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Original article

Comparative study of three herbal formulations against dengue vectors *Aedes aegypti*

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ABSTRACT

The efficacy of three formulations (i.e., natural lavender crude, essential oil, and gel) extracted from *Lavender angustifolia* was tested against vectors of the epidemic dengue virus, *Aedesaegypti*, to evaluate their larvicidal activity effect. The ethanolic extract of the lavender crude was prepared using a rotary evaporator, while the other extracts, such as essential oil and gel, were obtained from iHerb, a supplier of medicinal herbs in the US. The mortality rate of larvae was evaluated 24 h after exposure. Larvicidal activity of the lavender crude was 91% mortality at 150 ppm, 94% for essential oil at a concentration of 3000 ppm, and 97% for lavender gel at a 1000 ppm. Natural lavender crude was one of the most promising extracts tested against *Ae.aegypti* larvae, with lethal concentrations at LC_{50} and LC_{90} of 76.4 and 174.5 ppm post-treatment. The essential oil had the least effect on mosquito larvae, with LC_{50} and LC_{90} reaching 1814.8 and 3381.9 ppm, respectively. The lavender gel was moderately effective against *Ae. aegypti* larvae, with LC_{50} and LC_{90} values reaching 416.3 and 987.7 ppm after exposure. The occurrence of morphological abnormalities in the larvae treated with the three compounds, in turn, resulted in an incomplete life cycle. Therefore, our results indicated that natural lavender crude displayed the highest larvicidal activity against larvae, followed by gel and essential oil. Thus, this study concluded that lavender crude is an effective, eco-friendly compound that can be used as an alternative to chemical products to control vector-borne epidemic diseases.

1. Introduction

Dengue is a mosquito-borne viral disease that was first observed in 1950 in Pakistan (Fazal et al., 2013). Over the past few years, the diseases has become one of the most common worldwide, killing or debilitating millions of humans annuallysa, particularly in tropical and subtropical areas (Dias and Moraes, 2014; Mendes et al., 2017; Tauil, 2014). These areas have a humid and warm climate, which promotes the spread of *Aedes aegypti* (Julio et al., 2009), being the optimal conditions for its reproduction(Mendes et al., 2017). The *Ae. aegypti* mosquito is mainly responsible for transmitting several viral epidemic diseases, including chikungunya, dengue, Zika, and yellow fever among humans (Alyahya et al., 2021; Fernandez et al., 2018). The high incidence of the aforementioned viral diseases, in general, and dengue fever, in particular, is a global concern due to the rapid spread of the primary vector *Ae. aegypti* (Viana and Ignotti, 2013). Vector control is a major challenge because they can lay their eggs in humid and semi-humid environments, survive for several months in harsh conditions, and hatch until favorable conditions (Alyahya et al., 2021). A common vector control method is using synthetic chemical products with larvicidal/insecticidal properties, such as pyrethroids and organophosphorus compounds (Alyahya et al., 2021; El-Akhal et al., 2021; Macoris et al., 2014; Mendes et al., 2017). These methods have largely succeeded in vector control in some African areas over the past years (Bhatt et al., 2015). Unfortunately, however, the frequent and extreme use of synthetic organic pesticides caused the development of *Ae. aegypti* resistance to synthetic larvicides/insecticides (Cadavid-Restrepo et al., 2012; Haddi et al., 2017). Furthermore, using pesticides leaves residues in the environment, causing water and soil pollution and exposing people's health and the environment to risks (Arias-Estévez et al., 2008; Hazarika et al., 2018). Therefore, there is a serious desire to find effective, eco-friendly alternative methods to control vectors and pathogens. More recently, natural plant extracts with larvicidal properties have received much attention as a promising

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alternative for vector control (Al-Rashidi et al., 2022; Al-Zahrani Mohamd et al., 2019; Alyaha et al., 2018; Alyahya et al., 2021; Barnawi et al., 2019; Bosly, 2022; Dias and Moraes, 2014; El-Akhal et al., 2021; Fazal et al., 2013; Mahyoub, 2018, 2021; Mendes et al., 2017; Pavela, 2009; Wangrawa et al., 2022).

Natural plant extracts are easily obtainable due to their prevalence and availability in several forms (such as crude, gels, and essential oils) and their lower cost than synthetic products (Fazal et al., 2013; Hazarika et al., 2018; Mahyoub, 2021). Since ancient times, certain essential oil extracts have been used as repellents for adult mosquitoes when applied to the skin in human communities (Alavez-Rosas et al., 2022; Ansari et al., 2000; El-Sheikh et al., 2016; Lee, 2018; Sutthanont et al., 2022). However, several recent studies have evaluated the effectiveness of essential oils as insecticides and larvicides (Alavez-Rosas et al., 2022; Ansari et al., 2000; Asadollahi et al., 2019; Barazandeh, 2002; Chantraine et al., 1998; Dias and Moraes, 2014; El-Akhal et al., 2021; Fazal et al., 2013; Fekadu et al., 2009; Fernandez et al., 2018; Hazarika et al., 2018; Lavor et al., 2012; Magalhães et al., 2010; Manimaran et al., 2012; Martins et al., 2019; Pavela, 2009; Smigielski et al., 2018; Sutthanont et al., 2022, 2019). Besides, the crude natural extracts derived from marine and terrestrial plants were tested as larvicidal (Al-Rashidi et al., 2022; Al-Hakimi et al., 2022; Alyaha et al., 2018; Alyahya et al., 2021; Barnawi et al., 2019; El-Sheikh et al., 2016; Mahyoub et al., 2016a; Mahyoub et al., 2016b; Mahyoub, 2019; Mahyoub et al., 2017). For instance, citronella and eucalyptus oils were widely used as insect repellents to protect the body from any bites (Batish et al., 2008; Kongkaew et al., 2011). The same essential oil extracted from the leaves of the eucalyptus plant was a good inhibitor against *Ae. aegypti* larvae (Fazal et al., 2013). Neem oil is also an effective larvicide as it inhibits the normal development of the larvae into a pupa (Fazal et al., 2013). A study conducted by Pugazhvendan and Elumali tested some oils against *An. Stephensi, Cx. Quinquefasciatus* and *Ae. aegypti* and indicated that eucalyptus, clove, and eucalyptus oils showed larvicidal activity against those mosquitoes (Pugazhvendan and Elumali, 2013). In a recent local study by Aljameeli, essential oils extracted from four plants – lemon, lavender, neem, and peppermint – were tested to evaluate larvicidal efficacy against *Ae. aegypti* larvae. This study showed that lemon oil had the highest larvicidal activity, followed by peppermint, lavender, and neem (Aljameeli, 2023). The larvicidal activity of oils extracted from the same plant differs according to the location and season (Fernandez et al., 2018; Pandey et al., 2013) due to the difference in their chemical composition (Mendes et al., 2017). Larvicides obtained from plant extracts have received wide attention from many studies due to the increasing demand for effective and eco-friendly alternatives such as (Al-Rashidi et al., 2022; Al-Zahrani Mohamd et al., 2019; Al-Hakimi et al., 2022; Aljameeli, 2023; Alyahya et al., 2021, 2021; Ansari et al., 2000, 2000; Barnawi et al., 2019; Bosly, 2022; El-Sheikh et al., 2016; Mahyoub et al., 2016b; Mahyoub, 2021, 2019, 2018, 2013; Mahyoub et al., 2017). However, the crude extracted from the *L. angustifolia* has not been assessed against the *Ae. aegypti* mosquito larvae. Therefore, this study aims to evaluate the larvicide efficacy of three formulations (lavender crude, lavender essential oil, and lavender gel) of *Lavender angustifolia* on *Ae. aegypti*, to develop effective and safe alternatives for vector control instead of other synthetic products.

2. Material and methods

2.1. Rearing the strain in the laboratory

The *Ae. aegypti* colony was established from eggs obtained from Dengue Research Unit, Biological sciences department, College of Science, KAU, and placed in 20 × 30 × 5-cm white enamel trays are relatively filled with tap water for hatching. After hatching eggs and the emergence of larvae, they were reared in controlled abiotic conditions (at 25 ± 2 °C, relative humidity about 70 ± 10%, and 14:10 h, light/dark cycle) without exposure to any pesticides or pathogens (Alyaha et al., 2018; Sutthanont et al., 2019). Larvae were fed daily with a mixture of rusks, milk, and yeast in a ratio of (1:1:1) and sometimes ground fish food (Goldfish flakes) according to the methods described in (Mahyoub, 2013) with some modifications. The feeding continued until the pupae emerged. After that, the pupae were transferred from the rearing containers into plastic jars (250 ml), kept in breeding cages (30 × 30 × 30 cm). After the adults emerged, they were fed a 10% glucose solution for 72 h before the blood feeding (Subramaniam et al., 2012). *Aedes* females were fed blood three times per week using the Hemotek membrane feeding system (Model: PS6220) to produce eggs. After two days of adequate blood feeding, the filter paper was placed into plastic containers with half-filled water and transferred to breeding cages to serve as oviposition substrates.

2.2. Preparation of the lavender angustifolia extract

Lavender angustifolia was obtained from a reliable medicinal herb supplier in Bangkok, Thailand. The plant (*L. angustifolia*) was finely ground using an electric grinder (Eidi et al., 2005). The powder was soaked in 70 % ethanol for three days with constant stirring to extract all the compounds in *L. angustifolia* into a solvent solution (ethanol). The soaked *lavender* was filtered using Whatman filter papers, and the filtered solution was kept for later use. This process was repeated thrice to ensure the extraction of all polar compounds. The filtered solution was evaporated to near dryness using a rotary evaporator to obtain a crude extract that was used to evaluate the biological activity of *lavender* against *Ae*. a*egypti* mosquito larvae.

2.3. Preparation of standard solutions for three formulations of L. angustifolia

Standard solutions of plant extract (*L. angustifolia*) were prepared in a dengue vector control unit, one ml of *lavender* extract (crude, stock solution) was dissolved in 98.5 ml of tap water, and 0.5 ml of Triton X-100 was added to lower the surface tension of the solution, it was considered as 1% stock Solution-I. Then one ml of standard Solution-I was taken and added to 99 ml of water to prepare Standard Solution-II. Five concentrations (1 ppm, 5 ppm, 10 ppm, 30 ppm, and 50 ppm) of stock solution-II (0.01%, 100 ppm) were prepared to test their effectiveness against *Ae. aegypti* mosquito larvae, however, these concentrations were insufficient to reach the median lethal concentration of LC_{50}; thus, the concentrations were increased to 50 ppm, 75 ppm, 100 ppm, 125 ppm, and 150 ppm. Three replicates were conducted simultaneously with 60 larvae per concentration separately.

Standard solutions of *L. angustifolia* essential oil (EO) were obtained from iHerb, a supplier of medicinal herbs in the United States, Korea, and Hong Kong. Standard solutions of *L.angustifolia* essential oil were prepared by adding 1 ml of oil (stock solution) to a 100 ml flask containing 99 ml of tap water; it was considered 1% (10^4 ppm) stock solution-I. Five conc. (1 ppm, 5 ppm, 10 ppm, 30 ppm, 50 ppm) of the prepared stock solution-II (0.01%, 100 ppm) were used to test their effectiveness against *Ae. aegypti* mosquito larvae. These concentrations also did not reach the median lethal conc. $LC_{50\%}$, so they were increased several times until they reached the appropriate conc. of 1000 ppm, 1500 ppm, 2000 ppm, 2500 ppm, and 3000 ppm. Three replicates were conducted for each concentration separately.

Standard solutions of *L. angustifolia* gel were prepared using the same procedure as *lavender* oil, except that 0.5 ml of Triton X-100 were added into a 100 ml flask containing 98.5 ml of tap water (1% stock solution-I). Also, five concentrations (1 ppm, 5 ppm, 10 ppm, 30 ppm, 50 ppm) of the prepared stock solution-II were used; However, these concentrations were not fatal. Therefore, these concentrations were increased several times to become 200 ppm, 400 ppm, 600 ppm, 800 ppm, and 1000 ppm. Three replicates were made simultaneously using 60 larvae for each concentration.

2.4. Larval toxicity test

A laboratory strain from the third and fourth-instars was used to test the larvicidal activity. A series of concentrations required for tests were prepared for each of the plant extract, oil, and gel. Twenty larvae were kept in a plastic container containing 100 ml of a specific concentration to evaluate the efficacy of killing mosquito larvae and determine the larval mortality rate (0–100 %) after 24 h post-treatment (PT). Then the same procedure was applied to the series of other concentrations. The test larvae were supplied with the necessary food to avoid starvation factor. Three replicates were made for all the tested concentrations of the three extracts, and the percentage of mortality was observed for each replicate after 24 h PT. The larva is considered dead if it shows no swimming movements after 24 h, even when touched lightly with a needle. The dead larvae were counted using special needles to prick them in the neck or the siphon, and the larvae were stationary without movement. The larvicidal activity was assessed according to the standard protocol established by the WHO with some modifications (WHO, 2005). Control larvae were prepared by the same procedure using tap water only. Treated and control larvae and pupae were kept in a plastic bottle containing 70% ethanol to study morphological abnormalities under a stereomicroscope OPTIKA SZN-T (18-65X magnification).

2.5. Statistical analysis

Statistics parameters such as means and standard deviation (SD) were calculated using Microsoft Excel software (Office 2016). Mortality proportions were calculated using Microsoft Excel following equation (1), and Abbott's formula (Abbott, 1925) did not use to correct mortality because there were no mortalities in the control samples. Thus, the study did not need to make such corrections. Lethal concentrations at 50% (LC_{50}) and 90 % mortalities (LC_{90}) with upper and lower limits were estimated through a logistic regression model using probit analysis at confidence intervals of P less than 0.05 according to (Finney, 1971) by LDP line software. LDP Line is software used to illustrate the relationship in terms of stimulus and response in biological studies. The tested formulations' relative efficacies (RE) were calculated according to LC_{50} and LC_{90} using LDP Line software.

$$\text{Mortality rate}\,(\%) = \frac{\text{dead larvae}}{\text{total number of larvae}} \times 100 \qquad (1)$$

3. Results

3.1. Mortality

Three formulations of *L. angustifolia* extract, i.e., plant crude, essential oil, and gel at different concentrations of less/more than 0.1%, were tested and demonstrated efficacy against *Ae. aegypti* larvae within 24 h unevenly depending on the concentrations and type of tested formulations used on mosquito larvae. When exposed to these concentrations, the mortality rate for *Ae. aegypti* larvae were 100%, 24 h PT. However, no mortality was seen among control larvae.

The crude extracts displayed larvicidal activity against larvae 24 h after treatment, where the mortality ranged between 4 and 9 larvae at a concentration of 50 ppm with an average ± S.D. of 6 ± 2.6 (Table 1), representing 30% of the total larvae used at this concentration (Table 1). At 75 ppm, the mortality varied between 6 and 10 larvae, with an average of 9 ± 2.1, representing 42% of the total larvae (Fig. 1). At 100 ppm, it ranged from 12 to 15, with an average of 14 ± 1.5, representing 67% of the total larvae. At 125 ppm, mortality varied between 13 and 16 with a mean of 15 ± 1.5, accounting for around 74% of the total larvae used at this concentration. The 150 ppm concentration was more toxic against larvae, with mortality ranging from 17 to 19 larvae with an average of 19 ± 1.2, causing about 91 % mortality of larvae (Table 1). Table 1 shows that mortality rates of larvae increase gradually with the increasing concentrations (Fig. 1).

The essential oil) *L. angustifolia,* EO (exhibited good larvicidal activity against larvae of *Ae.aegypti* at high concentrations, the mortality ranged from 1 to 4 larvae at a concentration of 1000 ppm with an average of 3 ± 1.5, accounting for around 12% of the total larvae used at this concentration. Mortality ranged between 7 and 9 at a concentration of 1500 ppm with an average of 8 ± 1, representing about 40% of the total tested larvae. At 2000 ppm, the larval mortality varied between 8 and 11 larvae with a mean of 10 ± 1.5, causing about 49% of mosquito larval mortality. At 2500 ppm, mortality ranged from 12 to 15 larvae and averaged 14 ± 1.5, representing about 67% of the total tested larvae. Among the different concentrations of the essential oil, 3000 ppm was the highest effective on mosquito larvae, and mortality ranged between 18 and 19 larvae, with an average of 19 ± 0.6 representing about 94% of the total tested larvae. In contrast, concentrations < 1000 ppm were less effective against *Ae. aegypti* mosquito larvae (Fig. 2).

L. angustifolia gel showed moderate larval efficacy against larvae of *Ae.aegypti* compared to a plant extract and essential oil. At 200 ppm, the larval mortality varied between 2 and 5 larvae, with a mean of 4 ± 1.5 representing 19% of the total larvae. While at a concentration of 400 ppm, the mortality ranged between 6 and 9 larvae, with a mean of 8 ± 1.5 representing 39% of the total larvae (Table 1). Mortality at 600 ppm ranged from 13 to 14 larvae with a mean of 14 ± 1, which larviciding of 69% of *Ae. aegypti*. At 800 ppm, mortality ranged from 15 to 17 larvae with a mean of 16 ± 1 representing 80% of the total larvae (Fig. 3). The 1000 ppm concentration was more toxic against larvae, with mortality ranging from 18 to 20 larvae with a mean 19 ± 1.2, causing the death of 97 % of *Ae. aegypti* larvae (Table 1).

3.2. Larvicidal activity of extracts

The larvicidal effects of three formulations extracted from a medicinal plant of *L. angustifolia* were examined against *Ae. Aegypti* larvae. The results showed that the first formulation, plant extract, displayed LC_{50} and LC_{90} of 76.4 and 174.5 ppm, respectively, with 95% confidence lower interval of 69.5 and 152.9 ppm and upper interval of 82.6 and 210.99 ppm, respectively for larvicidal activity (Tables 2 & 3; Fig. 4). Whereas the second formulation, essential oil displayed LC_{50} and LC_{90} of 1814.8 and 3381.9 ppm, respectively with 95% confidence lower interval of 1363.5 and 3245.1 ppm and upper interval of 2295 and 6636.9 ppm, respectively for larvicidal activity against *Ae. aegypti* (L.) (Fig. 5). The third formulation, gel, displayed $LC_{50,}$ and LC_{90} of gel were 416.3 and 987.7 ppm, respectively, with 95% confidence lower intervals of 281.1 and 846.3 and upper intervals of 534.9 and 1867.7 ppm, respectively for larvicidal activity on *Ae. aegypti* larvae (Fig. 6). Thus, plant extract (*L. angustifolia*) exhibited the lowest LC_{50} and LC_{90} values to be the most effective compounds on *A. aegypti* (L.) compared

Table 1
Larvicidal efficacy of *L. angustifolia* extract, *L. angustifolia* essential oil (EO), and *L. angustifolia* gel against *Ae. aegypti* larvae after 24 h PT.

Formulations	Conc. (ppm)	Statistical analysis of larvicidal activity (LA)			
		Mortality means ± S.D.	Min-Max	Mortality %	Control
Plant extract	50	6 ± 2.6	4–9	30	00 ± 00
	75	9 ± 2.1	6–10	42	00 ± 00
	100	14 ± 1.5	12–15	67	00 ± 00
	125	15 ± 1.5	13–16	74	00 ± 00
	150	19 ± 1.2	17–19	91	00 ± 00
Essential oil	1000	3 ± 1.5	1–4	12	00 ± 00
	1500	8 ± 1	7–9	40	00 ± 00
	2000	10 ± 1.5	8–11	49	00 ± 00
	2500	14 ± 1.5	12–15	67	00 ± 00
	3000	19 ± 0.6	18–19	94	00 ± 00
Gel	200	4 ± 1.5	2–5	19	00 ± 00
	400	8 ± 1.5	6–9	39	00 ± 00
	600	14 ± 1	13–14	69	00 ± 00
	800	16 ± 1	15–17	80	00 ± 00
	1000	19 ± 1.2	18–20	97	00 ± 00

SD: standard deviation; EO: Essential oil.

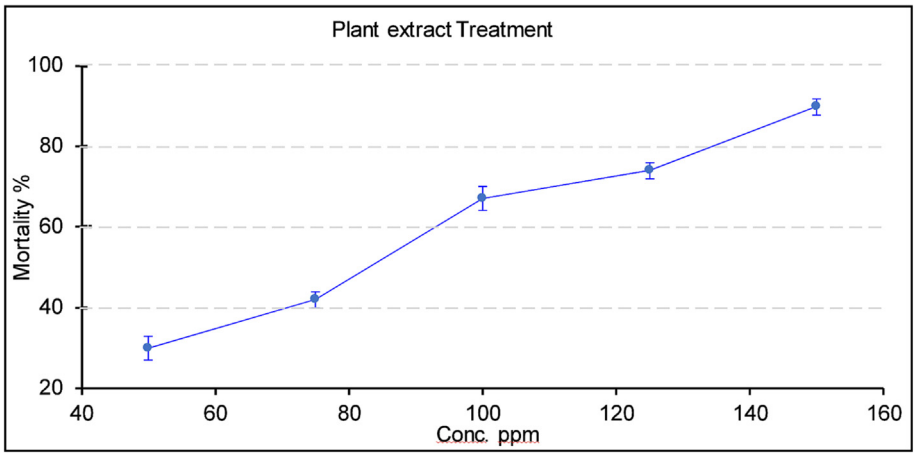

Fig. 1. Mortality rate of *Ae.aegypti* larvae after exposure to five different concentrations of plant extract (*L. angustifolia*) for 24 h of treatment.

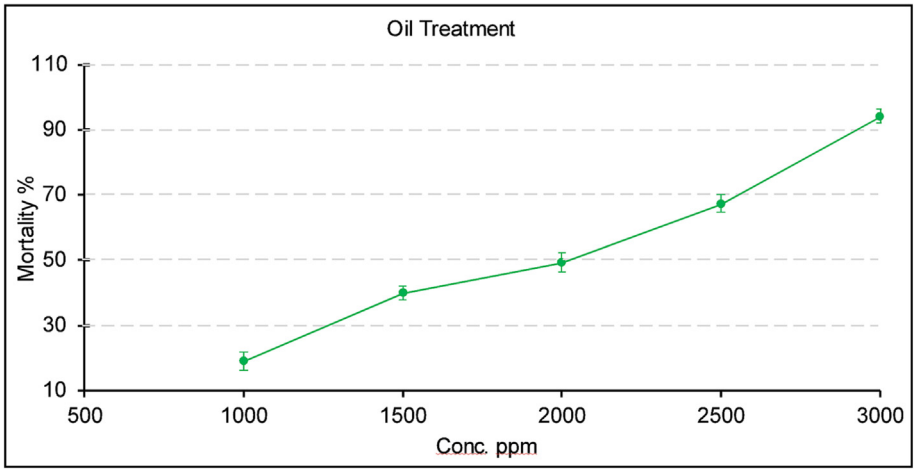

Fig. 2. Mortality rate of *Ae. aegypti* larvae after exposure to five different concentrations of essential oil extracted from *L. angustifolia* for 24 h of treatment.

to the other tested compounds. However, essential oil showed the least larvicidal activity among the other compounds.

The relative efficacies (RE_{50}) for these three formulations of plant extracts, essential oil, and gel according to LT_{50} values were 1, 23.8, and 5.5 times, respectively, while the RE_{90} according to LT_{90} values was 1, 19.4, and 5.7, respectively. The Chi-square, slope, and significance (*P*) were presented for all tested formulations (Tables 2 and 3).

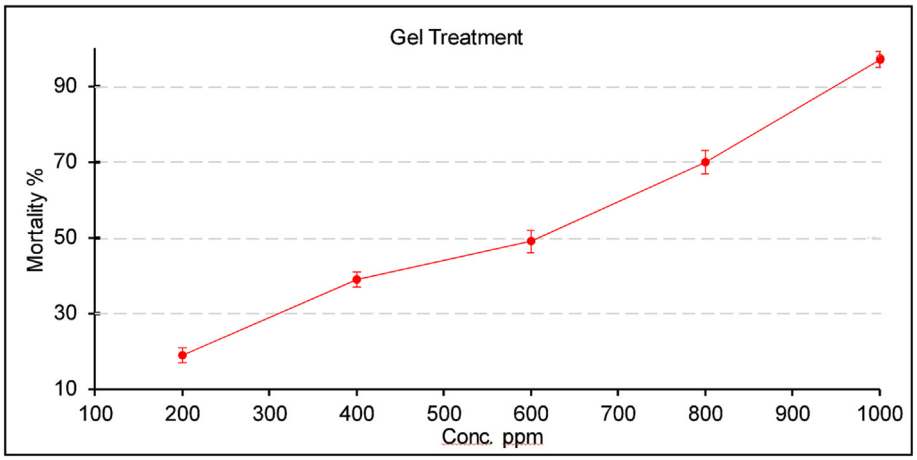

Fig. 3. Mortality rate of *Ae. aegypti* larvae after exposure to five different concentrations of gel extracted from *L. angustifolia* for 24 h of treatment.

Table 2
The larvicidal effects of *L. angustifolia* extract, *L. angustifolia* essential oil, and *L. angustifolia* gel on *Ae. aegypti* larvae after 24 h PT.

Formulations	LC_{50} (ppm)	LCI-UCI	LC_{90} (ppm)	LCI-UCI	Chi^2	slope	*p*-value
Plant extract	76.4	(69.5–82.6)	174.5	(152.9–210.99)	5.6	3.6 ± 0.4	$P < 0.1$
Essential oil	1814.8	(1363.5–2295)	3381.9	(3245.1–6636.9)	13.98	4.7 ± 0.4	$P < 0.003$
Gel	416.3	(281.1–534.9)	987.7	(846.34–1867.7)	11.3	3.4 ± 0.3	$P < 0.01$

LC50: 50% lethal concentration; LC90: 90% lethal concentration, LCI: lower Confidence Interval; UCI: Upper Confidence Interval.

Table 3
LC_{50} and LC_{90} of the larvicidal activity of *L. angustifolia*extract, *L. angustifolia* essential oil, and *L. angustifolia* gel on *Ae.aegypti* larvae after 24 h PT.

Formulations	LC_{50} (ppm) (95% CI)	RE_{50} (95% CI)	LC_{90} (ppm) (95% CI)	RE_{90} (95% CI)
Plant extract	76.4 (69.54–82.64)	1	174.5 (152.9–210.99)	1
Essential oil	1814.8 (1363.46–2295)	23.8	3381.9 (3245.1–6636.9)	19.4
Gel	416.3 (281.1–534.9)	5.5	987.7 (846.3–1867.7)	5.7

RR_{50}: 50% relative efficacy; 95% CI: 95% confidence interval; RR_{90}: 90% relative efficacy.

3.3. Morphological abnormalities of Ae.aegyptilarval

The exposure of *Ae. aegypti* mosquito larvae with plant extracts, oil, and gel extracted from the medicinal *L. angustifolia* plant killed many of these larvae. The results also showed the occurrence of many abnormalities in the morphological appearance of both live and dead larvae, in addition to hindering their growth and development from one stage to the next. Morphological effects that occurred over the whole body of larvae and pupae and were characterized under a stereomicroscope OPTIKA SZN-T (18-65X magnification) include shrinkage of the larval body rings, especially the abdominal rings, larval neck elongation, head deformities, thorax deformities, abdomen deformities, siphon swelling, siphon deformities, the disappearance of head appendages, partial or complete pigmentation of the larval body, larvae fail to develop into a pupa, an intermediate stage, the disappearance of the body antennae, larval cuticle abnormalities, incompletely developed pupa stages, and appearance of an unpigmented pupa called an "albino pupa" Fig. 7. In contrast, untreated control larvae showed a normal appearance with typical structures consisting of a head, thorax, abdomen, and all other well-developed organs. Moreover, some naturally developed into typical pupae (Fig. 8).

4. Discussion

The current study examined the larvicidal efficacy of three formulations extracted from plants at different concentrations against *Ae. aegypti* larvae to find effective and environmentally safe alternatives for vector control. Especially after recent studies revealed that prolonged, excessive, and unregulated use of pesticides has serious negative repercussions on the environment (Zhang et al., 2015) and makes mosquitoes acquire resistance to insecticides, and thus becomes a major obstacle to vector control such as *Ae. aegypti* and other mosquito species, causing the prevalence of vector-borne diseases and the inability to control them (Georghiou and Taylor, 1986; Smith et al., 2016). Recently, studies have begun to focus seriously on natural products in biological control because of their insecticidal properties that qualify them to be used as alternative insecticides to other synthetic products. They also do not leave hazardous effects on the environment, and their accessibility and lower cost compared to synthetic products make them an ideal and environmentally safe alternative to mosquito control (Al-Rashidi et al., 2022; Alyaha et al., 2018; Hazarika et al., 2018). In this study, we used three formulations extracted from the medicinal lavender plant (crude ethanolic plant extract, essential oil, and lavender gel). The results differed among them and were promising and encouraging as environmentally friendly alternatives. The medicinal lavender extract (*L. angustifolia*) showed the mortality rate of mosquito larvae exceeded 90% at a concentration of 150 ppm within 24 h of treatment.

Lavender *angustifolia* is an aromatic plant with more than 100 compounds; however, it contains about 65% linalool and linalyl acetate, widely used in the perfume industry (Barazandeh, 2002; El-Akhal et al., 2021; Reverchon et al., 1995). However, some studies differed in determining the proportions of these compounds in

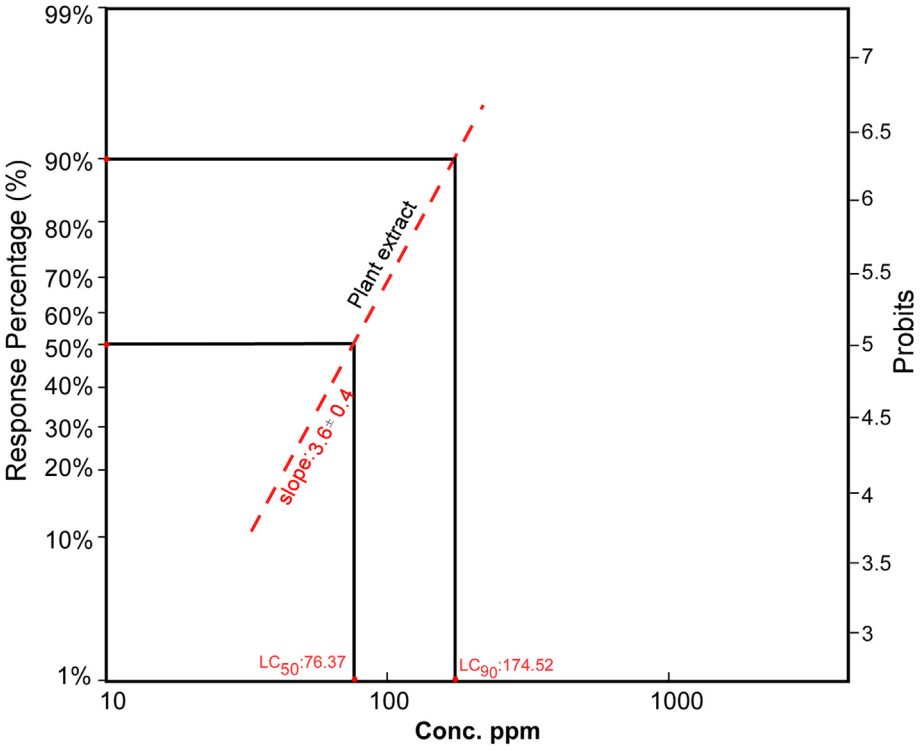

Fig. 4. Toxicity-Probit line (Ldp) of plant extract (*L. angustifolia*) on *Ae. aegypti* larvae after 24 h PT.

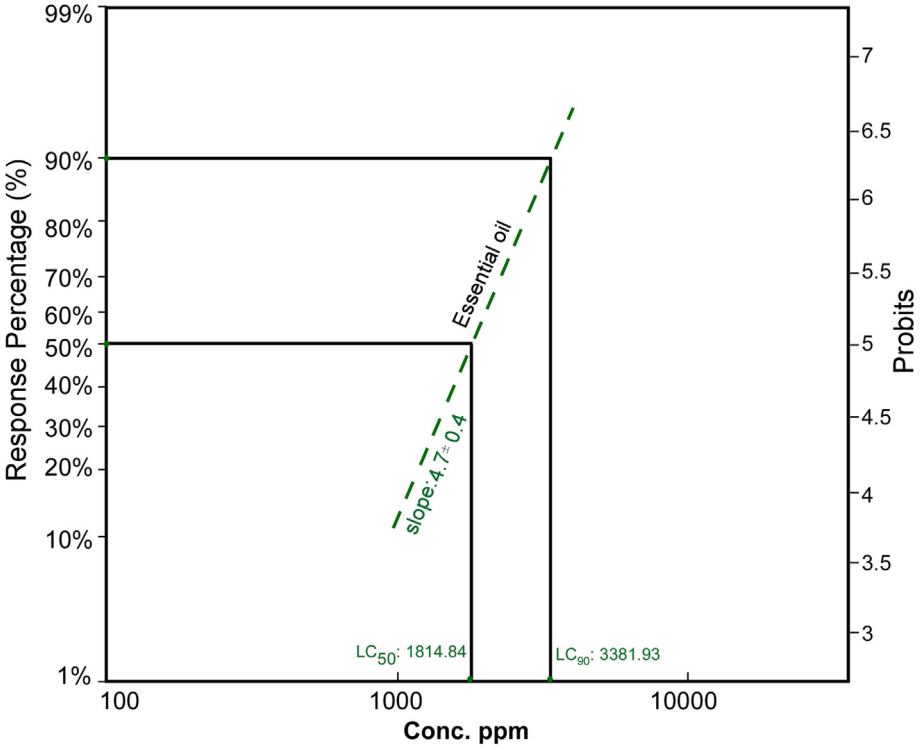

Fig. 5. Toxicity-Probit line (Ldp) of essential oil extract (*L. angustifolia)* on *Ae. aegypti* larvae after 24 h PT.

the lavender plant. For instance, Smigielski et al. documented that the concentration of linalool ranged between 26.50 and 34.70%, while the concentration of linalyl acetate ranged between 19.70 and 23.4% in the plant *L. angustifolia* (Smigielski et al., 2018). Another investigation conducted in India by (Babu et al., 2016) showed that the proportion of linalool and linalyl acetate was 36.10% and 19.90% in *L. angustifolia*, respectively. Several authors indicated that the differences in the levels of chemical compounds in this plant might be attributed to many factors such as climatic conditions, geographical, physiological age and genotype of the

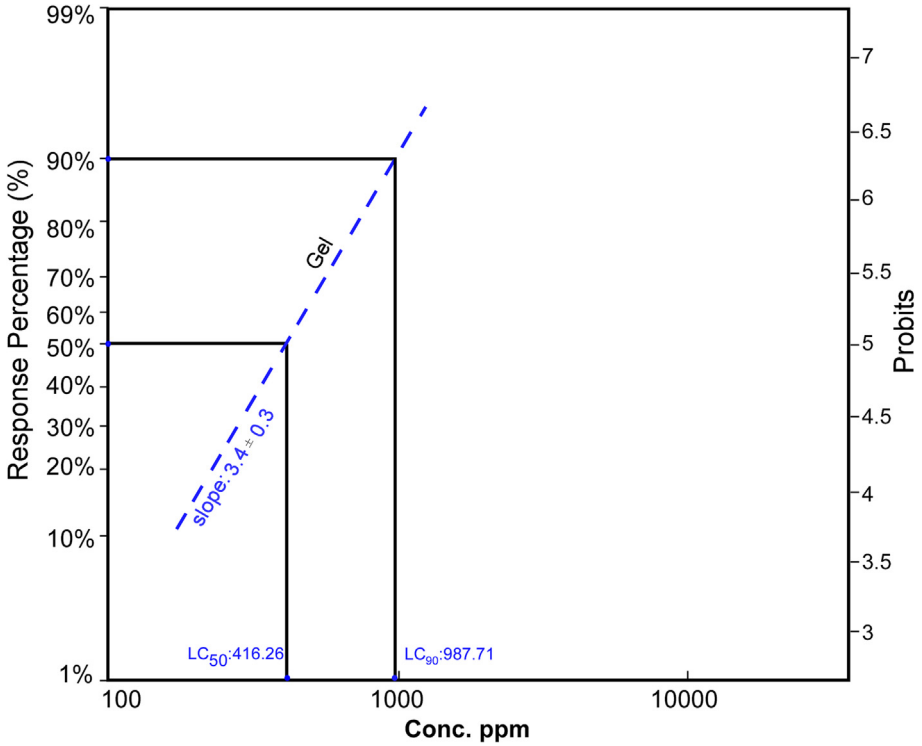

Fig. 6. Toxicity-Probit line (Ldp) of gel) *L. angustifolia*) on *Ae. aegypti* larvae after 24 h PT.

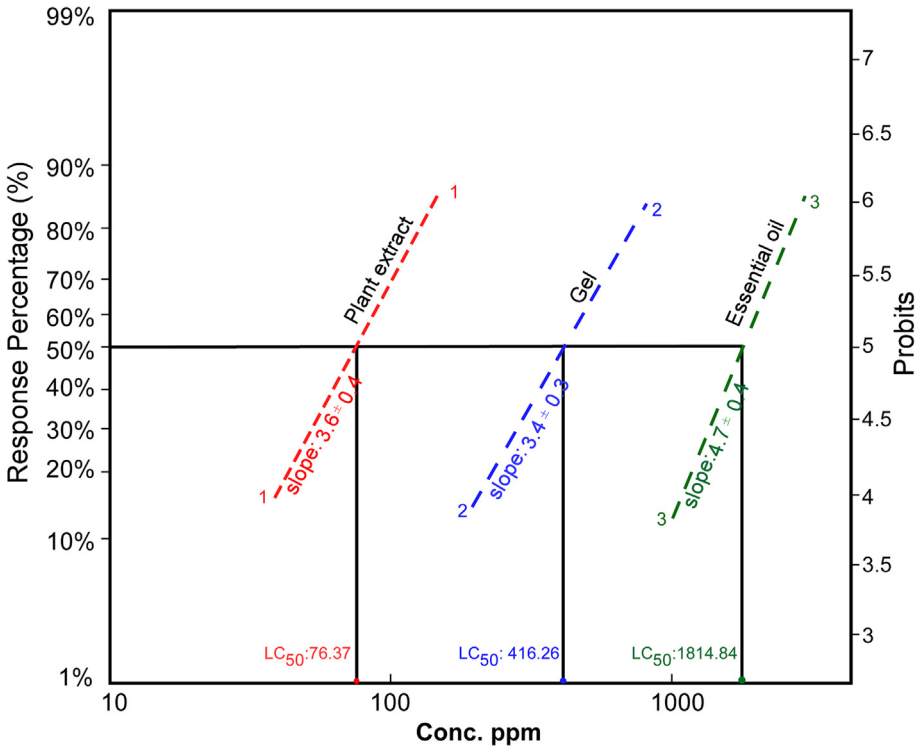

Fig. 7. Toxicity-Probit line (Ldp) of plant extract, essential oil, and gel of *L. angustifolia* on *Ae. aegypti* larvae after 24 h PT.

plant, location, and terrain characteristics, in addition to the harvest season of *L. angustifolia* (Bousta and Farah, 2020; Martins et al., 2019). In general, these volatile chemical compounds in lavender plant extract may be the major reason for the high mortality rate of *Ae. aegypti* larvae that exceeded more than 90% at a concentration of 150 ppm due to their toxic effects on the larvae, indicating that they can be used as safe alternatives in vector control rather than other synthetic products. El-Akhal et al. tested an extract of *L. angustifolia* growing in Morocco against *Cx. pipiens* mosquitoes. They reported that volatile compounds found in

Fig. 8. Microscopic images of larvae and pupae of *Ae. aegypti* treated with lavender extracts. Control (a-d) not showing morphological changes (normal larva and pupa). Treatment with extract at different concentrations ppm after 24 h PT (e-p). Morphological abnormalities of the larvae and pupae of the *Ae. aegypti* mosquito: **e-f:** (2) Larval neck elongation. **g**: (6) Pigmentation, (7) Appendages. **h**: (3) Deformed head, (5) Breakup abdomen. **i**: (9) Antennae. **j**: (4) Deformed thorax, (8) Deformed siphon, (10) Deformed cuticles. **k**: (6) Pigmentation. **l**: (11) shrinkage of the larvae. **m**: (3) Deformed head, (4) Deformed thorax, (9) Antennae, (11) shrinkage of the larvae, (13) Failed to develop into a pupa. **n**: (12 An intermediate stage, (14) Albino pupa. **o**: An intermediate stage, (14) Albino pupa. **p**: (13) Failed to develop into a pupa, (14) Albino pupa.

L. angustifolia showed toxic effects against *Cx. pipiens* larvae (El-Akhal et al., 2021). In addition, Tabari et al. indicated that the linalool compound has a strong toxic effect on mosquito larvae and eggs of *Cx. pipiens* mosquitoes upon exposure to a LC_{50} of 14.87 and 1.27 ppm, respectively (Tabari et al., 2017). In a similar study, Fujiwara et al. reported that linalool, the main constituent in lavender extract, had larvicidal activity after 24 h against *Ae.aegypti* larvae, in addition to detecting some morphological abnormalities in the larvae bodies that were exposed to sub-lethal concentrations of lavender extract that included elongation and abdominal curvature (Fujiwara et al., 2017). This is also consistent with our findings, where the exposure of *Ae. aegypti* larvae to lavender extract for 24 h caused the mortality of more than 90% of the larvae. Many morphological deformities occurred in the body of larvae and pupae, including neck elongation, head deformity, thorax deformity, abdominal breakup, larval body shrinkage, pigmentation, failure to develop into a pupa, Albino pupa, and swelling and siphon deformity. Research studies conducted by (Al-Rashidi et al., 2022; Alyaha et al., 2018; Alyahya et al., 2021) revealed the toxic effects of plant extracts when used

against *Ae. aegypti* larvae as well as associated morphological abnormalities.

In the current study, lavender extract recorded LC_{50} and LC_{90} values of 76.4 and 174.5 ppm, respectively, with LCI and UCI between 69.5 and 82.6 for LC_{50} and 152.9 and 210.99 ppm for LC_{90} against *Ae. aegypti* larvae after 24 h for larvicidal activity. El-Akhal et al. observed that the LC_{50} and LC_{90} values were 140 ppm and 450 ppm, respectively (El-Akhal et al., 2021). Pavela evaluated larvicidal activity on larvae of *Cx. Quinquefasciatus* of the plant derived from *L. angustifolia.* The author reported that the LC_{50} and LC_{90} were 121.60 and 337.20 ppm, respectively (Pavela, 2009). A study showed that the LC_{50} and LC_{90} for *L. angustifolia* against *Cx. quinquefasciatus* were 121.60 and 337.20 ppm, respectively (Pavela, 2009). *Lavender angustifolia* extract showed LC_{50} and LC_{90} values of 301.11 and 1437. 63 ppm, respectively, against the larvae of *Ae. aegypti* (Bosly, 2022). Several authors have developed criteria for evaluating the efficacy of mosquito larvicides based on plant extracts (Chantraine et al., 1998; Fekadu et al., 2009; Magalhães et al., 2010). For instance, Kiran et al. reported that plant extract compounds with LC_{50} less than 100 ppm showed a significant larvicidal effect (Kiran et al., 2006). Other authors reported that natural product compounds that showed an LC_{50} of less than 50 ppm were considered active, between 50 and 100 ppm were considered moderate, 100–750 ppm were considered effective, and more than 750 ppm were considered inactive (Komalamisra et al., 2005). The difference in LC_{50} and LC_{90} values in similar studies of the same plant extract and standard solutions may be due to environmental, developmental, experimental, or other factors (Al-Hakimi et al., 2022; Barnawi et al., 2019; Hazarika et al., 2018).

Lavender oil exhibited a 94% larvicidal activity on *Ae. aegypti* larvae at a concentration of 3000 ppm at 24 h PT. E. Oil extracted from *L. angustifolia* recorded the LC_{50} and LC_{90} values of 1814.8 and 3381.9 ppm, respectively, with LCI and UCI between 1363.5 and 2295 for LC_{50} and 3245.1 and 6636.9 ppm for LC_{90} against *Ae. aegypti* larvae after 24 h PT. Bosley tested oil derived from lavender against the larvae of *Ae. aegypti* and showed a mortality percentage of 87.2% at a concentration of 1,000 ppm (Bosly, 2022). Similar observations have been documented by Manimaran et al. with *L. angustifolia* oil, with a mortality rate of 68% recorded at a concentration of 1000 ppm against *Cx. quinquefasciatus* 24 h PT (Manimaran et al., 2012). However, our results showed considerably lower values than previous studies mentioned above, probably due to seasonal changes and different plant locations from one region to another, which may affect plant metabolism and, consequently, the difference in the chemical composition of oils. Morais stated that the chemical compounds of essential oils might change over the seasons (de Morais, 2009). Ozcan and Chalchat tested laurel leaves collected from seven different regions in Turkey and found variations in the chemical composition of each region's essential oils (Özcan and Chalchat, 2005). Specifically, the spatial and seasonal variations of the plant cause a change in the ratios of linalool content in the extracted essential oil, which has larvicidal activity against *Ae.aegypti* (Fernandez et al., 2018; Pandey et al., 2013). On the other hand, a study tested a wide range of plant oils as repellents for *Anopheles* mosquitoes, and the results showed that lavender oil was a good repellent and could be used for personal protection against *An.* mosquitoes (Asadollahi et al., 2019). In another study, essential oils extracted from lavender and rosemary showed effective repellent activity against adult *Cx. pipiens* mosquitoes (Choi et al., 2002).

Lavender gel exhibited 97% larvicidal activity against *Ae. aegypti* larvae at a concentration of 1000 ppm after 24 h PT. The gel extracted from *L. angustifolia* recorded the LC_{50} and LC_{90} values of 416.3 and 987.7 ppm, respectively, with LCI and UCI between 281.1 and 534.9 for LC_{50} and 846.34 and 1867.7 ppm for LC_{90} against *Ae. aegypti* larvae after 24 h PT.

Our findings for the three formulations derived from the lavender plant, i.e., plant extract (crude), essential oil, and gel, showed larvicidal activity on *Ae. aegypti* larvae; however, it was found that crude lavender extract displayed the highest efficacy on *Ae. aegypti* larvae with LC_{50} 76.4 ppm, followed by lavender gel with LC_{50} 416.3 ppm, and finally, essential oil LC_{50} 1814.8 ppm, indicating the different percentages of Linalool content in each formulation. Overall, the results of our study indicated that the crude plant extract was promising for use as an effective and low-cost alternative to control the larvae of the *Ae. aegypti* mosquito that transmits dengue fever.

5. Conclusion

The current study tested the larvicidal efficacy of three formulations (lavender crude, essential oil, and gel) extracted from *L. angustifolia* against *Ae. aegypti* larvae, to develop effective and safe alternatives for use in vector control programs instead of other synthetic products. The results showed that all three formulations had remarkably larvicidal properties, as indicated by larval mortality rates and morphological abnormalities of their bodies. The crude lavender extract recorded 91% mortality at low concentrations of 150 ppm, while lavender gel showed 97% at 1000 ppm and lavender essential oil showed 94% at 3000 ppm. Some morphological abnormalities in the larvae bodies exposed to different concentrations of the three lavender extracts included elongation, the curvature of the abdomen, failure to develop into a pupa, larvae shrinkage, pigmentation, and albino pupa. Therefore, the study results indicated that lavender extracts are promising and encouraging to be used as effective and less costly alternatives to control *Ae. aegypti* larvae; in particular, the crude extract showed the strongest larvicidal activity at low concentrations, followed by lavender gel and then lavender oil.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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