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## Effects of Castor Oil Nanoemulsion Extracted by Hexane on the Fourth Larval stage of *Culex quinquefasciatus* from Al Hawizeh Marsh/Iraq, and Non- Targeted Organism

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### Abstract:

The current study aims to show the importance of plant products as mosquitocides against *Culex quinquefasciatus*. Castor oil Nanoemulsions were subedit in various ratios including castor oil, ethanol, tween 80, and deionized water by using ultrasonication. Thermodynamic, centrifugation, PH, assay which 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 stable formula of castor oil nanoemulsion was characterized by transmission electron microscopy (TEM) and dynamic light scattering (DLS). Nanoemulsion droplets were spherical in shape and were found to have a Z-average diameter of 87.4nm. A concentration of castor oil nanoemulsion 250, 350, 450, and 550 ppm were tested as larvicidal agents and bulk emulsion 1000, 1500, 2000 , and 2500 ppm were tested also and compared, against the fourth instar larvae of *C. quinquefasciatus*. Castor oil nanoemulsion exhibited higher activity when compared to bulk emulsion. LC50 of castor oil nanoemulsion and castor bulk emulsion were found as 291.46 and 439.19ppm after 72 h, respectively. The toxic effects of castor oil bulk and Nano emulsion was tested against non-target organism Guppy fish and found to be not toxic at the concentration which used in the study.

**Keywords:** *Culex*, Guppy fish, Mosquitoes control, Nanotechnology, Plant products.

### Introduction:

Insects -borne diseases were and are still considered a great public health problem in different countries of the world. Medical insect pests are the most common in developing and poor resource countries and inflict vast encumbrance in terms of morbidity and mortality <sup>1,2</sup>.

In Iraq, there were 18 species of the genus *Culex* one of them is *Culex qeinquefasciatus* <sup>3</sup> this species is present in the marshes also. Al Hawizeh marsh is located in the south of Iraq to the south-eastern edge of the Tigris River particularly in Missan governorate and the national Iraq -Iran border passes through it, where the mosquitoes were collected. This marsh covers large areas and its richness and diversity in flora and fauna. Al Hawizeh marsh was listed as a RAMSAR site in 17/10/2007 <sup>4,5</sup>.

Mosquitoes have medical importance because its bite causes allergy and local inflammation in the place of bites and are in charge of the transmission of diverse of pathogen-borne diseases like malaria, filariasis, dengue, chikungunya, and encephalitis <sup>6</sup>. Diseases like this resulted in morbidity and mortality in many countries of the world. Filariasis is a serious disease caused by parasite transmitted to humans through the bite of the *Culex* species leading to epidemic diseases in countries of the Southeast Asia <sup>7</sup>. 51 million people were infected by this disease in 2018, a decline of about 74% since the start of WHO's Global Programme to eliminate lymphatic filariasis in 2000 <sup>8</sup>. It is confined to the rural plain and semi urban areas of these areas .Alternative methods for vector control becomes an important tool in prevent of disease, due to the development of

pesticide resistance in mosquitoes. Insecticides of botanical source become of a great importance for the control of insect vectors. Essential oils procured from plants have been considered prospective sources of bioactive substances<sup>9</sup>. Researches concerning insecticides based on botanical origin have been sophisticated in recent years as alternative methods for chemicals, even when using alternative chemicals such as citric acid and bicarbonate which have effects on mosquitoes larvae but their safety on environment is uncertain<sup>10</sup>. This is appropriate since yearly changes in susceptibility of various mosquitoes species have become resistant worldwide to chemical insecticides.

The use of plants with bio-potential products and secondary metabolites, have a significant role in mosquito management<sup>11</sup>. The larvicidal effects of three plant oil extracts of *Piper nigrum*, *Eucalyptus regnans*, and *Azadirachta indica* against *Culex pipiens* third instar larvae, were examined, the oil extract of *P. nigrum* was more effected than other plants oil extracts when examining the disorder signs in mid gut of the treated larvae<sup>12</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>13</sup>. Castor plants (*Ricinus communis* L.) which are considered medicinal plants that belong to the Euphorbiaceae family and grown all over the world because of its tolerance to various weather conditions and for its oil that has huge use in industrial, pharmaceutical, cosmetic and engine sector<sup>14</sup>. Castor oil consists of many fatty acids like Ricinoleic acid, which comprises more than 75% of the total fatty acids<sup>15</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>16-19</sup>.

The environmental condition such as light, air, high temperatures, and moisture caused the instability of plant essential and fix oils and that could 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 type of emulsions that have 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>20</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>21,22</sup>.

This study was done to formulate an alternatives to chemical pesticides in mosquito control by using plant products based on nanotechnology in the form of eco-friendly 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-targeted organisms.

## Material and Methods:

### Chemicals material

Materials like Hexan (Thomas Baker, India) as a solvent, Ethanol as co-surfactant, tween 80 (Thomas Baker, India) as surfactant, and deionized water, were used.

### Mosquito collection and rearing

Mosquito larvae were collected from Al Hawizeh marsh in the Abu Khasaf region in the Missan governorate / Iraq, by using small containers 1 L. 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. Mosquitoes samples were diagnosed as *Culex quinquefasciatus*. The experiments will be done in the laboratory of the Agricultural Research Directorate in Al Twaitha (Baghdad).

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 with 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 were reached pupae, the pupae were collected and placed in plastic containers containing 400 mL of distilled water till the adults emerge, which feed 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 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 transmitted with a plastic tea spoon to clean plastic containers of 500ml that contain 400ml of distilled water <sup>19</sup>.

#### Plant collection and extraction

Dried castor fruits were collected from castor trees in the center of agricultural research (EPA) in Abo Guraib, Baghdad / Iraq, in February 2020, the plant is diagnosed by Natural History Museum of Iraq. 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 hexane was put in the round flask which was exposed to heating with a heater at 65 °C, then the condenser was connected with the 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, surfactant, co- surfactant and deionized water was mixed with a magnetic stirrer hot plate 30°C 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 sonotrode with a probe diameter of 13 mm, which composed of piezoelectric crystal. The disruptive forces generated by 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 were used showed in Table 1.

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

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

#### Stability analysis

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

#### 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. An argon laser 1 ¼ 633 nm with variable intensity was implicated in the apparatus. Castor oil emulsion was diluted about 1000 times with Milli-Q water before analysis in order to avoid multiple light scattering effects and each analysis was made with three readings per sample <sup>23</sup>.

#### GC/MASS analysis

The fatty acid methyl esters (FAME) were prepared according to the modified method <sup>24</sup>. 1.8 ml of petroleum ether was used to dissolve 0.05 g of oil , to which 0.2 ml of sodium methylate was added and swirled to separate the mixture in two layers, then 1ml of the upper layer that contain the FAME was injected onto a Shimadzu GCMS QP2010 74707 30 m capillary column 0.25 mm i.e. 0.25 m film .The GCMASS temperature was programmed with an primary oven temperature of 70 °C rein time 5 min, which was increased at the rate of 10 °C/min to 300 °C rein time 5min, then the sample injected at temperature of 260 °C, the split ratio was 10. The GC MASS solution version NIST08 standard mass spectrometry library was used to analyze the data.

#### Transmission Electron Microscope (TEM) Test

The images of TEM were taken out by Hitachi H-7650B electron microscope programed at an accelerating voltage of 80 kV to get detailed

information about the morphology and particle size of the nanoemulsion. 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, then the extra phosphor tungstic acid was rub out <sup>23</sup>.

#### Larvicidal bioassay

Fourth larval stages were collected from the rearing containers and treated with several concentrations of nanoemulsion, following WHO Guidelines <sup>25</sup>. The larvae of *Culex quinquefasciatus* were placed in each of the four 125 ml containers containing the different concentrations of bulk emulsion, 1000, 1500, 2000, and 2500 ppm, twenty larvae for each container in three replicates. The control was distilled water only. Nanoemulsion also was investigated for its efficiency against *Culex quinquefasciatus* in concentrations of 250, 350, 450, and 550ppm. The larval mortality was observed after 24, 48, and 72 hours. Corrected mortality was measured by the Abbott formula, As follows:

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

#### Toxicity to non – target organism

*Poecilia reticulata*, Guppy fish was chosen as experience organism. The fishes were purchased from fish shop / Baghdad, then they kept three days at the room temperature of 27–28 °C and were provided with artificial diet (Siso, flake food fortified with vitamins and proteins. China). The experiments were done according to methods described by

Promsiri et al <sup>26</sup>, with slight modification. The toxicity was assessed by using the concentration of 2500 ppm for bulk emulsion and 550 ppm for nanoemulsion. Ten healthy fishes of *P. reticulata* were placed in a spherical, glass fishbowl containing 500 ml of either castor oil bulk and nano emulsion in three replicates. The control, with three replicates, consisting of 10 fishes for each replicate with distilled water only. The dead fishes were recorded at 24, 48 and 72 h period, and the percentage mortalities were calculated. The experiment were conducted at a room temperature of 27–28 °C, without water replenishment, and the fishes fed on artificial diet.

#### 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>27</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	F1	F2	F3
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

### 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 1. 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 diozinoid water 71%, surfactant tween 80, 14% w/w, and co-surfactant ethanol 5% w/w was shown to have the optimal percentage.

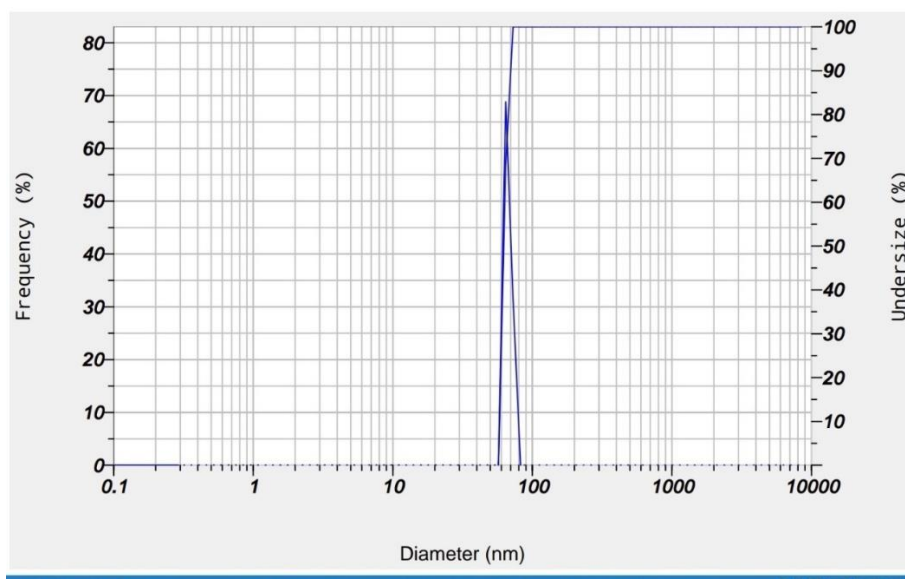


Figure 1. Nanoemulsion droplet size by Dynamic Light Scattering

### GC-Mass Analysis

This test shows that Ricinoleic acid is the main product of castor oil since its peak is the highest as in Fig 2 which shows the peaks of the presented fatty

acids. 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.

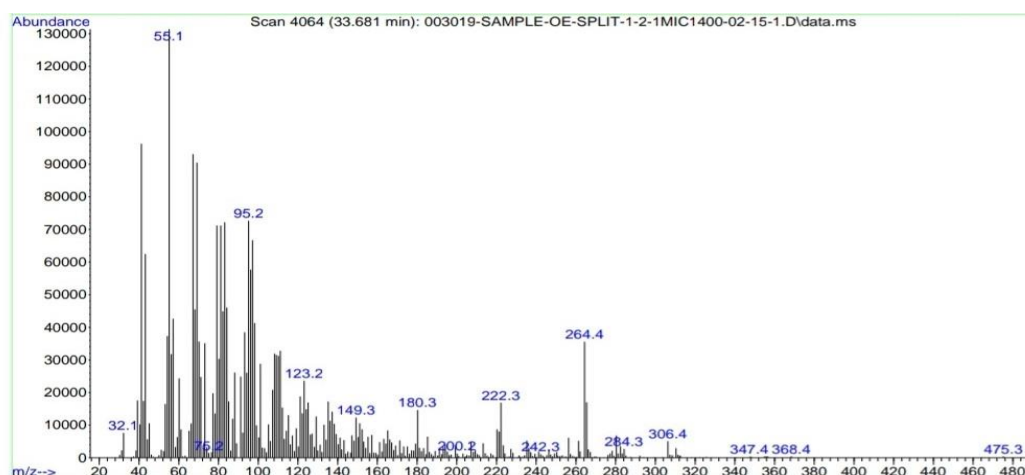


Figure 2. GC-MS Chromatogram of Castor bean oil.

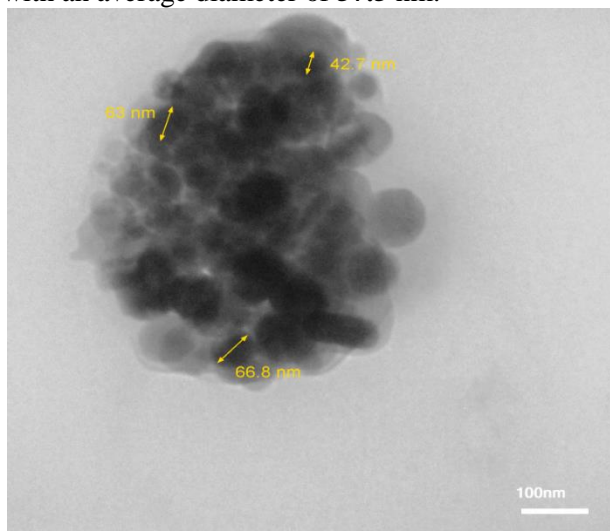
Table 3. Major compound identified of Castor bean oil by GC-MS

Compound	R.T. /min.	Molecular formula	Area %	Similarity index (SI)
Butylated hydroxyl toluene	13.550	C <sub>15</sub> H <sub>24</sub> O	0.458	43.2
Hexadecanoic acid, ethyl ester	28.531	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	2.479	81.7
13-Hexyloxacyclotridec-10-en-2-one	30.537	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	1.602	60.4
Linoleic acid ethyl ester	33.549	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	7.88	22.2
Ethyl Oleate	33.764	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>	15.72	10.9
Oleic Acid	34.195	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	6.04	14.4
Ricinoleic acid	39.377	C <sub>18</sub> H <sub>34</sub> O <sub>3</sub>	7.42	23.7
9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	43.801	C <sub>21</sub> H <sub>40</sub> O <sub>4</sub>	4.29	20.2
Heptanoic acid, docosyl ester	54.508	C <sub>29</sub> H <sub>58</sub> O <sub>2</sub>	6.29	24.8

R.T.: Retention Time

### TEM Test

TEM test is an important assay to detect the surface morphology of castor oil nanoemulsion, it supplies a high-resolution images of the in situ structure of the nanoemulsion<sup>23</sup> Fig 3 shows 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 57.5 nm.



**Figure 3.** TEM image of Castor oil nanoemulsion prepared by Ultrasonication method.

### 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 larval stage of *Culex quinquefasciatus* mosquitoes. The mortality rate reached an average of 80.20, 91.31, 91.21, 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 80.20% 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 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 43.0, 63.8, 73.9, and 91.4 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 91.4%, while the concentration 250ppm show the lowest 43.0% , 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 (LC 50) for bulk emulsion was reached 439.19 ppm while , lethal concentration 50 ( LC50) reached an average of 291.46 ppm for castor oil nanoemulsion as in Table 6 which shows the LC50 of both bulk and nanoemulsion which it for bulk emulsion twice more than for nanoemulsion. Figs 4 and 5 show a high correlation rate between the log of castor oil bulk emulsion and nanoemulsion concentration against *C. quinquefasciatus* mortality percent probit for 4<sup>th</sup> larval instar under study with high R<sup>2</sup> values 0.8535, 0.9585. The treated larvae appeared as in Fig. 6.

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

Conc. ppm	% mortality after			Mean
	24 h	48 h	72 h	
1000	46.67	67.19	80.20	64.69
1500	68.33	84.47	91.31	81.37
2000	66.67	81.05	91.21	79.64
2500	73.33	96.49	100	89.94
Control	0.00	3.33	5.00	2.78
Mean	51.00	66.51	73.54	
LSD0.05	Conc. = 6.195	time = 4.799	conc. × time = 10.730	

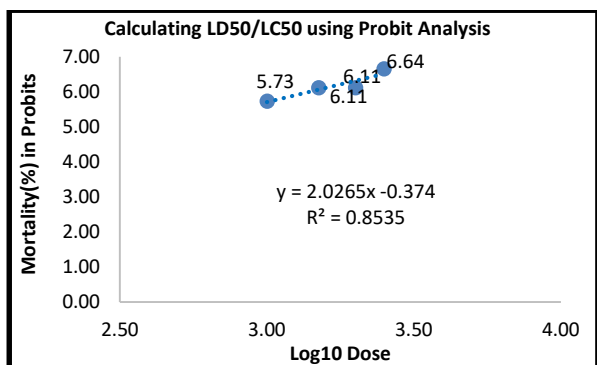
**Table 5.** Toxicity of castor oil nanoemulsion extracted by Hexane on 4<sup>th</sup> larval stage of *Culex quinquefasciatus*

Conc. ppm	% mortality after			Mean
	24 h	48 h	72 h	
250	3.4	16.8	43.0	21.1
350	10.1	37.2	63.8	37.0
450	20.1	45.7	73.9	46.6
550	32.4	56.1	91.4	59.9
Control	1.7	1.7	3.3	2.2
Mean	13.5	31.5	55.1	
LSD0.05	Conc. = 7.03	time = 5.45	conc. × time = 12.18	

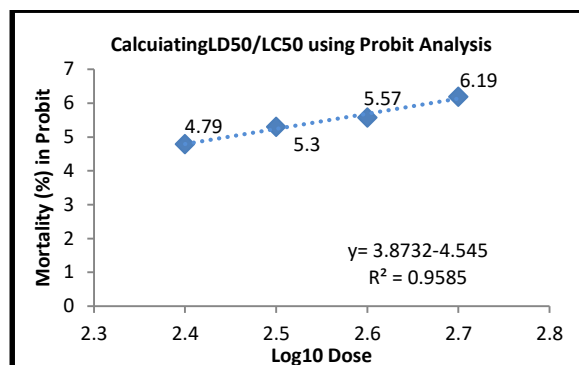


**Table 6. Lethal and sub lethal concentration of castor oil bulk and nano emulsion on 4th larval stage of *Culex quinquefasciatus***

TREAT	slope	LC25 (95% confidence limit)	LC50 (95% confidence limit)	LC90 (95% confidence limit)	Regression Equation	$\chi^2$
Castor oil	3.873	194.586	291.46	628.032	Y= 3.873X -4.545	0.958*
Nanoemulsion		151.56-249.82	227.02-374.19	489.17 - 806.31		
Castor oil bulk emulsion	2.027	200.06 113.50 – 352.62	439.19 249.17 – 774.12	1956.59 1110.05 –3448.70	Y= 2.027 X -0.374	0.994*



**Figure 4. Bulk emulsion of Castor oil extracted by Hexane**



**Figure 5. Nanoemulsion of Castor oil extracted by Hexane**



**Figure 6. Fourth larval stage of *Culex quinquefasciatus*, A: before treatment, B: after treatment by castor oil nanoemulsion. (picture 10 X)**

**Toxicity to non- target organisms**

Castor oil bulk emulsion 2500ppm and nanoemulsion 550ppm did not display any deadly effects on *Poecilia reticulata* (guppy fish ) after 24,

48 , and 72 hrs from exposer as viewed in Table. 7. The fishes were normally swim a fed when they removed from the treatment container and placed in cleaned water and monitor for 10 days.

**Table 7. Effects of bulk and nanoemulsion of castor oil extracted by hexane on Guppy fishes**

Treatment	Mortality % after		
Con. ppm	24h	48h	72h
Bulk emulsion 2500ppm	Zero	Zero	Zero
Nanoemulsion 550ppm	Zero	Zero	Zero
Control distal water only	Zero	Zero	Zero

## Discussion:

What the results achieved is to reach the mortality rate of 100% it required 2500ppm of castor oil bulk emulsion, while it required 550ppm of castor oil nanoemulsion, to reach the same rate. This is approximately five times higher compared to the nanoemulsion. These results show the importance of nanotechnology in building materials with high efficiency and lower concentrations than bulk materials 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>28</sup>. Seed and leaf extracts of *R. communis* were examined as insecticides against *Spodoptera frugiperda*, castor extracts contain ricinine and ricinolic acid which represent the major ingredients of the *R. communis* and they are responsible for the insecticidal activity of the extracts<sup>29</sup>.

Researchers proposed that insect death caused by oils is caused by anoxia or interferences in normal respiration, resulting in suffocation due to the presence of ricinolic acid. Sugmar et. al. mention that the larvicidal activity of eucalyptus oil nanoemulsion demonstrated high efficiency compared to the bulk emulsion, the homogenate of treated larvae displays a decrease in total protein content and inhibition in enzyme activity, subsequently the biological activity of mosquitoes<sup>30</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>31</sup>. The effects of plant extracts pesticides on non- target organisms were studied, *Carissa carandas* synthesised Ag nanoparticles were found to be safer to non- target organisms in LC50 ranging from 1097.87 to 1783.34 µg/ml for *Anisops bouvieri*, *Diplonychus indicus*, and *Gambusia affinis*, although the LC50 of the same nanoparticles were 14.33, 15.69 and 16.95 respectively which show a high toxicity against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*<sup>32</sup>. Seed extracts of *Ricinus communis* displayed high mortality rate against the larvae of *Anopheles culicifacies* and *Aedes aegypti* at concentration of 16, 32, 64 ppm respectively for 24 h while they were not toxic for non- target organism *Poecilia reticulata*<sup>19</sup>. Al Qahtani et al by using *Rubus ellipticu* biosynthesis Ag nanoparticles proved that this pesticide were not toxic to non- target organisms such *Anisops bouvieri*, *Diplonychus indicus*, and *Gambusia affinis*<sup>33</sup>. *Carlina acaulis* root essential oil has high larvicidal activity against *Culex quinquefasciatus* larvae but does not has toxic effects against *Daphnia magna* adults<sup>34</sup>.

## Conclusion:

From the results, it can appear that castor oil nanoemulsion has natural insecticidal efficacy and is eco-friendly. 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 is an efficient alternative in the control of vector-borne diseases caused by mosquito larvae and considered safe for environment.

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

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## تأثيرات المستحلب النانوي لزيت الخروع المستخلص بواسطة الهكسان على الطور اليرقي الرابع لـ *Culex quinquefasciatus* من هور الحويضة/العراق، و كائن غير مستهدف

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### الخلاصة:

تهدف الدراسة الحالية الى اظهار اهمية المنتجات النباتية كمبيدات للبعوض ضد *Culex quinquefasciatus* . المستحلب النانوي لزيت الخروع حضر بالعديد من التوليفات , مكونا من زيت الخروع و الايثانول و توين 80 و الماء منزوع الايونات بواسطة الموجات فوق الصوتية . فحوصات الديناميكية الحرارية و الطرد المركزي و PH اثبتت ان التوليفة المكونة من 10 مل زيت الخروع و 5 مل ايثانول و 14 مل توين 80 و 71 مل ماء منزوع الايونات هي الاكثر ثباتا من التوليفات الاخرى . توليفة المستحلب النانوي لزيت الخروع تم تشخيص خصائصها بواسطة المجهر الالكتروني النافذ ( TEM ) و الاستطارة الديناميكية للضوء ( DLS ) . قطيرات المستحلب النانوي كانت كروية الشكل و معدل اقطارها هو 87.4 nm . تراكيز من المستحلب النانوي لزيت الخروع ( 250, 350, 450 , and 550 ppm ) تم اختبارها كعوامل ابادة يرقية و ايضا المستحلب الخام لزيت الخروع ( 1000, 1500, 2000 , and 2500 ppm ) قد اختبر وقورنا , ضد الطور اليرقي الرابع للكيلوكس . المستحلب النانوي لزيت الخروع اظهر فعالية عالية مقارنةً بالمستحلب الخام . LC50 للمستحلب النانوي و المستحلب الخام كانت 291.46 ppm و 439.19ppm بعد 72 ساعة على التوالي. التأثير السام للمستحلب الخام و النانوي لزيت الخروع قد اختبر ضد كائن غير مستهدف وهو اسماك الكوبي وقد وجد انه غير سام بالتراكيز المستخدمة في الدراسة.

**الكلمات المفتاحية:** كيلوكس، سمك الكوبي، مكافحة البعوض، تقنية النانو، المنتجات النباتية.