BIODIVERSITAS
Volume 25, Number 3, March 2024
Pages: 990-997

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250311

Effectiveness of *Foeniculum vulgare* leaf essential oil nanoemulsion as a natural insecticide against *Aedes aegypti* larvae and mosquitoes

ARUM TRIYAS WARDANI[1,3], TRIANA KUSUMANINGSIH[1,♥], AHMAD AINUROFIQ[2]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia. Tel./fax.: +62-271-669376, ♥email: triana_kusumaningsih@staff.uns.ac.id

[2]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia

[3]Institute Vector and Reservoir Control Research and Development, Ministry of Health. Jl. Hasanudin No. 123, Salatiga 50721, Central Java, Indonesia

Manuscript received: 2 January 2024. Revision accepted: 14 March 2024.

Abstract. *Wardani AT, Kusumaningsih T, Ainurofiq A. 2024. Effectiveness of* Foeniculum vulgare *leaf essential oil nanoemulsion as a natural insecticide against* Aedes aegypti *larvae and mosquitoes. Biodiversitas 25: 990-997.* The control of dengue hemorrhagic fever (DHF) was by breaking the chain of transmission by controlling *Aedes aegypti* larvae and mosquitoes. One alternative to controlling mosquito larvae is to use natural materials because they are more environmentally friendly and do not cause harmful residual effects. This study used a *Foeniculum vulgare* Mill. (fennel) leaf as a natural insecticide to kill *Ae. aegypti* larvae and mosquitoes. Steam distillation was done to extract essential oils from the *Foeniculum vulgare* Mill. leaves. *F. vulgare* leaf essential oil was made as nanoemulsion with tween 80, PEG 400, essential oil, and aqua demineralized. GC-MS results showed that the main ingredients in *F. vulgare*. leaf essential oil were anethole, estragole, bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methyl ethyl)-,α-pinene, cyclohexene, 1-methyl-5-(1-methyl ethenyl)-, (R)-.The current study aimed to determine the effectiveness of an *F. vulgare* essential oil nanoemulsion as a larvicidal, adulticidal *Ae.aegypti* mosquito. The study results obtained LC_{50} values for each formula I, II, and III were 0.098, 0.317, 0.157 µL/mL, and LC_{90} values for each formula I, II, and III were 0.158, 0.416, 0.245 µL/mL. Fennel leaf essential oil nanoemulsion was tested on adult *Ae. aegypti* mosquitoes aged 3-5 days. The results of the study obtained LC_{50} values for each formula I, II, III were 7.768, 10.575, 5.069 g/m^3, and LC_{90} values for each formula I, II, III were 15.191, 27.565, 8.892 g/m^3. The difference in the effectiveness of each formula may be due to the large number of essential oils in the formula. *F. vulgare* leaf essential oil could be an environmentally friendly natural insecticide with less residual effect.

Keywords: *Aedes aegypti*, essential oil, *Foeniculum vulgare*, insecticide, nanoemulsion

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is an illness transmitted by vectors, caused by the dengue virus belonging to the *Flavivirus* genus within the Flaviviridae family. The transmission of the dengue virus occurs through the bite of a female *Aedes aegypti* mosquito (World Health Organization 2020). In 2022, there were 143,266 dengue cases, with 1,237 deaths in Indonesia. Cases and deaths due to dengue fever have increased compared to 2021, which is as high as 73,518 cases and 705 deaths. The dengue viruses have been identified as important pathogens that can lead to serious health complications and potentially fatal consequences (Murugesan et al. 2023). Several indicators are used to monitor activities in dengue disease control. The dengue incidence rate per 100,000 population shows an increasing trend of 27 in 2021 and 52.1 in 2022 (Indonesian Ministry of Health 2023). So, it is necessary to reduce dengue cases, for example, by vector control.

One of the efforts to overcome this disease's spread is cutting the mosquito's life cycle. The step that can take to break the life cycle of mosquitoes is at the level of larvae and mosquitoes. Chemical control of mosquitoes can be done, for example, by using insecticides. The use of insecticides for vector control can be done by several methods depending on the purpose. Several chemical insecticides are used for vector control (Indonesian Ministry of Health 2012). Management at the larval or larval stage usually uses temephos, and at the mosquito stage, it usually uses insecticides from various classes of insecticides and multiple formulations on the market. Continuous use of chemical insecticides can cause resistance to both mosquitoes and mosquito larvae (Valle et al. 2019; Palomino et al. 2022). As Sayono et al. (2023) reported, the populations of *Ae. aegypti* resistant to cypermethrin 0.05% and temephos 0.02% are spread in several dengue endemic areas in Central Java, Indonesia.

Insecticides made from natural ingredients are more environmentally friendly, do not have harmful residual effects, and do not cause death from other non-target organisms. Natural insecticides can be obtained from a plant or plant parts. Insecticidal plants usually contain compounds or metabolites that kill larvae or repel mosquitoes. Plant metabolites that can be utilized for natural insecticides are essential oils, the secondary metabolites of an aromatic plant with a strong aroma and volatile properties (Kant and Kumar 2022).

This study will evaluate alternative insecticides derived from natural ingredients of fennel (*Foeniculum vulgare*

Mill.) leaves, with characteristic aroma. Fennel essential oil also has antibacterial effects against *Micrococcus luteus* and *Staphylococcus aureus* bacteria (Khammassi et al. 2023) and antioxidant potentials (Gu et al. 2023; Khammassi et al. 2023). Fennel plant also has properties as an insecticide (Modise and Ashafa 2016; Pavela et al. 2016; Al-Mekhlafi et al. 2021; Safa and Lynda 2023). Fennel plant essential oil also kills *Aedes caspius* mosquito larvae with LC_{50} values of 37.76 μL/100 mL and LC_{90} of 70.49 μL/100 mL with the main content found in fennel essential oil, namely camphor of 38.2% and fenchone of 28.24% (Safa and Lynda 2023). Plant essential oils are considered alternatives to conventional pesticides and have been well-documented for their toxicity to stored product insect pests (Vendan et al. 2017). This study will evaluate an insecticide product based on fennel (*F. vulgare*) leaf essential oil as an emulsion. The emulsion type is water-based oil to be used for larvicide, that water is where mosquito larvae live.

The purpose of utilizing fennel (*F. vulgare*) leaf essential oil is to larvicide or kill mosquito larvae where the habitat is in water. Before carrying out formulations, the physicochemical ingredient properties must be investigated (Ainurofiq et al. 2021). The selection of surfactants and co-surfactants is important to influence the stability of an emulsion (Ahmad et al. 2019). Relying solely on surfactants is inadequate for reducing the surface tension between the oil and water phases; therefore, a co-surfactant is necessary (Syapitri et al. 2022). This study utilized Tween 80 as the surfactant and PEG 400 as the co-surfactant in formulating the emulsion containing fennel leaf essential oil.

MATERIALS AND METHODS

Tools

Moreover, several tools were used, including analytical balance (Ohaus), laboratory glassware, magnetic stirrer (Thermo Scientific), ultrasonicator (Qsonica Ultrasonicator), distillator, Gas Chromatography-Mass Spectra (Thermo Scientific), Particle Size Analyzer (Malvern Zetasizer NanoZs), test chamber, aspirator, paper cup, plastic cup, timer, moisture temperature gauge.

Materials

Fresh fennel (*F. vulgare*) leaves (from Semarang District, Indonesia), tween 80 (CV Subur Kimia Jaya, Bandung), PEG 400 (CV Subur Kimia Jaya, Bandung), and aqua demineralized (Jaya Santosa Yogyakarta).

Insects

Ae. aegypti larvae and mosquitoes were provided by the laboratory colonies of Institute Vector and Reservoir Control Research and Development (IVRCRD) Salatiga. Larvicidal assays were conducted on 3^{rd}-4^{th} instar larvae. Adulticidal activity was conducted on female mosquitoes, 3-5 days old, susceptible, and full of sugar solution, not full of blood, before being used for the test.

Procedure

Distillation of essential oil

Fennel (*F. vulgare*) leaf distillation was conducted by steam distillation; fresh fennel leaves were collected from Semarang Regency, Indonesia. Fresh fennel leaves were cut into small pieces, and chopped fennel leaves were put in the distillation pan. Fennel leaves were vaporized until no more essential oils were dripping, and the essential oils drip were collected in containers (Abdellaoui et al. 2020). Next, water was mixed with essential oils, separated from anhydrous $MgSO_4$, and stored in dark vials at 4°C until analysis. The essential oil yield was calculated using the following formula:

$$\text{Essential oil yield} = \frac{\text{Volume of essential oil (mL)}}{\text{Mass of dry matter (Kg)}}$$

Analysis of physical parameters of essential oils

The essential oil obtained is then analyzed with physical parameters such as color, smell, refractive index, and optical rotation.

Analysis of the content of chemical compounds of essential oils using GC-MS

The essential oil of fennel leaves was dissolved in ethanol in a ratio of 1:2; GC-MS determined the Chemical content of fennel essential oil. A GC-MS instrument (Trace 1310 Gas Chromatograph, ISQ LT Single Quadropole Mass Spectrometer Thermo Scientific, USA) was used with an HP-5 MS UI Column (length 30 mm, I.D 0.25 mm, film 0.25µm), and the carrier gas used was helium. The injector temperature was set at 300°C, and the split flow was 50 mL/min, the split ratio was 50, the front inlet flow was 1.00 mL/min, the purge flow was 3 mL/min, the gas saver flow was 5 mL/min, gas saver time was 5 min.

Preparation of emulsion formulations

The fennel leaf essential oil emulsion formulation was prepared by mixing tween 80 and PEG 400 with a ratio as in Table 1. A mixture of essential oils, surfactants, and cosurfactants with a magnetic stirrer at a speed of 600 rpm for 30 min, then aqua demineralized was added slowly and mixed with a magnetic stirrer at a speed of 600 rpm for 30 min, then ultrasonicated at a frequency of 20 kHz (Ahmad et al. 2019; Campolo et al. 2020). The sonication time was 15 minutes for Formula I and 5 minutes for Formula II and Formula III.

Table 1. Fennel leaf essential oil nanoemulsion formula

Materials (%) / Emulsion	Fennel leaf essential oil	Tween 80	PEG 400	Aqua demineralized
Formula I	25	5	5	ad 100
Formula II	10	20	10	ad 100
Formula III	20	20	10	ad 100

Physical characterization and evaluation of fennel leaf essential oil nanoemulsion

Organoleptic test. Organoleptic tests were carried out by observing odor, color, clarity, and the presence or absence of separation in both phases.

Determination of emulsion droplets. Determination of particle size, particle size distribution, and zeta potential using the Particle Size Analyzer (PSA) instrument (Senapati et al. 2016). Analysis using Malvern Zetasizer NanoZS instrument (UK). Nanoemulsion diluted with water (Formula I: 2 times dilution of 100 uL nanoemulsion in 10 mL water, Formula II: 100 uL nanoemulsion in 10 mL water, Formula III: 2 times dilution of 100 uL nanoemulsion in 10 mL water)

Emulsion type test by dilution. Emulsion-type testing was carried out by dilution. The sample was dissolved in the aqueous phase in ratio of emulsion : water was 1 : 50. If the sample was completely dissolved in the aqueous phase, the emulsion type belongs to the oil-in-water emulsion (Deore et al. 2019).

Test the effectiveness of emulsion on Aedes aegypti mosquito larvae. *Aedes aegypti* instar 3^{rd}-4^{th} prepared before testing the mosquito larvae from laboratory colonies of the Institute Vector and Reservoir Control Research and Development (IVRCRD) Salatiga. Each test concentration was repeated 5 times with 25 larvae in each experiment. First, plastic cups were prepared on the test site and put the water and emulsion into the test beaker according to the test ratio. Mosquito larvae were lowered to contact with fennel oil emulsion for 24 hours, and the number of dead mosquito larvae was recorded (World Health Organization 2005). The mortality rate of the larvae was determined after 24 hours, larvae were declared dead if they did not respond to stimuli or did not ascend to the solution surface (Murugesan et al. 2023).

Test the effectiveness of emulsion on Aedes aegypti mosquito. Female *Ae. aegypti* mosquitoes were prepared before testing, aged 3-5 days, previously satisfied of sugar solution. Mosquitoes come from Institute Vector and Reservoir Control Research and Development (IVRCRD) Salatiga laboratory colonies. A 70 x 70 x 70 cm glass chamber was prepared before testing, and 20 mosquitoes were put into the glass chamber and waited for 20 minutes for the test. Next, each emulsion formula was sprayed into a test cage filled with mosquitoes. Observations were made for 20 minutes by counting and recording fainting/paralyzing test insects within each predetermined period in the glass chamber. After 20 minutes, 20 mosquitoes that have been tested in a glass chamber both alive and fainted/paralyzed then transferred into paper cups, given a sugar solution, and reared/held for 24 hours in the holding room. Observations are made by counting the number of dead, fainted, and living mosquitoes in the paper cup after 24 hours (World Health Organization 2009).

Data analysis

Larvae and mosquito mortality were analyzed with probit using the SPSS 16 computer program to determine its LC_{50} and LC_{90} values.

RESULTS AND DISCUSSION

Distillation of essential oil

Essential oil was obtained from the distillation of fresh fennel leaves carried out by steam distillation and the average yield of essential oil was 2.6 mL/Kg. The fennel plant and fennel leaf essential oil are presented in Figure 1. The characteristics of fennel leaf essential oil can be observed in Table 2.

Figure 2 and Table 3 show the analysis of the GCMS of fennel essential oil and the phytocompounds contained in the essential oil of fennel leaf are presented in Figure 3.

Formulation of fennel leaf essential oil

An emulsion of fennel leaf essential oil is made as many as 3 formulas. The appearance of the resulting emulsion can be seen in Figure 4 and Table 4.

Test emulsion type

The results of the fennel leaf essential oil emulsion type test can be seen in Table 5.

Determination of droplet size and potential Zeta

The droplet size and zeta potential were analyzed with a Particle Size Analyzer. The PSA characterization determines droplet size and potential zeta to examine the emulsion stability. The droplet size and zeta potential of the emulsion can be seen in Table 6.

Test the effectiveness of emulsion on *Aedes aegypti* mosquito larvae

Emulsion effectiveness tests were conducted on *Ae. aegypti* instar 3-4 larvae. The test results of the effectiveness of fennel leaf essential oil emulsion against *Ae. aegypti* larvae, and the LC_{50} and LC_{90} values can be seen in Table 7.

Table 2. Characteristics of fennel leaf essential oil

Test	Result
Color	pale yellow
Smell	fresh, typical fennel oil
Refractive index	1.5279
Optical rotation	+9.5 º

Figure 1. A. Fennel plant, B. fennel leaf essential oil

Table 3. Identify possible compounds contained in the essential oil of fennel leaf

Compound	Retention time	Percentage (%)	Similarity Index (SI)	Molecular formula	Molecular weight (g/mol)
Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	6.45	7.58	939	$C_{10}H_{16}$	136
α-Pinene	6.68	6.26	917	$C_{10}H_{16}$	136
Cyclohexene, 1-methyl-5-(1-methylethenyl)-. (R)-	9.31	2.87	897	$C_{10}H_{16}$	136
Fenchone	11.01	2.33	924	$C_{10}H_{16}O$	152
Estragole	14.25	22.41	950	$C_{10}H_{12}O$	148
Anethole	16.77	52.56	964	$C_{10}H_{12}O$	148
Others (37 compounds)	-	5.99	-	-	-

Table 4. Physical parameters of fennel leaf essential oil emulsion

Formula	Appearance	Separation
Formula I	Milky	No separation
Formula II	Transparent	No separation
Formula III	Milky	No separation

Table 5. Emulsion type of fennel leaf essential oil

Formula	Type of emulsion
Formula I	oil in water
Formula II	oil in water
Formula III	oil in water

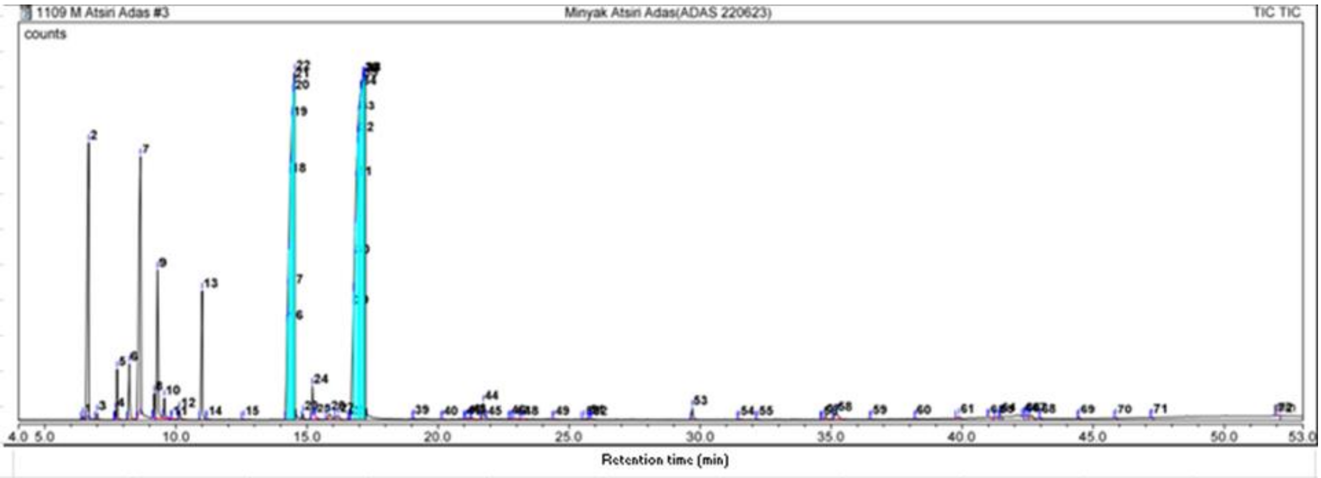

Figure 2. Chromatogram of fennel leaf essential oil

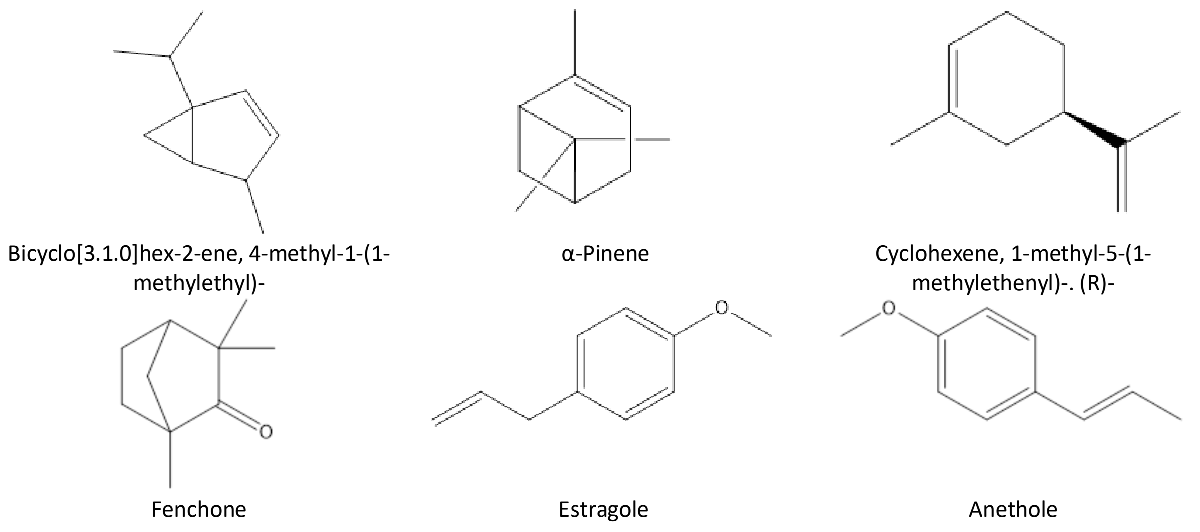

Figure 3. Phytocompounds contained in the essential oil of fennel leaf

Test the effectiveness of emulsion on *Aedes aegypti* mosquito

The effectiveness of fennel leaf essential oil nanoemulsion was tested on female *Ae. aegypti* mosquitoes. The mosquitoes were 3-5 days old, previously satisfied of sugar solution. The test results of the effectiveness of fennel leaf essential oil emulsion against *Ae. aegypti* mosquitoes and LC_{50} and LC_{90} values can be seen in Table 8.

Discussion

The essential oil of fennel leaves has a characteristic aroma, and the specific gravity of fennel leaf essential oil on water exposure at distilling the fennel leaf essential oil layer leads to the above-water layer. Table 3 on the GC-MS analysis showed the five highest percentage contents in fennel leaf essential oil were anethole (52.56%), estragole (22.41%), bicyclo[3.1.0]hex-2-ene. 4-methyl-1-(1-methyl ethyl)-(7.58%), α-pinene (6.26%), cyclohexene, 1-methyl-5-(1-methyl ethenyl)-. (R)-(2.87%). Milenković et al. (2022) reported that the main components in fennel leaf essential oil were anethole (51.4%), methyl chavicol (9.3%), p-Cymene (6.5%), α-phellandrene (5.9%) and β-phellandrene (4.9%). The fennel plants have characteristics with a dominant anethole content, and the yield in this study was 2.6 mL/Kg, as Khalid et al. (2015) reported. In addition, the yield of essential oil from *F. vulgare* leaves from Tafilatet (Southeast of Morocco) was 2% (w/w) (Khammassi et al. 2023). The yield of essential oil leaves collected from different regions of Tunisia varied significantly between populations from 0.68 to 1.91% (w/dw). Moreover, Rahimmalek et al. (2014) reported the yield of essential oil of fennel leaves ranged from 0.65% to 2.03%. In addition to the leaves, other parts of fennel that can be used as essential oil are seeds. Fennel seed essential oil can also be used as a raw material for fragrance (Kusumaningsih et al. 2004). The main components in fennel essential oil were trans-anethole, fenchone, estragole (methyl chavicol), para-anisaldehyde, and a-phellandrene (Rather et al. 2016). The main components of the Egyptian fennel essential oil were estragole (51.04%), limonene (11.45%), l-fenchone (8.19%) and trans-anethole (3.62%). In comparison, the main components of Chinese fennel essential oil were trans-anethole (54.26%), estragole (20.25%), l-fenchone (7.36%), and limonene (2.41%) (Ahmed et al. 2019). The characteristic of fennel plants is the presence of anethole content; this can be known from leaves and seeds that contain anethole. Essential oils isolated from aromatic herbs are variable and may differ in yield and chemical components. Additionally, several influence factors on essential oils variability were harvest time and season, post-harvest processing, extraction method, and geographical region (dos Santos et al. 2016; de Sá Filho et al. 2022; Noppawan et al. 2022).

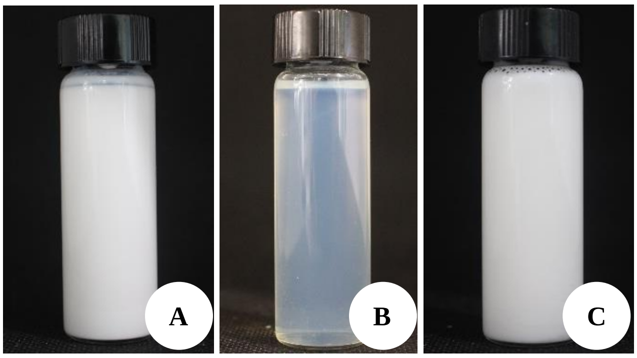

Figure 4. A. Emulsion Formula I, B. Formula II, C. Formula III

Table 6. Droplet size and emulsion zeta potential

Formula	Droplet size (nm)	Polydispersity Index	Zeta potential (mV)
Formula I	184.90	0.362	-33.30
Formula II	40.90	0.220	-19.07
Formula III	353.93	0.419	-17.37

Table 7. The effectiveness of fennel leaf essential oil formula against *Aedes aegypti* larvae

Emulsion	Dosage (µL/mL)	24-Hour mortality (%)	LC_{50} (µL/mL) 24 hour	LC_{90} (µL/mL) 24 hour	*p*-value	SS	DF	MS	F
Formula I	0.05	10.40 ± 11.5239	0.098	0.158	0.000	2022.400	20	101.120	94.595
	0.10	28.00 ± 18.762							
	0.15	94.40 ± 4.561							
	0.20	100.00 ± 0.000							
	0.25	100.00 ± 0.000							
Formula II	0.05	0.00 ± 0.000	0.317	0.416	0.000	89.600	24	3.733	2138.629
	0.10	0.00 ± 0.000							
	0.15	0.00 ± 0.000							
	0.20	4.80 ± 3.347							
	0.25	7.20 ± 3.347							
	0.50	100.00 ± 0.000							
Formula III	0.05	0.00 ± 0.000	0.157	0.245	0.000	2854.400	24	118.933	75.255
	0.10	5.60 ± 4.561							
	0.15	56.80 ± 11.100							
	0.20	69.60 ± 19.718							
	0.25	90.40 ± 13.446							
	0.50	100.00 ± 0.000							

Note: LC: Lethal Concentration, *p*-value: Probability value (p-value≤0.05, significantly effective), SS: Sum of Squares, DF: Degree of Freedom, MS: Mean Square, F: Fisher distribution

Table 8. The effectiveness of fennel leaf essential oil formula against *Aedes aegypti* mosquitoes

Emulsion	Dosage (g/m^3)	24-Hour mortality (%)	LC_{50} (g/m^3) 24 hour	LC_{90} (g/m^3) 24 hour	*p*-value	SS	DF	MS	F
Formula I	4.08	8.33 ± 2.887	7.768	15.191	0.000	234.567	6	39.094	130.257
	8.16	60.00± 10.000							
	16.33	89.76 ± 2.991							
Formula II	8.16	32.98 ± 11.548	10.575	27.565	0.000	907.032	6	151.172	7.523
	12.24	66.17 ± 3.697							
	16.33	67.22 ± 17.506							
Formula III	4.08	30.00 ± 5.000	5.069	8.892	0.000	375.181	6	62.530	38.243
	6.12	69.12 ± 9.494							
	8.16	84.82 ± 8.511							

Note: LC: Lethal Concentration, *p*-value: Probability value (*p*-value≤0.05: significantly effective), SS: Sum of Squares, DF: Degree of Freedom, MS: Mean Square, F: Fisher distribution

The differences in the formulation of materials used in the experiment have different results. Differences in the composition of materials and the manufacturing time can cause this. Tween 80 was chosen as a surfactant because it is non-toxic and stable. The long alkyl chain length of Tween 80 (C18) provides a greater dissolving capacity for hydrophobic solutes (Christy et al. 2017); Tween 80 is a non-ionic surfactant that affects emulsion stability. PEG 400, functioning as a co-surfactant, possesses hydroxyl and short ethylene groups which enable it to reduce the interfacial tension between the oil and water phases by penetrating the interfacial area. The groups are able to penetrate the interfacial area between the oil and water phases (Adi et al. 2019).

The nanoemulsion in this study was made using the ultrasonication technique. Ultrasonication is one of the popular methods for preparing the nanoemulsion; it is an energy-efficient process, an easy and straightforward manipulating system, has low production cost, and produces stable emulsions (Singh and Pulikkal 2022). Ultrasound emulsification uses an acoustic field to disperse one liquid into another immiscible liquid. The key effect of ultrasound is cavitation, which involves the rapid formation of vapor bubbles in a liquid by reducing the pressure at ambient temperature. The presence of ultrasonication can reduce the particle size of an emulsion (Pongsumpun et al. 2020; Yang et al. 2023).

The formula produced during the study was oil-in-water-based. An oil-in-water emulsion is an emulsion in which the oil phase is dispersed in the aqueous phase (Marzuki et al. 2019); this follows the study objective to make an emulsion with an oil-in-water type. Tween 80 is a surfactant that plays a role in lowering the inter-phase interface voltage as one of the surfactants that can cause stable emulsions at specific compositions and produce emulsions with oil-in-water types (Roldan-Cruz et al. 2016). To create an oil-in-water emulsion, it's essential for the surfactant to possess a Hydrophilic-Lipophilic Balance (HLB) greater than 10. Tween 80, with an HLB of 15, meets this criterion, making it suitable as a surfactant for nanoemulsion production (Smail et al. 2021). Therefore, this study aims to make oil-in-water nanoemulsions according to the application purpose to be an emulsion easily dispersed evenly in water for mosquito larvae living habitat. All three formulas in this study produce an oil-in-water type emulsion.

Droplet size and size distribution are important characteristics of the nanoemulsion system. A good nanoemulsion has a droplet size of <200 nm, which is important in manufacturing nanoemulsions. Formula I and Formula III exhibit a milky appearance where the globular size is above 100 nm. In comparison, Formula II showed a transparent appearance with a globule size below 100 nm. The emulsion droplet size also depends on several factors, such as homogenizer types, manufacturing temperature, energy intensity, time, oil concentration, surfactant type, and surfactant concentrations (Syapitri et al. 2022). The differences between each formula in this study are the concentration of essential oils, surfactants, co-surfactants, and sonication time. This difference leads to differences in the particle size of each emulsion formula evaluated.

Zeta potential is important to determine the surface tension of an emulsion and to predict the stability of an emulsion. Zeta potential is the electrostatic voltage that occurs in a field particle surface due to the interaction between charges on the surface particle with the environment of the particle medium. The potential zeta charge generates an electrical repulsive force between droplets to prevent aggregation; a poor potential zeta value indicates that the repulsive strength between particles is getting weaker so that it can undergo aggregation, affecting the emulsion's stability. Furthermore, good emulsion stability has a potential zeta value of more than +30 mV and less than -30 mV (Kumar et al. 2021). Formula I is predicted to be the most stable formula among all the three formulas because the zeta potential value was less than -30 mV.

The polydispersity index identifies the uniformity of droplet size in a sample, the range of droplet size distribution, and the emulsion aggregation. The polydispersity index value of a sample inversely correlates with the uniformity of particle size in that sample; thus, a lower value indicates higher particle size uniformity. A good polydispersity index value is below 0.5 and above 0.5, indicating that the globules' distribution is nonuniform. The lower the index value polydispersity, the higher the uniformity of globular size on the emulsion. All formulas have a polydispersity index below 0.5 and good uniformity. In this formula, using Tween 80 can increase droplet stability in oil-in-water emulsion systems (Christy et al. 2017).

Essential oils are formulated as nanemulsions to be easily dispersed in water, the main habitat of mosquito larvae. When the nanoemulsion droplets are evenly dispersed on the liquid, the nanoemulsion is likely effective in killing the larvae because the behavior of the larvae moves in all directions. The efficacy test of the fennel leaf essential oil nanoemulsion formula was conducted on instar 3rd mosquito larvae. The results showed that fennel leaf essential oil nanoemulsion effectively killed *Ae. aegypti* mosquito larvae for all formulas; the essential oil content of fennel leaves causes the mortality of mosquito larvae by nanoemulsion. The largest content in fennel leaf essential oil is anethole. As reported by Soonwera et al. (2022), anethole effectively kills *Ae. aegypti* and *Ae. albopictus* mosquito larvae. This larvicide is said to be effective if, for 24 hours, it can kill mosquito larvae by 90% (World Health Organization 2005). Each formula has a different amount of essential oil content. Each formula has a different effectiveness in killing mosquito larvae. The study results obtained LC_{50} values for each formula I, II, III were 0.098, 0.317, 0.157 µL/mL, and LC_{90} values for each formula I, II, III were 0.158, 0.416, and 0.245 µL/mL, respectively. The smaller the Lethal Concentration (LC) value, the more effective it is. The most effective formula in killing *A.e aegypti* mosquito larvae is formula I because the LC value is the smallest. This could be because Formula I contains more essential oils than Formula II and III, even though Formula I has a bigger droplet size than Formula II.

Fennel leaf essential oil nanoemulsion was tested on adult *Ae. aegypti* mosquitoes aged 3-5 days. The results of the study obtained LC_{50} values for each formula I, II, III were 7.768, 10.575, 5.069 $g/m^{3,}$ and LC_{90} values for each formula I, II, III were 15.191, 27.565, 8.892 g/m^3, respectively. The study results showed that the death with the smallest dose was formula III, and the mechanism of mosquito death is likely due to substances contained in fennel leaf essential oil. Shaari et al. (2021) reported that *Ae. aegypti* was susceptible to the newly developed oil-based nanoemulsion insecticide, indicating a high potential for mosquito control. The substance inhibits the acetylcholinesterase enzyme resulting in the phosphorylation of serine amino acids at the esoteric center of the enzyme concerned, causing paralysis to mosquito death.

In conclusion, the nanoemulsion of *F. vulgare* leaf essential oil has larvicidal and adulticidal activity against *Ae. aegypti* larvae and mosquitoes. The concentration of essential oil in the nanoemulsion formulation affects the larvicidal and adulticidal of the mosquito. The best formula is based on stability and effectiveness against *Ae. aegypti* larvae and mosquitoes was formula I of the three formula. The formulation of *F. vulgare* essential oil has potential as a natural insecticide for controlling *Ae. aegypti* larvae and mosquitoes.

ACKNOWLEDGEMENTS

The authors would like to thank the Ministry of Research, Technology and Higher Education, who provided financial support through the 2023 Masters Thesis Research program with Contract Letter Number: 1280.1/UN27.22/ PT.01.03/2023. We also sincerely thank the Department of Chemistry Faculty of Mathematics and Natural Science, Sebelas Maret University, Surakarta, Indonesia, and Institute Vector and Reservoir Control Research and Development (IVRCRD) Salatiga, Ministry of Health Indonesia for providing the place and tools to conduct this research.

REFERENCES

Abdellaoui M, Bouhlali EDT, Derouich M, El-Rhaffari L. 2020. Essential oil and chemical composition of wild and cultivated fennel (*Foeniculum vulgare* Mill.): A comparative study. S Afr J Bot 135: 93-100. DOI: 10.1016/j.sajb.2020.09.004.

Adi AC, Christanto C, Rachmawati H, Adlia A. 2019. Vitamin E-based folic acid nanoemulsion: Formulation and physical evaluation for oral administration. Pharm Nanotechnol 7 (4): 304-313. DOI: 10.2174/2211738507666190717154040.

Ahmad N, Ahmad R, Al-Qudaihi A, Alaseel SE, Fita IZ, Khalid MS, Pottoo FH. 2019. Preparation of a novel curcumin nanoemulsion by ultrasonication and its comparative effects in wound healing and the treatment of inflammation. RSC Adv 9 (35): 20192-20206. DOI: 10.1039/c9ra03102b.

Ahmed AF, Shi M, Liu C, Kang W. 2019. Comparative analysis of antioxidant activities of essential oils and extracts of fennel (*Foeniculum vulgare* Mill.) seeds from Egypt and China. Food Sci Hum Wellness 8 (1): 67-72. DOI: 10.1016/j.fshw.2019.03.004.

Ainurofiq A, Maharani A, Fatonah F, Halida HN, Nurrodlotiningtyas T. 2021. Pre-formulation study on the preparation of skin cosmetics. Sci Technol Indones 6 (4): 273-284. DOI: 10.26554/sti.2021.6.4.273-284.

Al-Mekhlafi FA, Abutaha N, Al-Doaiss AA, Al-Keridis LA, Alsayadi AI, Mohamed RAEH, Wadaan MA, Ibrahim KE, Al-Khalifa MS. 2021. Target and non-target effects of *Foeniculum vulgare* and *Matricaria chamomilla* combined extract on *Culex pipiens* mosquitoes. Saudi J Biol Sci 28 (10): 5773-5780. DOI: 10.1016/j.sjbs.2021.06.024.

Campolo O, Giunti G, Laigle M, Michel T, Palmeri V. 2020. Essential oil-based nanoemulsions: Effect of different surfactants, sonication and plant species on physicochemical characteristics. Ind Crops Prod 157: 112935. DOI: 10.1016/j.indcrop.2020.112935.

Christy GP, Arimurni DA, Wahyudi MDP, Martien R, Tunjung WAS. 2017. Formulation and characterization of kaffir lime oil nanoemulsion. Biosci Biotechnol Res Asia 14: 915-922. DOI: 10.13005/bbra/2525.

de Sá Filho JCF, de Castro Nizio DA, de Oliveira AMS, Alves MF, de Oliveira RC, Luz JMQ, de Lima Nogueira PC, de Fátima Arrigoni-Blank M, Blank AF. 2022. Geographic location and seasonality affect the chemical composition of essential oils of *Lippia alba* accessions. Ind Crops Prod 188: 115602. DOI: 10.1016/j.indcrop.2022.115602.

Deore SK, Surawase RK, Maru A. 2019. Formulation and evaluation of o/w nanoemulsion of ketoconazole. Res J Pharm Dosage Forms Technol 11 (4): 269-274. DOI: 10.5958/0975-4377.2019.00045.4.

dos Santos CP, Pinto JAO, dos Santos CA, Cruz EMO, de Fátima Arrigoni-Blank, Andrade TM, de Alexandria Santos, Alves PB, Blank AF. 2016. Harvest time and geographical origin affect the essential oil of *Lippia gracilis* Schauer. Ind Crops Prod 79: 205-210. DOI: 10.1016/j.indcrop.2015.11.015.

Gu J, Chen F, Zheng Z, Bi L, Morovvati H, Goorani S. 2023. Novel green formulation of copper nanoparticles by *Foeniculum vulgare*: Chemical characterization and determination of cytotoxicity, anti-human lung cancer and antioxidant effects. Inorg Chem Commun 150: 110442. DOI: 10.1016/j.inoche.2023.110442.

Indonesian Ministry of Health. 2012. Pedoman Penggunaan Insektisida (Pestisida) dalam Pengendalian Vektor. Kementerian Kesehatan RI, Jakarta. [Indonesian]

Indonesian Ministry of Health. 2023. Profil Kesehatan Indonesia 2022. Kementerian Kesehatan RI, Jakarta. [Indonesian]

Kant R, Kumar A. 2022. Review on essential oil extraction from aromatic and medicinal plants: Techniques, performance and economic analysis. Sustain Chem Pharm 30: 100829. DOI: 10.1016/j.scp.2022.100829.

Khalid S, Mohamed B, Mhamed R, Tariq BED, Fatima J, Laila N, Lhoussaine ER. 2015. Antifungal potential of the seed and leaf *Foeniculum vulgare* Mill essential oil in liquid and vapor phase against phytopathogenic fungi. J Appl Pharm Sci 5 (11): 50-54. DOI: 10.7324/japs.2015.501108.

Khammassi M, Ayed RB, Loupasaki S, Amri I, Hanana M, Hamrouni L, Jamoussi B, Khaldi A. 2023. Chemical diversity of wild fennel essential oils (*Foeniculum vulgare* Mill.): A source of antimicrobial and antioxidant activities. S Afr J Bot 153: 136-146. DOI: 10.1016/j.sajb.2022.12.022.

Kumar N, Verma A, Mandal A. 2021. Formation, characteristics and oil industry applications of nanoemulsions: A review. J Petrol Sci Eng 206: 109042. DOI: 10.1016/j.petrol.2021.109042.

Kusumaningsih T, Handayani DS, Makruf A. 2004. Synthesis of perfume compound etil p-anisat from anethole. Biofarmasi 2 (2): 58-63. DOI: 10.13057/biofar/f020203. [Indonesian]

Marzuki NHC, Wahab RA, Hamid MA. 2019. An overview of nanoemulsion: Concepts of development and cosmeceutical applications. Biotechnol Biotechnol Equip 33 (1): 779-797. DOI: 10.1080/13102818.2019.1620124.

Milenković A, Ilić Z, Stanojević L, Milenković L, Šunić L, Lalević D, Stanojević J, Danilović B, Cvetković, D. 2022. essential oil yield, composition, antioxidant and microbial activity of wild fennel (*Foeniculum vulgare* Mill.) from monte negro coast. Horticulturae 8 (11): 1015. DOI: 10.3390/horticulturae8111015.

Modise SA, Ashafa AOT. 2016. Larvicidal, pupicidal and insecticidal activities of *Cosmos bipinnatus*, *Foeniculum vulgare* and *Tagetes minuta* against *Culex quinquefasciatus* mosquitoes. Trop J Pharm Res 15 (5): 965-972. DOI: 10.4314/tjpr.v15i5.10.

Murugesan R, Vasuki K, Kaleeswaran B. 2023. A green alternative: Evaluation of *Solanum torvum* (Sw.) leaf extract for control of *Aedes aegypti* (L.) and its molecular docking potential. Intelligent Pharmacy (November). DOI: 10.1016/j.ipha.2023.11.012.

Murugesan R, Vasuki K, Ramadevi S, Kaleeswaran B. 2023. Rosmarinic acid: Potential antiviral agent against dengue virus - in silico evaluation. Intelligent Pharm 2023: 1-12. DOI: 10.1016/j.ipha.2023.12.006.

Noppawan P, Bainier C, Lanot A, McQueen-Mason S, Supanchaiyamat N, Attard TM, Hunt AJ. 2022. Effect of harvest time on the compositional changes in essential oils, cannabinoids, and waxes of hemp (*Cannabis sativa* L.). R Soc Open Sci 9 (6): 211699. DOI: 10.1098/rsos.211699.

Palomino M, Pinto J, Yañez P, Cornelio A, Dias L, Amorim Q, Martins AJ, Lenhart A, Lima JBP. 2022. First national-scale evaluation of temephos resistance in *Aedes aegypti* in Peru. Parasit Vectors 15 (1): 254. DOI: 10.1186/s13071-022-05310-x.

Pavela R, Žabka M, Bednář J, Tříska J, Vrchotová N. 2016. New knowledge for yield, composition and insecticidal activity of essential oils obtained from the aerial parts or seeds of fennel (*Foeniculum vulgare* Mill.). Ind Crops Prod 83: 275-282. DOI: 10.1016/j.indcrop.2015.11.090.

Pongsumpun P, Iwamoto S, Siripatrawan U. 2020. Response surface methodology for optimization of cinnamon essential oil nanoemulsion with improved stability and antifungal activity. Ultrason Sonochem 60: 104604. DOI: 10.1016/j.ultsonch.2019.05.021.

Rahimmalek M, Maghsoudi H, Sabzalian MR, Pirbalouti AG. 2014. Variability of essential oil content and composition of different Iranian fennel (*Foeniculum vulgare* mill.) accessions in relation to some morphological and climatic factors. J Agric Sci Technol 16 (6): 1365-1374.

Rather MA, Dar BA, Sofi SN, Bhat BA, Qurishi MA. 2016. *Foeniculum vulgare*: A comprehensive review of its traditional use, phytochemistry, pharmacology, and safety. Arab J Chem 9 (S2): S1574-S1583. DOI: 10.1016/j.arabjc.2012.04.011.

Roldan-Cruz C, Vernon-Carter EJ, Alvarez-Ramirez J. 2016. Assessing the stability of Tween 80-based O/W emulsions with cyclic voltammetry and electrical impedance spectroscopy. Colloids Surf A: Physicochem Eng Asp 511: 145-152. DOI: 10.1016/j.colsurfa.2016.09.074.

Safa KB, Lynda A. 2023. determination of chemical characterization of *Foeniculum vulgare* Mill essential oil composition and its toxicological effects against mosquito (*Aedes caspius*, Pallas, 1771). Cutting Edge Res Biol 3: 66-78. DOI: 10.9734/bpi/cerb/v3/4117B.

Sayono, Nurullita U, Handoyo W, Tyasningrum WS, Chakim I, Budiharjo A. 2023. Bioassay and molecular detection of insecticides resistance of *Aedes aegypti*, vector of dengue in Central Java Province, Indonesia. Biodiversitas 24: 300-307. DOI: 10.13057/biodiv/d240136.

Senapati PC, Sahoo SK, Sahu AN. 2016. Mixed surfactant based (SNEDDS) self-nanoemulsifying drug delivery system presenting efavirenz for enhancement of oral bioavailability. Biomed Pharmacother 80: 42-51. DOI: 10.1016/j.biopha.2016.02.039.

Shaari A, Yunus R, Raman IA, Omar D, Shahar MK, Biak DRA, Karnia D, Aulia A. 2021. Field efficacy of palm oil-based nanoemulsion insecticides against *Aedes aegypti* in Malaysia. Acta Tropica 224: 106107. DOI: 10.1016/j.actatropica.2021.106107.

Singh IR, Pulikkal AK. 2022. Preparation, stability and biological activity of essential oil-based nanoemulsions: A comprehensive review. OpenNano 8: 100066. DOI: 10.1016/j.onano.2022.100066.

Smail SS, Ghareeb MM, Omer HK, Al-Kinani AA, Alany RG. 2021. Studies on surfactants, cosurfactants, and oils for prospective use in formulation of ketorolac tromethamine ophthalmic nanoemulsions. Pharmaceutics 13 (4): 467. DOI: 10.3390/pharmaceutics13040467.

Soonwera M, Moungthipmalai T, Aungtikun J, Sittichok S. 2022. Combinations of plant essential oils and their major compositions inducing mortality and morphological abnormality of *Aedes aegypti* and *Aedes albopictus*. Heliyon 8 (5): e09346. DOI: 10.1016/j.heliyon.2022.e09346.

Syapitri H, Panduragan SL, Babu S, Purwandari V, Thaib CM. 2022. Preparation of black cumin extract nanoemulsion using the oil phase of Virgin Coconut Oil (VCO) tween 80 and peg 400 surfactants. Malays J Med Health Sci 18 (6): 2-5.

Valle D, Bellinato DF, Viana-Medeiros PF, Lima JBP, de Jesus Martins Junior A. 2019. Resistance to temephos and deltamethrin in *Aedes aegypti* from Brazil between 1985 and 2017. Mem Inst Oswaldo Cruz 114: e180544. DOI: 10.1590/0074-02760180544.

Vendan SE, Manivannan S, Sunny AM, Murugesan R. 2017. Phytochemical residue profiles in rice grains fumigated with essential oils for the control of rice weevil. PLoS One 12 (10): e0186020. DOI: 10.1371/journal.pone.0186020.

World Health Organization. 2005. Guidelines for laboratory and field testing of mosquito larvicides. World Health Organization Communicable Disease Control, Prevention and Eradication Who Pesticide Evaluation Scheme. www.who.int/publications/i/item/WHO-CDS-WHOPES-GCDPP- 2005.13.

World Health Organization. 2009. Guidelines for efficacy testing of household insecticide products. World Health Organization. www.who.int/publications/i/item/WHO-HTM-NTD-WHOPES-2009.3.

World Health Organization. 2020. Dengue and severe dengue. www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue.

Yang Z, He Q, Ismail BB, Hu Y, Guo M. 2023. Ultrasonication induced nano-emulsification of thyme essential oil: Optimization and antibacterial mechanism against *Escherichia coli*. Food Control 148: 109652. DOI: 10.1016/j.foodcont.2023.109652.