqueous phase (deionized water)	74 ml	71 ml	67 ml

#### Characterization of a castor oil nanoemulsion

The preliminary stability of the castor oil nanoemulsion was evaluated at 24 h by centrifuging at 4500 rpm for 30 min. Samples showing layer separation were eliminated. Without layer separation, samples were stored at 25 °C for four weeks, then at 21 °C for 48 hours, then at 44 °C, then at 4 °C. Viscosity and pH were measured<sup>20</sup>.

#### GC/MASS analysis

The fatty acid methyl esters (FAME) were prepared according to the modified method<sup>21</sup>. 0.05 g of oil was dissolved in 1.8 ml of petroleum ether, to which 0.2 ml of sodium methylate was added and vortexed to separate the two layers, and 1L of the upper layer containing the FAME was injected onto a Shimadzu GCMS QP2010 74707 30 m capillary column (0.25 mm i.e. 0.25 μm film). The GCMS temperature was programmed with an initial oven temperature of 70 °C (hold time 5 min), which was increased at the rate of 10 °C/min to 300 °C (hold time 5min) and the sample injection temperature was 260 °C, with a split ratio of 10. The GC MASS solution version NIST08 standard mass spectrometry library was used to analyze the data.

#### Transmission Electron Microscope (TEM) Test

TEM images were obtained on a Hitachi H-7650B electron microscope operating at an accelerating voltage of 80 kV to obtain detailed information about the morphology and particle size of the nanoemulsion particles. For the preparation of samples, about 10 mL of diluted nanoemulsion sample (100 times) was dropped on the copper-coated carbon grid for 1 minute, and the extra sample was wiped off with a capillary tube. Then about 10 mL of 2% phosphor tungstic acid (PTA) solution (pH 6.4) was added for staining for 1 minute, and the extra phosphor tungstic acid was wiped off with a capillary tube<sup>20</sup>.

#### Analysis of nanoemulsion droplet size by Dynamic Light Scattering (DLS) Test

Droplet size (z-average diameter) and size distribution (PDI) of nanoemulsions were measured by dynamic light scatter (DLS) using the HORIBA Zetasizer Nano-SZ-100V2 (HORIBA Scientific Instruments, Japan) at 298 K. The instrument contains an argon laser (1/4 633 nm) with variable intensity. The emulsion was diluted about 1000 times with Milli-Q water before measurements in order to avoid multiple light scattering effects and each measurement was made with three readings per sample<sup>22</sup>.

#### Larvicidal bioassay

Fourth larval stages were collected from the rearing containers and were treated with different concentrations of nanoemulsion, following WHO Guidelines<sup>23</sup>. Twenty larvae of *Culex*

*quinquefasciatus* were placed in each of the four 125 ml containers containing the different concentrations of nanoemulsion, 250, 350, 450, and 550ppm. The control was distilled water only. Each treatment was performed in three replicates. The bulk emulsion was also investigated for its efficacy against *Culex quinquefasciatus* (1000, 1500, 2000, and 2500 ppm). The larval mortality was observed after 24, 48, and 72 hours. Corrected mortality was measured by the Abbott formula, as follows:

$$\% \text{ corrected mortality} = \frac{\% \text{mortality in treatment} - \% \text{mortality in control}}{100 - \% \text{mortality in control}} \times 100$$

#### Enzyme inhibition assay

Acetylcholinesterase activity was measured according to a method by Huang et al.<sup>24</sup> with slight modification, they treated the fourth larval stage of mosquitoes with castor oil nanoemulsion in concentrations of 250ppm and 550ppm for 48 h. Each treatment was performed in three replicates with 20 larvae in each replicate, and the control was with distal water only. The treated larvae were washed fully with distilled water and then dried with filter paper. Then the larvae were homogenized utilizing Disodium phosphate buffer (PH7.0, 20 mM) by using a pestle. Then the larval homogenized was exposed to centrifugation at 440rpm for 30 min. The resulting supernatant was kept at 4°C on ice. .0.5ml of supernatant mixed with 0.5 of Acetylcholine chloride (2.6 mM) as a substrate and 1ml of sodium phosphate buffer (PH 7.0, 20 mM), the mixture placed in an incubator at

25°C for 10 min , after which 4ml of 0.3% Fast blue B salt was added to stop the reaction . The optical density was measured on the samples and blanks by running them through a spectrophotometer at 405nm. The percentage inhibition of the enzyme activity by Castor oil Nanoemulsion was calculated as follows:

$$\% \text{ Enzyme inhibition} = \frac{\text{OD of control larvae} - \text{OD of treated larvae}}{\text{OD of control larvae}} \times 100 \quad (26)$$

#### Statistical analysis

The experiments were carried out according to factorial experiments using a completely randomized design (CRD), and the differences between the means of the treatments were tested according to the value of the least significant difference at the probability level of 0.05. The results were analyzed by the statistical program Genstat. The lethal and sub-lethal concentrations were determined by a probit analysis software program<sup>25</sup>.

#### Results:

##### Stability Evaluation of Nanoemulsions

Depending on stability tests, the F2 formula of castor oil nanoemulsion was chosen, because it is more stable than other formulas which show phase separation in all stability tests as shown in Table 2. This feature provides long-term stability to the nanoemulsion.

**Table 2. Formulas of castor oil nanoemulsion**

Composition	Formula 1	Formula 2	Formula 3
Castor oil	10 ml	10 ml	10 ml
Co – surfactant ethanol	5 ml	5 ml	5 ml
Surfactant tween 80	11 ml	14 ml	18 ml
Deionized water	74 ml	71 ml	67 ml
Appearance	White	Translucent	White
Appearance after centrifugation	Phase separation	No phase separation	Phase separation
Appearance after stability tests at different temperatures	Phase separation	No phase separation	Phase separation
PH	6.6	6.3	6.5
Viscosity	155mpa.s	165mpa.s	195mpa.s

#### GC -MS result

This test shows that Ricinoleic acid is the main product of castor oil since its peak is the highest in Fig. 1, in addition to the presence of other

acids such as Oleic acid , linoleic acid , linolenic acid , palmitic acid , stearic acid Heptanoic acid, and many others as shown in Table 3 .

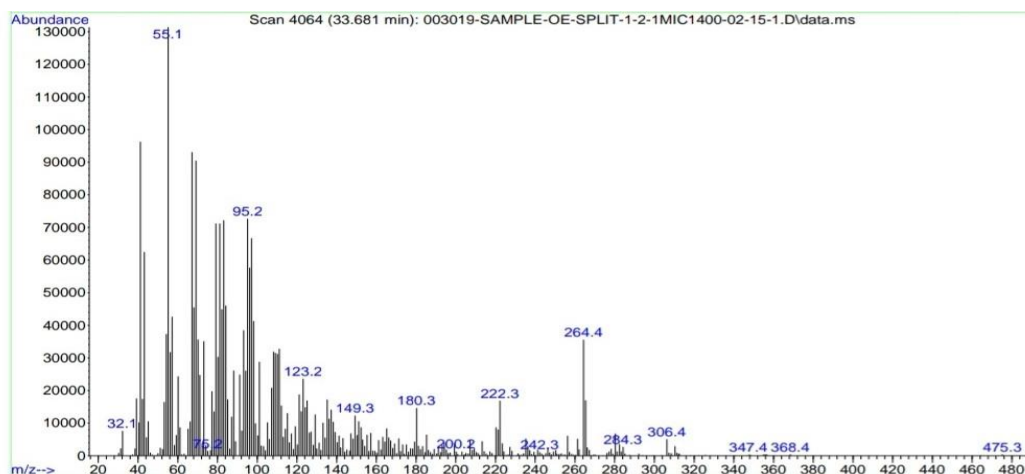


Figure 1. GC-MS Chromatogram of Castor bean oil

Table 3. Major compound identified of Castor bean oil by GC-MS

Compound	R.T /min.	Molecular formula	Area %
Butylated hydroxyl toluene	13.550	C <sub>15</sub> H <sub>24</sub> O	2.92
Benzen,( 1 butyl hexyl )	14.18	C <sub>16</sub> H <sub>26</sub>	8.32
Benzene, (1-pentyl hexyl)	17.049	C <sub>17</sub> H <sub>28</sub>	12.84
Benzene, (1-pentyl heptyl)-	20.144	C <sub>18</sub> H <sub>30</sub>	30.30
Benzene, (1-hexyl heptyl)-	23.283	C <sub>19</sub> H <sub>32</sub>	25.50
Hexadecanoic acid, ethyl ester	28.531	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	15.84
13-Hexyloxacyclotridec-10-en-2-one	30.537	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	10.31
Linoleic acid ethyl ester	33.549	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	50.17
Ethyl Oleate	33.764	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>	100.00
Oleic Acid	34.195	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	38.45
Ricinoleic acid	39.377	C <sub>18</sub> H <sub>34</sub> O <sub>3</sub>	47.21
9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	43.801	C <sub>21</sub> H <sub>40</sub> O <sub>4</sub>	27.28
Heptanoic acid, docosyl ester;	54.508	C <sub>29</sub> H <sub>58</sub> O <sub>2</sub>	40.03

### TEM Test

The transmission electron microscope is an important way to analyze the surface morphology of castor oil nanoemulsion, as it provides A high-resolution view of the in situ structure of the nanoemulsion <sup>24</sup>. The surface morphologies of droplets that appeared as bright circles, and the castor oil nanoemulsion showed both regular and irregular spherical shapes with an average diameter of 84 nm Fig.2.

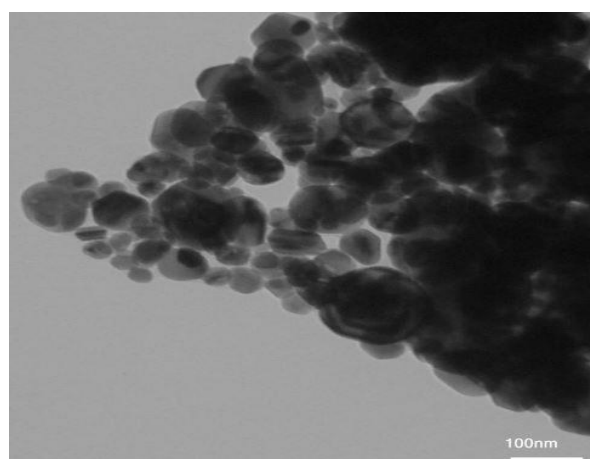
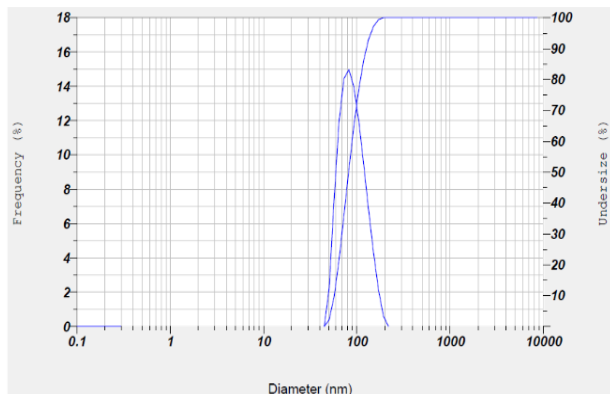


Figure 2. TEM image of Castor oil nanoemulsion prepared by ultrasonication method

### Dynamic Light Scattering (DLS)

The mean particle diameter and particle size distribution of the Castor oil nanoemulsion were measured using dynamic light scattering techniques Fig.3. According to the DLS findings, due to the nanoemulsion components, the mean particle

diameter was found to be 87.4 nm. Based on these findings, the castor oil nanoemulsion with castor oil (10% w/w), aqueous phase dioizinoic water (71%), surfactant tween 80 (14% w/w), and co-surfactant ethanol (5% w/w) was shown to have the optimal percentage.



**Figure 3. Nanoemulsion droplet size by Dynamic Light Scattering**

**Larvicidal bioassay**

The results reported in Table 4 show the efficacy and toxicity of four different concentrations of castor oil bulk emulsion against the fourth larvae stage of *Culex quinquefasciatus* mosquitoes. The mortality rate reached an average of (80.7, 96.5, 94.7, and %100) at concentrations of (1000, 1500,

2000, and 2500 ppm) respectively after 72 hours of treatment, with a significant difference between the concentrations at 0.05. According to Table 4, a concentration of 2500ppm resulted in a 100% mortality rate, while a concentration of 1000ppm resulted in an 80.75% mortality rate. The results showed the importance of the time factor in increasing the rates of mortality with a significant difference. The results also revealed that the interaction between the studied factors had a significant effect on increasing the mortality rates with a significant difference as well. The lethal concentration 50 (LC 50) was reached (409.37 ppm) as shown in Table 6. The results in Table 5 indicate the efficacy and toxicity of four different concentrations of castor oil nanoemulsion, as the mortality rates reached an average of (48.3, 74.1, 79.3, and 100) at concentrations of (250, 350, 450, and 550 ppm) respectively after 72 hours of treatment, with a significant difference between the concentrations, the concentration (550 ppm) showed the highest mortality rates (100%), while the concentration (250ppm) show the lowest (48.3%), the results showed the importance of the time factor in the increasing the rate of mortality with a significant difference as well, the lethal concentration 50 (LC50) reached an average of (268.21 ppm) as in table 6.

**Table 4. Toxicity of castor oil bulk emulsion on 4<sup>th</sup> larval stage of *Culex quinquefasciatus***

Concentration Ppm	% mortality after			mean
	24 h	48 h	72 h	
1000	46.7	65.3	80.7	64.2
1500	86.7	93.1	96.5	92.1
2000	78.3	87.9	94.7	87.0
2500	91.7	98.3	100.0	96.6
Mean	75.8	86.2	93.0	
LSD 0.05	Conc. = 7.04      time = 6.09			conc. × time = 12.19

**Table 5. Toxicity of castor oil nanoemulsion on 4<sup>th</sup> larval stage of *Culex quinquefasciatus***

Concentration Ppm	% mortality after			mean
	24 h	48 h	72 h	
250	7.3	17.2	48.3	24.3
350	15.3	34.5	74.1	41.3
450	22.0	41.4	79.3	47.6
550	35.6	82.8	100.0	72.8
Mean	20.1	44.0	75.4	
LSD 0.05	Conc. = <b>8.67</b> time = <b>7.50</b>			conc. × time = <b>15.01</b>

**Table 6. lethal and sub- lethal concentration of castor oil bulk and nanoemulsion on the 4<sup>th</sup> larval stage of *Culex quinquefasciatus*.**

TREAT	slope	LC25 (95% confidence limit)	LC50 (95% confidence limit)	LC90 (95% confidence limit)	Regression Equation	$\chi^2$
Castor oil emulsion bulk	2.651	192.45 107.00-346.114	409.37 227.62 -736.24	1717.69 955.087-3089.19	y=2.054x -0.352	0.988*
Castor oil Nano emulsion	5.298	198.99 162.625-243.508	268.21 219.19- 328.19	472.892 386.46 -578.66	Y= 5.298 x - 7.866	0.951*

### Enzyme inhibition assay

The effect of castor oil nanoemulsion on the acetyl cholinesterase activity in the treated fourth larval stage showed maximum inhibition at 550ppm (25.73 ± 0.58), while with 250ppm it was (18.65 ± 0.39), with a significant difference between the two concentrations as shown in Table 7 .

**Table 7. Effect of different concentrations of Castor oil Nano emulsion on the percent inhibition of acetylcholinesterase enzymatic activity in the fourth 4th stage of *Culex quinquefasciatus* after 48 h.**

Concentration(ppm)	Absorbance(nm)	%enzyme Inhibition
0	0.579	-
250	0.471	18.65 ± 0.39 b
550	0.430	25.73 ± 0.58 a

P= 0.0182 sig.dif.  
t=7.30 , df=2

### Discussion:

We noticed from the results that to reach the mortality rate of 100% it required 550ppm of castor oil nanoemulsion, while it required 2500ppm of castor oil bulk emulsion to reach the same rate. This is approximately five times higher compared to the nanoemulsion. These results agree with those of Sogan et. al. in using castor oil nanoemulsion against *Anopheles culicifacies* and achieving the superiority of nanoemulsion compared with bulk emulsion<sup>26</sup>. The insecticidal activity of the seed and leaf extract of *R. communis* has been studied against *Spodoptera frugiperda*, and it was found that castor oil and ricinine are the major ingredients of the *R. communis* which are responsible for the insecticidal activity of the seed<sup>27</sup>.

It has been proposed that insect death caused by oils is caused by anoxia or interferences in normal respiration, resulting in suffocation due to the presence of ricinoleic acid. Sugmar et. al. mention that the larvicidal activity of eucalyptus oil nanoemulsion showed higher activity when compared to bulk emulsion, and the treated larval homogenate showed a decrease in total protein content and a reduction in enzyme activity, this inhibition in enzyme activity will effects all the process that this enzyme worked with it, subsequently the biological activity of the mosquitoes<sup>28</sup>. Also the effects of plant extracts were seen in other insects such as the fly house in which the effects of plant extracts showed great effects on the biological activity of the insects<sup>29</sup>.

### Conclusion:

It is believed that castor oil nanoemulsion is eco-friendly and has natural pesticide activity. The reason for the high efficiency of the nanoemulsion is the small size of the particles, subsequently the large surface area, compared to the bulk emulsion, and this causes the high efficiency in causing mortality rate to *Culex quinquefasciatus* with low concentrations compared to the bulk emulsion. This is very important from an environmental and economic point of view. It can be concluded that castor oil nanoemulsion can be used as a safe and effective alternative in the control of vector-borne diseases caused by mosquito larvae.

### 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 Baghdad.

### Authors' contributions statement:

H I Al S and SA K conceived of the presented idea and supervised the findings of this work. While, A. A. A. did all the experiments and verified the analytical methods. All authors discussed the results and contributed to the final manuscript.

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## سمية المستحلب النانوي و التقليدي لزيت الخروع في يرقات الطور الرابع لبعوض الكيولكس *Culex quinquifasciatus* تحت الظروف المختبرية

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### الخلاصة:

البعوض الكيولكس مثل نوع *Culex quinquefasciatus* هو الناقل الاولي للعديد من المسببات المرضية مثل الفلاريا و التهاب الدماغ الياباني و فايروس غرب النيل في العديد من الدول حول العالم . ان التطور الحاصل في الحقول العلمية مثل التقنيات النانوية قاد الى استخدام هذه التقنيات في برامج السيطرة على الحشرات ومنها البعوض، من خلال استخدام البناء الحيوي (الاخضر) للمستحلبات النانوية المستندة على المنتجات النباتية مثل زيت نبات الخروع. المستحلب النانوي لزيت الخروع أُعد بنسب متعددة محتويًا على، زيت الخروع و الايثانول و التوين 80 و الماء منزوع الايونات بواسطة جهاز الالتراسونيك . من خلال تحاليل الديناميكية الحرارية فقد تم تأكيد ان الصيغة ( 10 مل ) من زيت الخروع و ( 5 مل ) من الايثانول و ( 14 مل ) من التوين 80 و ( 71 مل ) من الماء منزوع الايونات هي الاكثر استقراراً من الصيغ الاخرى . المستحلب النانوي لزيت الخروع تم تشخيصه بواسطة المجهر الالكتروني النافذ و ديناميكية الاستطارة الضوئية، فكانت قطيرات المستحلب النانوي كروية الشكل و ذات معدل حجم 93 نانوميتر. استخدمت تراكيز مختلفة من المستحلب النانوي لزيت الخروع (250، 350، 450، 550) جزء بالمليون واختبرت فعاليتها كعوامل ابادة يرقية، و كذلك استخدمت تراكيز من المستحلب الخام لزيت الخروع (1000 ، 1500 ، 2000، 2500) جزء بالمليون قد اختبرت ايضاً، وتمت مقارنتها. المستحلب النانوي اظهر فعالية عالية مقارنةً بالمستحلب الخام، حيث ان الجرعة المميتة للنصف 50LC للمستحلب النانوي والمستحلب الخام كانت 21، 268 و 37,409 جزء بالمليون خلال 72 ساعة وعلى التوالي . اختبار كيميائي حيوي قد أُجري لليرقات المعاملة لفحص تأثير المستحلب النانوي لزيت الخروع على الخصائص البايوكيميائية لليرقات، فالمزيج المتجانس لليرقات اظهر تثبيط في فعالية انزيم الكولين ايستريز .

**الكلمات المفتاحية:** زيت الخروع، بعوض الكيولكس *Culex quinquifasciatus* ، مستحلب نانوي ، مستخلص نباتي، مكافحة الحشرات .