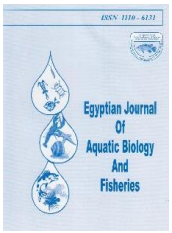
Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(2): 915 – 933 (2024)
www.ejabf.journals.ekb.eg
DOI: 10.21608/EJABF.2024.351928



Assessment of the Toxicological and Biological Effects of the Essential Oil of *Lavandula angustifolia* and its Nanoemulsion against the Aquatic *Culex pipiens* Larvae (Diptera: Culicidae)

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ARTICLE INFO

Article History:
Received: March 10, 2024
Accepted: April 14, 2024
Online: April 26, 2024

Keywords:
Culex pipiens,
Essential oils,
Lavender,
Lavender nanoemulsion

ABSTRACT

Plants and their derivatives, such as essential oils (EOs), have been mainly used in recent decades as promising insecticides instead of the conventional insecticides. Lavender essential oil and its nanoemulsion were tested as larvicides against the *Culex pipiens* larvae to assess their toxicological and biological effects on the tested population. The bioassay test showed that both lavender essential oil and its nanoemulsion were effective against *Cx. pipiens* larvae,with median lethal doses ($LC_{50,s}$) 133.85 and 480.78ppm after 24 hours of treatment for lavender essential oil and its nanoemulsion, respectively. GC-mass spectroscopy results indicated that the lavender essential oil is mainly composed of 1-Propanol, 2-(2-hydroxypropoxy) (25.13%), 2-Propanol, 1,1'-oxybis (21.7%), 1-Propanol, 2,2'-oxybis (11.93%), Linalool (10.81%), Linalyl acetate (10.35%), 2-Butanol, 3,3'-oxybis (5.12%), and Eucalyptol (4.8%). While, the lavender essential oil nanoemulsion is mainly composed of Linalool (38.32%), 2-Propanol, 1,1'-oxybis (15%), 1-Propanol, 2-(2-hydroxypropoxy) (11.24%), (+)-2-Bornanone (9.78%), 1-Propanol, 2,2'-oxybis (9.46%), and Linalyl acetate (8.29%). Biology tests confirmed that both lavender essential oil and its nanoemulsion caused larval, pupal, and pupal-adult intermediate malformations.

INTRODUCTION

Mosquitoes transmit numerous human and animal diseases, and their control represents a public health challenge worldwide **(Riaz *et al.*, 2012)**. In Egypt, eleven *Culex* species are widespread throughout the country. Among these species, the house mosquito, *Culex pipiens* L. (Diptera: Culicidae), is the most common mosquito insect in rural and urban zones, and it is the main vector of the Rift Valley fever virus, the Filariasis virus (*Wuchereria bancrofti*), and the Western Nile virus **(Zahran *et al.*, 2017; Shahat *et al.*, 2020a)**. Although chemical insecticides are highly efficacious, mosquito

control is facing a threat due to the development of resistance to these chemical insecticides, resulting in rebounding vectorial capacity **(Bream *et al*., 2018)**. Moreover, the use of synthetic insecticides in controlling agricultural pests has caused unintended damage to both human life and the environment. More than 98% of insecticides and 95% of herbicides sprayed have been found to affect non-target species through air, water, bottom sediments, and food **(Yazdani *et al*., 2013)**.

From this point of view, researchers diverted their attention toward the plant kingdom to find alternative agents possessing bioactive chemicals that may act as potential insecticides **(Shahat *et al*., 2020b)**. Plants and their derivatives, such as essential oils (EOs), play an important role in the protection of plants **(Nazzaro *et al*., 2013)**. EOs, such as lavender oil, are a natural product that can effectively act as an insecticide and repellent against mosquito larvae **(Choi *et al*., 2002)**. Moreover, since EOs usually have low toxicity to mammals, high biodegradability, and are often inexpensive, they are regarded as very promising substances for the formulation of highly toxic and eco-friendly pest control products **(Conti *et al*., 2010; Bedini *et al*., 2019)**. Essential oils are defined as any volatile oil(s) that have strong aromatic components that provide distinctive odor, flavor, or scent to a plant **(Jayakumar *et al*., 2016)**. They are documented for the exhibition of acute toxicity, anti-feeding, and oviposition deterrents against a wide variety of insectpests **(Hanan, 2013)**.

The EO obtained from *Lavandula angustifolia* flowers is primarily composed of linalyl acetate, linalool, lavandulol, 1,8-cineol, lavandulyl acetate, and camphor **(Jianu *et al*., 2013)**. Lavender, *L. angustifolia*, is an aromatic plant of the Lamiaceae family widely distributed in the Mediterranean area, and its EO was found to have medicinal, antibacterial, antifungal, and pesticide activities, hence it can act perfectly in controlling mosquito species **(Germinara *et al*., 2017)**. The mechanism of action of lavender essential oil on the bodies of insects also recorded disruption of the molecular events of morphogenesis and alteration in the behavior **(Rattan, 2010)**. Pupal death might be due to multiple mechanisms of action, including the oil blocking the moulting of pupae into adults **(Suryanarayanamurthy *et al*., 1997)**.

In addition, lavender nanoemulsion is expected to give more promising results than the lavender oil itself due to its good stability, rapid digestibility, protection against degradation, controlled release, and high capability of enhancing drugs' bioavailability **(Samie & Naser, 2020)**.

The objective of this research was to conduct an evaluation of the toxicological and biological effects induced by the essential oil extracted from *L. angustifolia* and its nanoemulsion on *Cx. pipiens* larvae.

MATERIALS AND METHODS

Colony maintenance

The *Culex pipiens* culture was reared in an insectary in the Research and Training Center on Vectors of Diseases (RTC), Faculty of Science, Ain Shams University, following the standard methods of **Gerberg *et al*. (1994)**. Insects were reared under conditions of optimum temperature (27± 1°C) and relative humidity (70± 5 %) with a constant photoperiod (light: dark = 12: 12h). Pupae were collected periodically from the water and placed in mosquito rearing mesh cages (30 × 30 × 30cm). Afterward, adults were kept in cages and provided with a 10% glucose solution for post-emergence. Blood-fed females (on pigeons for twenty minutes) were allowed to integrate the blood meals. Females were given admission to oviposition sites containing small plastic containers filled with water as egg deposition places. Eggs were allowed to hatch in sterilized water. Newly hatched larvae were reared in plastic trays and fed every two days with a tiny amount of fish food. Late 3rd instar larvae were used for larval bioassays.

Essential oil extraction

The commercially available essential oil of *Lavandula angustifolia* was obtained from Nefertari Company in Cairo. The essential oil was extracted from the flowers on cold according to the method of **Li *et al*. (2018)**.

Gas chromatography–mass spectrometry analysis

The essential oil of *Lavandula angustifolia* constituents were analyzed by a gas chromatography–mass spectrometry (GC-MS) (Agilent Technologies) equipped with a gas chromatograph (7890B) and mass spectrometer detector (5977A) at the Central Laboratories Network, National Research Center, Giza, Egypt. The sample was diluted with hexane (1:19, v/ v). The GC was equipped with a fused silica capillary column (HP5MS; 5% phenyl: 95% methylpolysiloxane; 30m × 0.25-mm internal diameter and 0.25-µm film thickness). Helium was used as the carrier gas at rate of 1ml/ min. The GC operating conditions were as follows: injector volume, 1µl; split ratio, 1:30; and injector and detector temperature, 280 and 220°C, respectively. The column temperature was isothermally maintained at 40°C for 1 minute before being programmed to increase to 150°C at a rate of 4°C/ min. It was then held at this temperature for 6 minutes before being further programmed to rise to 210°C at the same rate. After reaching 210°C, it was held for 1 minute. The ion source temperature was maintained at 230°C. The effluent of the GC column was directly introduced into the source of the MS. The MS operating conditions were as follows: solvent delay time: 3min; ionization voltage: 70eV, and m/ z range, 50 to 550. The identification of different constituents of garlic oil was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

Lavandula angustifolia nanoemulsion preparation

Lavandula angustifolia nanoemulsion (5%) was developed according to the method described in **Mohafrash *et al.* (2020)**. The ratios 1:1 of EO and Tween 80 were used. The dropping of the organic phase was performed under stirring at 400rpm for 30min, and deionized water was added drop by drop. Then, the emulsion was subjected to sonication for 10 minutes.

Lavandula angustifolia nanoemulsion characterization

Droplet size and zeta potential: The particle size and zeta potential were determined by dynamic light scattering (DLS) according to the method of **Mossa *et al.* (2018)**. The measurments were conducted at the National Research Center, Cairo, Egypt using Particle Sizing Systems, Inc., Santa Barbara, Calif., USA, at a temperature of 30°C, using the 632.8nm laser wavelength with an angle of 90° and zeta potential with an external angle of 13.8°.

Morphology of lavender nanoemulsion: Transmission electron microscopy (TEM) at the regional center for Mycology and Biotechnology (RCMB), AlAzhar University was used to determine the particles shape. One drop of emulsion was negatively stained with ethanol and was positioned on a copper grid. The TEM micrographs were acquired using a transmission electron microscope (JEOL JEM-1400Plus) with a tungsten source and operating at 80kV.

Physicochemical characterization was conducted following the methods described by **Golemanov *et al.* (2006)** and **Mossa *et al.* (2018, 2019)**. The pH value of the nanoemulsion was determined by immersing the pH electrode into the undiluted emulsion using a pH meter. Additionally, centrifugation and turbidity assessments were carried out by subjecting the nanoemulsion to centrifugation at 10,000rpm for 30 minutes at a temperature of 25°C, utilizing a Heraeus Labofuge 400R (Kendro Laboratory Products GmbH, Germany). Viscosity measurements were conducted by Ostwald viscometer at room temperature to ensure the mixing of the nanoemulsion solution. Furthermore, the thermal stability of the nanoemulsion formulation was evaluated by storing it for 3 months at both room temperature and 4°C in closed tubes, allowing for observation of any potential phase separation or creaming phenomena. These physicochemical analyses contribute to a comprehensive understanding of the nanoemulsion's properties and stability under different conditions.

Toxicological evaluation

The toxicological effects of *Lavandula angustifolia* essential oil and its nanoemulsion on *Cx. pipiens* larvae were assessed according to the standards outlined by the **WHO (2005)**. Three replicates of twenty larvae of 3rd larval instars were taken and treated with different concentrations of the essential oil: 100, 125, 150, 175, 200 and 225ppm. Additionally, another set of larvae were treated with varying concentrartions of the essential oil nanoemulsion: 200, 400, 500, 600 and 800ppm. The untreated larvae

were used as control, and replicates were maintained at the same time and under the same conditions. In order to feed the larvae, all the assay units were supplemented with a diet of finely ground fish food. Mortality data were recorded after 6, 12, 24 and 48 hours in a probit regression line to calculate LC_{25}, LC_{50}, LC_{90}, and the slope function.

Biological evaluation

Applying the LC_{25} concentration of the lavender oil and its nanoemulsion to 300 third larval instars for 24 hours and noticing the biological changes and malformations that could happen until they complete their life cycle. The untreated larvae were used as a control. Larval period, pupal period, adult emergence ratio, and sex ratio were calculated.

Statistical analysis

The data was analyzed with SPSS 19 software, followed by a one-way analysis of variance (ANOVA) and Tukey's HSD test. The results were stated as means ± SE of untransformed data and considered significantly different at $P< 0.01$. Probit analysis was conducted to calculate the estimated LC_{50} values with their limits, using Probit analysis software.

RESULTS

1. Gas chromatography–mass spectrometry analysis

Gas chromatography analysis showed that the lavender essential oil is mainly composed of 1-Propanol, 2-(2-hydroxypropoxy) (25.13%), 2-Propanol, 1,1'-oxybis (21.7%), 1-Propanol, 2,2'-oxybis (11.93%), Linalool (10.81%), Linalyl acetate (10.35%) , 2-Butanol, 3,3'-oxybis (5.12%) and Eucalyptol (4.8%), as shown in Table (1).

The lavender essential oil nanoemulsion is mainly composed of Linalool (38,32%), 2-Propanol, 1,1'-oxybis (15%), 1-Propanol, 2-(2-hydroxypropoxy) (11.24%), (+)-2-Bornanone (9.78%), 1-Propanol, 2,2'-oxybis (9.46%) and Linalyl acetate (8.29%), as shown in Table (2).

There were nine common components between the lavender essential oil and its nanoemulsion Eucalyptol, 1-Propanol, 2-(2-hydroxypropoxy)-, 1-Propanol, 2,2'-oxybis-, Linalool, (+)-2-Bornanone, Isoborneol, endo-Borneol, Linalyl acetate and Coumarin in different concentrations (Fig. 2), indicating their chemical structure, as shown in Fig.(1).

Table 1. Gas chromatography–mass spectrometry analysis of *Lavandula angustifolia* essential oil

Peak	RT	Area sum %	Name	Formula	Molecular weight
1	2.724	0.14	Propylene Glycol	$C_3H_8O_2$	76.09
2	6.962	4.8	Eucalyptol	$C_{10}H_{18}O$	154.25
3	7.187	21.7	2-Propanol, 1,1'-oxybis-	$C_6H_{14}O_3$	134.17
4	7.525	25.13	1-Propanol, 2-(2-hydroxypropoxy)-	$C_6H_{14}O_3$	134.17
5	7.62	11.93	1-Propanol, 2,2'-oxybis-	$C_6H_{14}O_3$	134.17
6	7.947	5.12	2-Butanol, 3,3'-oxybis-	$C_8H_{18}O_3$	162.22
7	8.06	10.81	Linalool	$C_{10}H_{18}O$	154.25
8	8.754	4.18	(+)-2-Bornanone	$C_{10}H_{16}O$	152.23
9	8.92	0.4	Isoborneol	$C_{10}H_{18}O$	154.25
10	9.057	0.75	endo-Borneol	$C_{10}H_{18}O$	154.25
11	10.297	10.35	Linalyl acetate	$C_{12}H_{20}O_2$	196.29
12	10.772	0.31	Isobornyl acetate	$C_{12}H_{20}O_2$	196.29
13	11.585	1.14	Alpha.-Terpinyl acetate	$C_{12}H_{20}O_2$	196.29
14	12.784	0.67	Coumarin	$C_9H_6O_2$	146.1427
15	14.624	0.26	Diethyl Phthalate	$C_{12}H_{14}O_4$	222.24
16	18.987	2.06	Musk ketone	$C_{14}H_{18}N_2O_5$	294.3

Table 2. Gas chromatography–mass spectrometry analysis of *Lavandula angustifolia* essential oil nanoemulsion

Peak	RT	Area sum %	Name	Formula	Molecular weight
1	6.855	15.1	2-Propanol, 1,1'-oxybis-	$C_6H_{14}O_3$	134.17
2	6.956	1.04	Eucalyptol	$C_{10}H_{18}O$	154.25
3	7.152	11.24	1-Propanol, 2-(2-hydroxypropoxy)-	$C_6H_{14}O_3$	134.17
4	7.235	9.46	1-Propanol, 2,2'-oxybis-	$C_6H_{14}O_3$	134.17
5	8.012	38.32	Linalool	$C_{10}H_{18}O$	154.25
6	8.73	9.78	(+)-2-Bornanone	$C_{10}H_{16}O$	152.23
7	8.902	1.29	Isoborneol	$C_{10}H_{18}O$	154.25
8	9.033	2.77	endo-Borneol	$C_{10}H_{18}O$	154.25
9	10.274	8.29	Linalyl acetate	$C_{12}H_{20}O_2$	196.29
10	12.784	2.7	Coumarin	$C_9H_6O_2$	146.1427

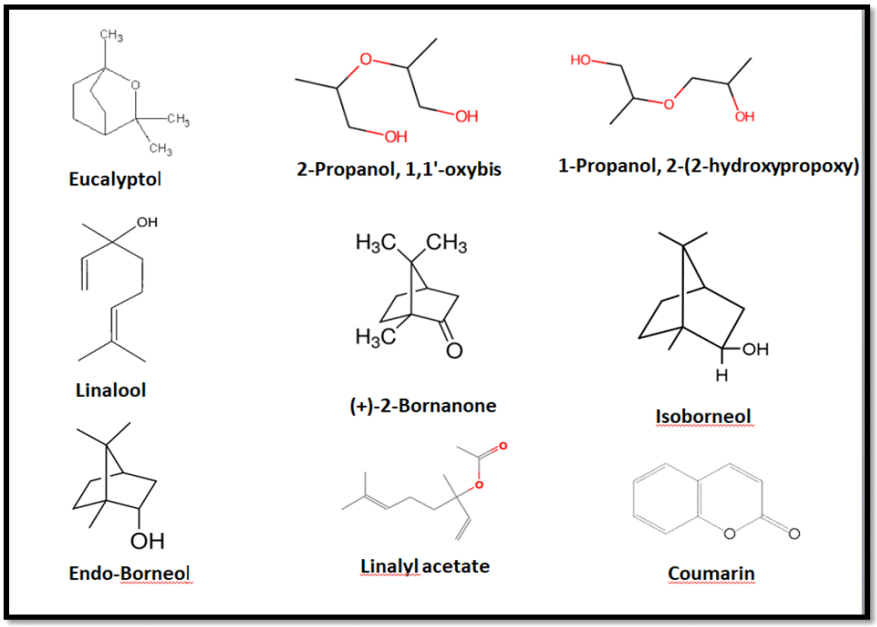

Fig. 1. Chemical structure of the common components in *Lavandula angustifolia* essential oil and its nanoemulsion

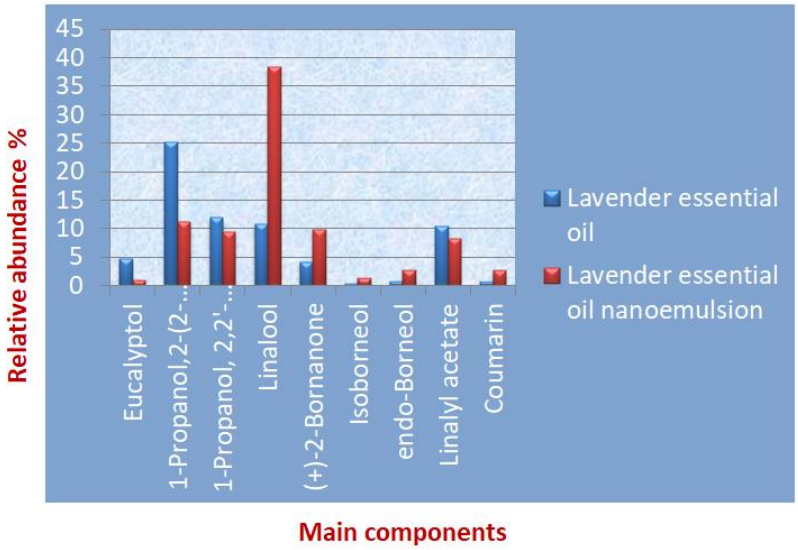

Fig. 2. Relative abundance of the main components in both *Lavandula angustifolia* essential oil and its nanoemulsion

2. Nanoemulsion characterization

2.1. Droplet size, zeta potential and morphology

Zeta potential, particle size, and polydispersity index (PDI) of the lavender EO nanoemulsion at room temperature were determined, with an average zeta potential of -15mv (Fig. 3), which is considered approximately neutral. Particle size and size distribution were determined by dynamic light scattering (DLS) and were found to be

12.7± 5.2nm (Fig. 4). The morphology of the nanoemulsion formulation was determined by TEM. From the TEM images of nanoemulsion (Fig. 5), it was observed that the particles were uniformly distributed, spherical in shape, and non-aggregated with a size of about 7nm. Even after being diluted 100 times with water, this nanometric size persisted for a considerable amount of time, demonstrating the system's compatibility with aqueous fluids.

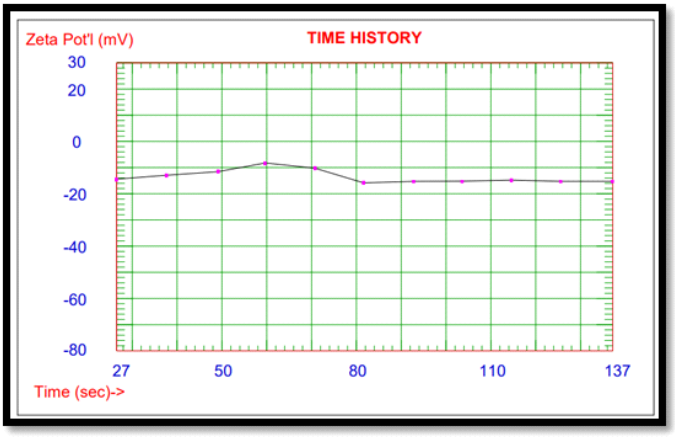

Fig. 3. Average zeta potential of *Lavandula angustifolia* oil nanoemulsion particles

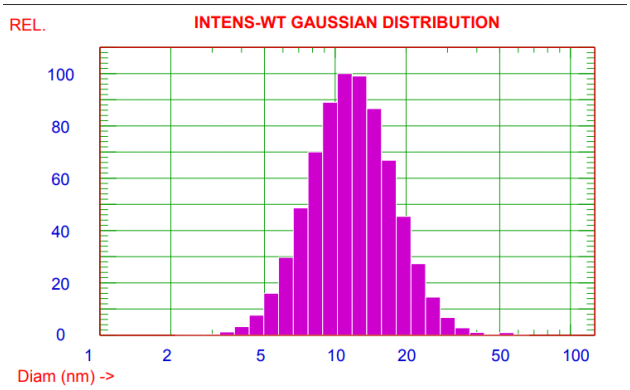

Fig. 4. Droplet size of *Lavandula angustifolia* oil nanoemulsion

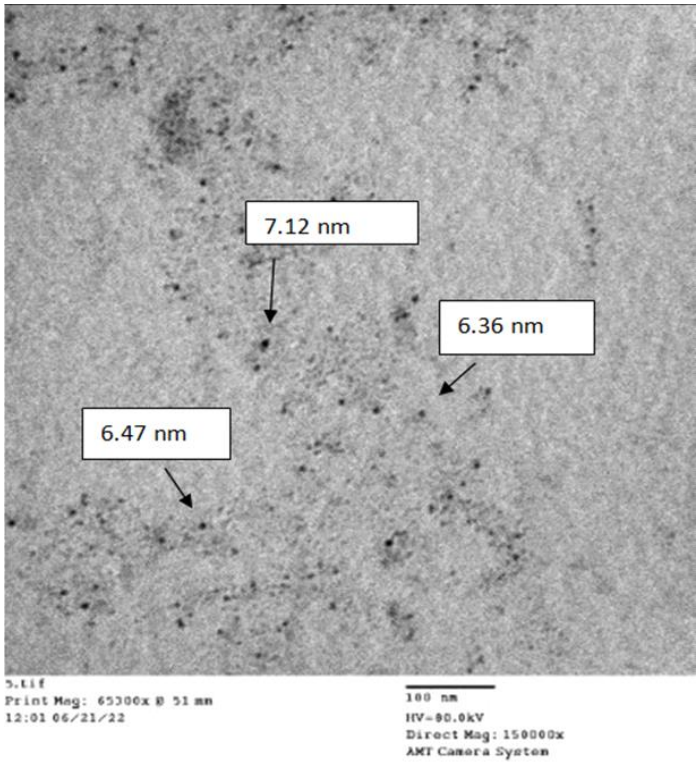

Fig. 5. Transmission electron microscopy (TEM) image of *Lavandula angustifolia* essential oil nanoemulsion indicating the shape and size of the particles

2.2 Physicochemical characterization

The undiluted nanoemulsion exhibited a pH value of 6.5, indicating a neutral to slightly acidic nature. Centrifugation of the nanoemulsion revealed no evidence of phase separation or turbidity, as depicted in Fig. (6). The viscosity of the lavender nanoemulsion was measured at 0.995 cPoise, signifying an effective mixing between the phases of the mixture. Thermal stability assessments were daily conducted during the initial week, followed by weekly evaluations for up to 3 months, and no separation or creaming features were observed in both instances. These comprehensive analyses attest to the favorable physicochemical properties and stability of the lavender nanoemulsion over an extended period.

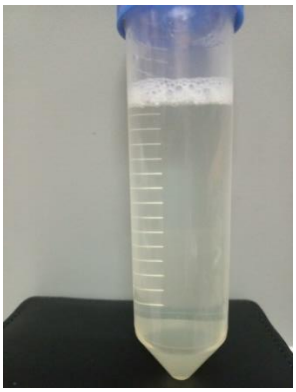

Fig. 6. The undiluted *Lavandula angustifolia* nanoemultion after centrifugation

3. Toxicological assay

Different concentrations of lavender oil and its nanoemulsion were applied to the late 3rd instar larvae of *Cx. pipiens*. The measured percentage mortalities are shown in Tables (3, 4). Data from Tables (1, 2) indicate that, as the concentration of any compound increased, the mortality percentages significantly ($P< 0.05$) increased. The LDP line program results declared that the median lethal concentration (LC_{50}) for the lavender oil and its nanoemulsion after 24 hours of the application were 133.9 and 468.3ppm, respectively (Figs. 7, 8). From the bioassay test, the *Lavandula angustifolia* essential oil and its nanoemulsion were effective as larvicides against the 3rd instar larvae, but contrary to expectations, lavender nanoemulsion was found to be less toxic than the bulk oil itself, as shown in Fig. (8). The toxicity of the lavender oil increased with time as a latened effect.

Table 3. Mortality percentages of 3rd instar *Culex pipiens* larvae exposed to different concentrations of the lavender oil *Lavandula angustifolia*

Conc.	Mortality percentage (%) of *Lavandula angustifolia* oil			
	6 hrs	12 hrs	24 hrs	48 hrs
200	5.0±0.0	8.3±1.7	16.7±1.7	18.3±1.7
400	23.3±3.3	33.3±3.3	53.3±4.4	53.3±4.4
500	40.0±5.0	48.3±3.3	60.0±5.8	66.7±3.3
600	58.3±3.3	63.3±4.4	76.7±3.3	86.7±4.4
800	88.3±9.3	91.7±8.3	96.7±3.3	98.3±1.7
Control	0.0±0.0	1.7±1.7	3.3±1.7	3.3±1.7
LC_{25}	131	121.5	107.22	104.98
LC_{50}	160.5	150.57	133.85	127.3
LC_{95}	263.2	254.2	229.9	203.76
Slope±SE	7.66±0.63	7.2±0.6	7±0.56	8.1±0.68

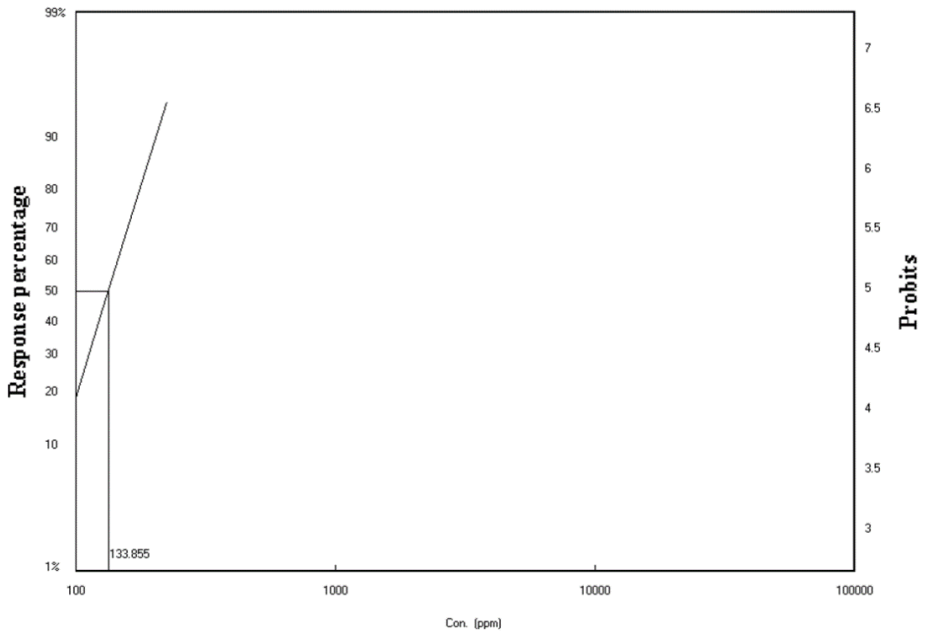

Fig. 7. Toxicity regression line of *Lavandula angustifolia* oil against 3rd instar larvae of *Culex pipiens*

Table 4. The percentages of mortality for 3rd instar *Culex pipiens* larvae exposed to different concentrations of the nanoemulsion of lavender oil *Lavandula angustifolia*

Conc.	**Percentage of mortality (%) for *Lavandula angustifolia* nanoemulsion oil**			
	6 hrs	**12 hrs**	**24 hrs**	**48 hrs**
200	3.3±1.7	3.3±1.7	20.0±2.9	50.0±2.9
400	15.0±5.0	20.0±2.9	35.0±2.9	55.0±5.8
500	20.0±5.0	28.3±1.7	40.0±5.8	60.0±7.6
600	30.0±5.0	40.0±7.6	65.0±7.6	80.0±5.8
800	50.0±5.0	80.0±7.6	80.0±7.6	95.0±2.9
Control	0.0±0.0	1.7±1.7	3.3±1.7	5.0±0.0
LC_{25}	512.49	432.4	274.079	122
LC_{50}	882.9	622.22	480.785	258
LC_{95}	3326.5	1511.9	1893.11	1603.9
Slope±SE	2.86±0.41	4.27±0.46	2.76±0.32	2.1±0.29

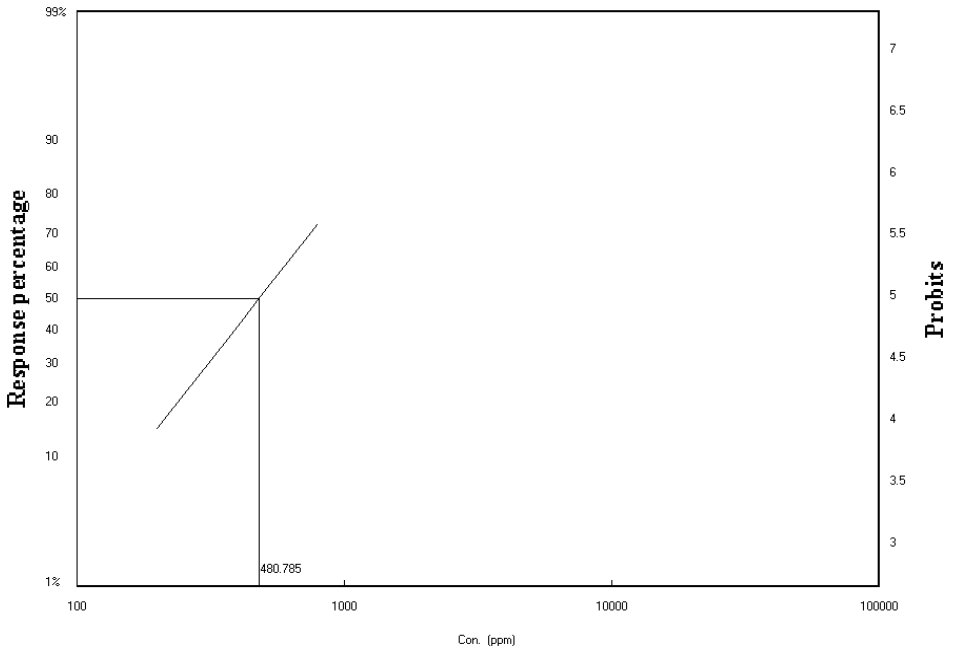

Fig. 8. Toxicity regression line of the *Lavandula angustifolia* oil nanoemulsion against 3rd instar larvae of *Culex pipiens*

4. Biological assay

Application of the LC_{25} concentration of lavender oil on the third larval instars of *Cx. Pipiens* caused larval, pupal, and pupal-adult intermediate malformations.

The lavender oil caused the treated larvae to be darker in color, as shown in Fig. (9). The pupae that resulted from the treated larvae suffered different malformations, with a smaller cephalothorax, losing its coma shape, and becoming dark in color, as shown in Fig. (10). While the adults failed to emerge from the pupal stage, as presented in Fig. (11).

On the other hand, the lavender oil nanoemulsion also showed larval-pupal, pupal, and pupal-adult intermediate malformations despite, the relatively accelerating growth time of the life cycle. Treatment with the lavender oil nanoemulsion caused some larvae to fail reaching the pupal stage completely, and they became stuck in its puparium, as shown in Fig. (9). The lavender oil nanoemulsion, also turned some of the treated pupae albino and lost their pigmentation, as presented in Fig. (10). Treated larvae in later stages failed to hatch from the pupa, as exhibited in Fig. (11).

The biology test indicated that, the lavender bulk oil reduced adult emergence by 29% compared in control and relatively reduced the percentage of males in the population, while the mean larval and pupal periods did not show a significant difference compared to the control. On the contrary, treatment with the lavender oil nanoemulsion increased the adult emergence by 12% compared to the control, while decreasing the

mean larval and pupal periods. The male percentage was slightly higher than the control, as shown in Table (5).

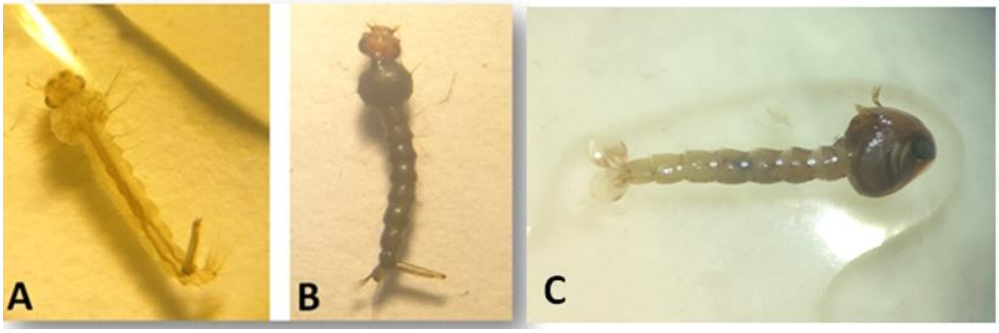

Fig. 9. *Culex pipiens* larvae showing: **(A)** Normal larva, **(B)** Treated larva with *Lavandula angustifolia* oil, **(C)** Larval-pupal intermediate treated with *Lavandula angustifolia* oil nanoemulsion

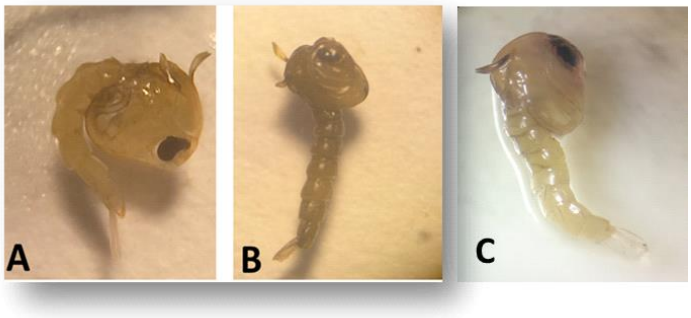

Fig. 10. *Culex pipiens* pupae showing: **(A)** Normal pupa, **(B)** Treated pupa with *Lavandula angustifolia* oil, and **(C)** Treated pupae with *Lavandula angustifolia*oil nanoemulsion

Table 5. Effect of LC_{50} of the *Lavandula angustifolia* essential oil and its nanoemulsion on the biological aspects of the 3rd larval instars of *Culex pipiens*

Conc. Ppm	% larval mortality	Mean larval period	% pupation	Mean pupal period	% Adult percentage	Sex ratio Male: Female
Control	17.83	10	82.17	6	81.4	1.4 : 1
Lavender oil	36.59	11	63.4	5.5	57.7	1.2 : 1
Lavender oil nanoemulsion	13	8.3	97.5	4.5	91.7	1.5:1

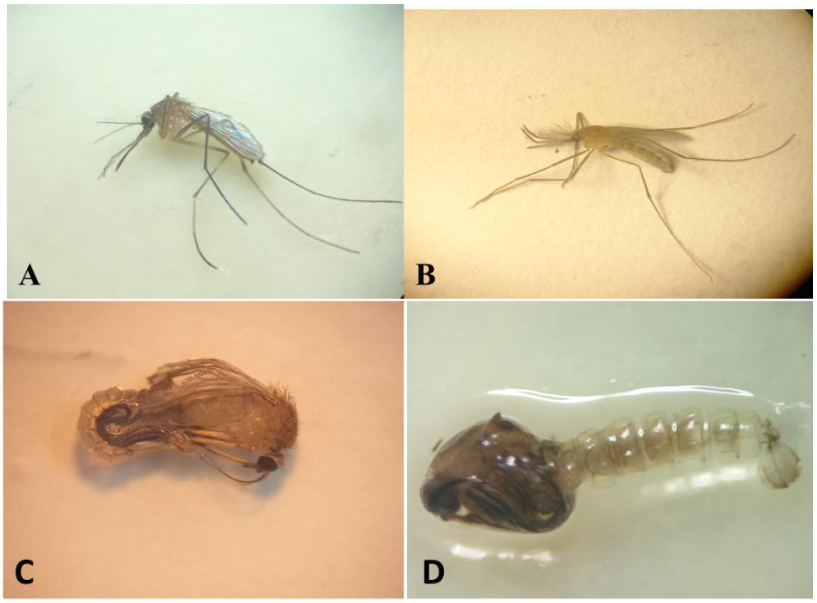

Fig. 11. *Culex pipiens* adults and pupal-adult intermediates showing: **(A)** Normal female adult, **(B)** Normal male adult, **(C)** Pupal-adult intermediate treated with *Lavandula angustifolia* bulk oil, and **(D)** Pupal-adult intermediate treated with *Lavandula angustifolia* oil nanoemulsion

DISCUSSION

The mechanism of action of lavender essential oil on the bodies of insects recorded disruption of the molecular events of morphogenesis and alteration in behavior **(Rattan, 2010)**. **Saad *et al.* (2019)** elucidated that lavender essential oil affects the nervous system. Pupal mortality might be due to multiple mechanisms of action, including the oil blocking the moulting of pupae into adults **(Suryanarayanamurthy *et al.*, 1997)**. Lavender nanoemulsion was also expected to give more promising results than the lavender oil itself owing to its good stability, rapid digestibility, protection against degradation, controlled release, and high capability of enhancing drugs' bioavailability **(Samie & Naser, 2020)**.

Lavandula angustifolia GC mass results widely ranged from test to another, our results indicated that the dipropylene glycol isomers were the major component of our *Lavandula angustifolia* essential oil, while linalool, linayl acetate and euclaptol were found in smaller percentages. The current findings coincide with those of **Erland and Mahmoud (2016)**; they found that *Lavandula angustifolia* EO contained about 10% of both linalool and linayl acetate. The GC mass major components of the *Lavandula angustifolia* EO were linalool and linayl acetate in percentages of 35.2 and 33.4%, respectively **(Bedini *et al.*, 2019)**. Moreover, **Dong *et al.* (2020)** postulated that, linalool and linayl acetate occupied the highest percentages of the *Lavandula angustifolia* EO GC mass results (19.71 and 26.61%, respectively). It was noticed that, *Lavandula*

angustifolia EO nanoemulsion GC mass results showed little percentages of both dipropylene glycol isomers and linayl acetate than the bulk oil itself, and higher percentage of linalool (38.32%). **Fouad *et al.* (2023)** disagreed with these results, they mentioned that *Lavandula officinalis* nanoemulsion linalool percentage decreased to 1.62m, while the main components were (+)-2-Bornanone and Eucalyptol in percentages of 36.36 and 33.48%, respectively.

Contrary to expectations, lavender essential oil showed higher toxicity levels than its nanoemulsion. The laboratory tests declared that the lavender essential oil was more effective against the 3rd larval instars of *Culex pipiens* than its nanoemulsion, which showed a latent effect. The LC_{50} values were 133.9 and 468.3ppm after 24 hours of treatment for the lavender essential oil and its nanoemulsion, respectively. This is consistent with what was achieved by **Pavala (2009)**, who said that the LC_{50} of *Lavandula angustifolia* essential oil against *Culex quinquefasciatus* larval instars after 24 hours of treatment was 121.6ppm. In accordance with this, **Giatropoulos *et al.* (2018)** proved that *Lavandula angustifolia* was effective against *Aedes albopictus* third to fourth larval instars with a LC_{50} of 142.9mg/ l. **El-Akhal *et al.* (2021)** also indicated that the LC_{50} effect of *Lavandula angustifolia* against *Culex pipiens* larvae after 24 hours of treatment is 140µg/ ml.

On the other hand, the lavender essential oil nanoemulsion did not show the expected effect as its relatives from the family Lamiaceae did. **Anjali *et al.* (2010)** applied both the bulk EO of permethrin and its nanoemulsion on the larvae of *Culex quinquefasciatus* and found that permethrin nanoemulsion was more toxic than the bulk oil, with LC50s of 0.117 and 0.715mg/ L, respectively, after 24 hours of exposure. **Sundararajan *et al.* (2018)** observed the larval mortality of the nanoemulsion *Ocimum basilicum* EO on the *Cx. quinquefasciatus* larvae, and they found that the LC_{50} value after 24 hours of exposure was 46.95ppm. As an extension of this, **Lucia *et al.* (2020)** exposed *Ae. aegypti* mosquito larvae to different formulations of Thymol EO nanoemulsion, and they found that Thymol EO nanoemulsion containing 100% thymol in the oil phase proved more efficient than the oil itself, with the lowest LC_{50} (11.1ppm). From another point of view, **Mohafrash *et al.* (2020)** proved that *Mentha spicata* EO nanoemulsion was more toxic against *Culex pipiens* larvae by 71.46% than the essential oil itself.

The biology test of the lavender essential oil showed more than an appearance of malformations, such as the blacking of larvae, pupae with smaller cephalothorax, and the failure of adults to emerge from the pupal stage. That is what was agreed upon by **Hanan (2013)**, who mentioned that lavender essential oil's mode of action as a promising alternative larvicide is to affect the insect's internal immature organs, causing malformations. **Khater (2021)** treated *Lucilia cuprina* (order Diptera) third larval instars with *Mentha longifolia* (family Lamiaceae). Her results showed that *Mentha longifolia* causes high levels of toxicity towards maggots and a significant change in the sex ratio in adults, in addition to appearing to cause adult malformation.

The lavender essential oil nanoemulsion also caused severe malformations in the different stages of *Culex pipiens*, including larval-pupal, pupal, and pupal-adult intermediate malformations. These results are consistent with those of **Gupta *et al.* (2022)**, who applied thyme oil nanoemulsion to the *A. stephensi*, *A. aegypti*, and *Cx. tritaeniorhynchus* larvae and found that the control was well-developed and segments, while in the treated larvae, the segments were separated as fragments. Furthermore, the bodies of the larvae exhibited constriction, blackening, and shrinkage in comparison to the control group.

CONCLUSION

In conclusion, the investigation into the mechanism of action of *Lavandula angustifolia* essential oil and its nanoemulsion against *Culex pipiens* larvae has provided valuable insights. The essential oil demonstrated higher toxicity levels than its nanoemulsion counterpart, contrary to initial expectations. The observed malformations in mosquito stages treated with both the essential oil and its nanoemulsion underscore the potential disruptive effects on insect development. While *Lavandula angustifolia* essential oil emerges as a promising natural insecticide, the nanoemulsion's unexpected lower efficacy suggests the need for further research to optimize its formulation and enhance insecticidal effectiveness. The findings contribute to our understanding of the complexities involved in utilizing botanical extracts for insect control. In the field of pest management, *Lavandula angustifolia* essential oil stands out as a potential candidate for eco-friendly alternatives. Future research should focus on refining nanoemulsion formulations to harness the full potential of *Lavandula angustifolia* essential oil against insect pests. This study contributes to the ongoing exploration of sustainable and effective strategies for insect control in public health and agriculture.

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