JFSP Vol.11, No.1, January-April 2025, Page: 11-17
pISSN: 2549-9068, eISSN: 2579-4558
Jurnal Farmasi Sains dan Praktis
(JFSP)
http://journal.unimma.ac.id/index.php/pharmacy

EFFECTIVENESS TEST OF OIL PALM LEAF EXTRACT (*Elaeis guineensis* Jacq.) AS A MOSQUITO BIOLARVACIDE *Aedes aegypti*

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https://doi.org/10.31603/pharmacy.v%vi%i.10275

Article info:
Submitted : 25-09-2023
Revised : 01-04-2025
Accepted : 11-04-2025

Publisher:
Universitas Muhammadiyah Magelang

ABSTRACT
Dengue Hemorrhagic Fever (DHF) is a disease that is transmitted by female mosquito species *Aedes aegypti* which has spread all over the world. Efforts to control the *Aedes aegypti* mosquito vector by using natural biolarvicides using oil palm leaf extract. The purpose of this study was to determine the effectiveness of oil palm leaf extract (*Elaeis guineensis* Jacq.) as a biolarvicide for the *Aedes aegypti* mosquito. This study employs 600 *Aedes aegypti* larvae in an experimental post-test only control group design. Concentrations of oil palm leaf extract (**OPLE**) ranging from 1000 to 4000 ppm, using temephos as a positive control and aquadest as a negative control. The mortality of *Aedes aegypti* larvae was calculated every 1 hour during the 48-hour observation period. SPSS was used to analyse data to determine the concentrations of LC_{90} and LT_{50}. The results of this study indicate the probit analysis value (LC_{90}) of *Aedes aegypti* larvae is 2190 ppm with time (LT_{50}) the fastest concentration of killing 4000 ppm with a time of 37,183 hours. Based on the results of research, oil palm leaf extract (*Elaeis guineensis* Jacq.) has a biolarvicidal effect on the larvae of the third instar *Aedes aegypti* mosquito.

Keywords: *Aedes aegypti*; Biolarvicide; Oil palm leaf extract; *Elaeis guineensis* Jack.

1. INTRODUCTION

The disease known as *dengue hemorrhagic fever* (DHF), which is spread by mosquitoes, has recently become widespread in the entire world. Female mosquitoes, often of the *Aedes aegypti* species but occasionally also of the *Aedes albopictus* species, are the carriers of the dengue virus. The majority of dengue cases typically occur in tropical and subtropical nations, particularly in metropolitan areas (Kularatne & Dalugama, 2022). In 2022, there will be 143,000 dengue cases in Indonesia, with a death rate of 0.86%. To address this issue, the government has implemented a number of programs, including the G1R1J (*One House One Jumantik Movement*) and COMBI (*Communication for Behavioral Impact*) programs (Kemenkes RI, 2022).

Aedes aegypti mosquito eradication efforts include physical, biological, and chemical eradication. This will stop the spread of the dengue virus (Kemenkes RI, 2017). Temephos is a common chemical control agent. Regular usage of temephos can harm human health and result in pollution if ingested, it can poison people. In order to eliminate mosquito larvae while not harming the ecosystem, a natural substance is consequently required. Palm Oil is a plant that is easy to find in Indonesia. One of them is that the leaves of oil palm plants are often useless and thrown away by the community. Not many people know that oil palm leaves contain many secondary metabolic contents that have the potential to act as biolarvicides (Mohamed, 2014). The advantage of oil palm leaves as a potential source is their abundance, particularly in Southeast Asia. This abundance makes oil palm leaves an easily accessible and renewable resource for various applications. Furthermore, the use of oil palm leaves contributes to sustainable waste management by utilizing a by-product of the palm oil industry (Kong dkk., 2014).

Phytochemical screening for compounds in oil palm leaves that have secondary metabolism such as tannins, alkaloids, saponins, steroids, terpenoids and flavonoids (Zumaro *et al*., 2021). Several studies have shown the potential of extracts from several parts of the oil palm plant (*Elaeis guineensis* Jacq). Male palm flower extract at 5% in lotion has been proven to be effective against mosquito bites up to 92% (Arsita *et al*., 2022). Palm leaf extract has been proven to be effective as a sunscreen and safe to use topically (Yusof, 2016) as well as being an anti-acne agent (Wahyudi *et al*., 2020). The irritation test of palm leaf extract shows the safe category for eye and skin irritation tests (Yusof *et al*., 2018). The toxicity test of palm leaf extract is safe for use up to a dose of 2 g/kgBB (Anyanji *et al*., 2013). Therefore, this study aims to test palm leaf extract as a safe biolarvicide as an alternative substitute for temephos.

2. METHODS

2.1. Tools and Chemicals

Volumepipette (*Pyrex*), test tube (*Pyrex*), plastic cup, glass cup (*pyrex*), analytical balances (*ohaus*), water bath, Spatula, porcelain cups, glass wear (*Pyrex*), *Aedes aegypti* larvae instar III, Etanol 70% (*Merck*), temephos, and leaf of oil palm from Tanah Bumbu, South Kalimantan.

2.2. Plant Material and Extraction

The determination was conducted at the Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, where the plant was identified as *Elaesis guineensis* Jacq. Extraction of 250 grams of *Elaesis guineensis* leaf using 12 liters of 70% ethanol. Subsequently, the obtained liquid extract was evaporated using a water bath at a temperature of 60°C. Phytochemical test were conducted qualitatively as discribed by previous study to asses tanin, saponon, flavonoid, alkaloid, steroid, and terpenoid (Yin *et al*., 2013).

2.3. Animal preparation

Aedes aegypti larvae instar III were prepared from Instar II about 2-3 days. Total of 600 *Aedes aegypti* larvae instar III was used in this study divided into six group (positive control, negative control, and 4 group of extract with 1000-4000 ppm). The test was repeated 4 replications for each group. The standard water temperature for larvae life is 25-30˚C. The optimal pH for larvae development is between 5.8-8.6. The optimum average temperature for the development of mosquito larvae is 25°-27°C. Suitable air humidity is about 70% - 89% (Marlina *et al*., 2021).

2.4. Biolarvacide test

The solution test of oil palm *Elaeis guineensis* leaf extract (**OPLE**) made according to concentrations of 1000 ppm, 2000 ppm, 3000 ppm, and 4000 ppm, the distilled water test solution (normal media) is put into a measuring cup until it reaches 100 ml, and the test solution temephos with a concentration of 1%. 25 *Aedes aegypti* larvae were put into several transparent plastic cups for each group. Observations were made to count the number of deaths of *Aedes aegypti* mosquito larvae every hour for 48 hours. The test was carried out by repeating four times. The data analysis technique uses probit analysis to determine LC_{90} and LT_{50}.

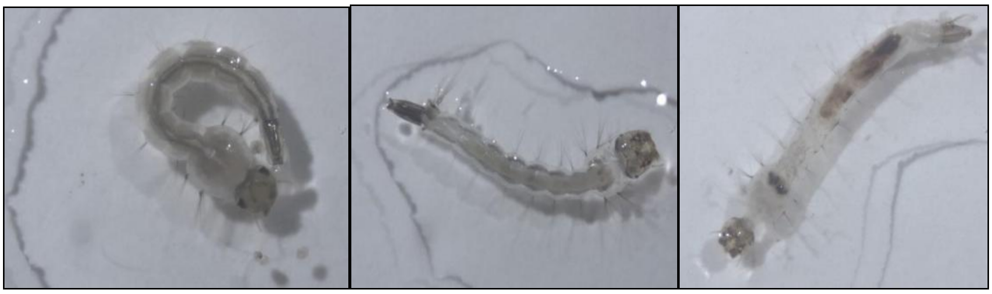
3. RESULTS AND DISCUSSION

Extraction process carried out on flowers produced a thick extract of 18 grams, resulting in a yield of 7.2%. The results of the phytochemical test for extract compounds are (**Table 1**):

Table 1. Extract Phytochemical test

Group of Compound	Result
Tanin	+
Saponin	+
Flavonoid	+
Alkaloid	+
Steroid	-
Terpenoid	-

The tannin, alkaloid and flavonoid content of **OPLE** is very important in this test (**Table 1**). The tannin content has anti-irritant, antimicrobial and anti-parasitic effects (Febrina *et al*., 2020). Saponins have a mechanism of action by reducing the surface tension of the mucous membrane of the digestive tract of *Aedes aegypti* mosquito larvae so that the walls of the digestive tract become corrosive and ultimately damaged. The digestive process of Aedes aegypti larvae by binding proteins in the insect's digestive system (Maula, 2022). Flavonoid content has an impact on biological systems. Flavonoids are often referred to as biological modifiers. This compound can provide antimicrobial and anticancer effects (Yin *et al*., 2013). As respiratory toxins, flavonoids have this role. Flavonoids impair the larva's respiratory system by entering the body of the larva through the syphon (Kurniawan *et al*., 2015). Mechanism of alkaloids by preventing the larva from eating and acting as stomach poison. Alkaloids are hypothesised to interfere with the acetylcholine enzyme's ability to function, which leads to an accumulation of acetylcholine and disarray in the muscle cells' impulse delivery mechanism. The *Aedes aegypti* mosquito will finally undergo seizures and pass away (Tamtama *et al*., 2023).



(A) (B) (C)

Figure 1. *Aedes aegypti* larvae**,** (A) Larva before test; (B) Negative control; (C) After OPLE intervention

Observation of third instar *Aedes aegypti* mosquito larvae was carried out for 48 hours as a benchmark for the effectiveness of a larvicide (**Figure 1**). Death of third instar *Aedes aegypti* mosquito larvae in this study was the condition when the larvae were declared dead with signs of not responding to stimulation and not moving. Biolarvicide test results are as follows (**Table 2**).

Table 2. Number of Larvae Deaths

Concentration of OPLE	Number of larvae	Number of Larvae Deaths						
		Replication				Total	Average	Percentage (%)
		I	II	III	IV			
Control (-)	25	0	0	0	0	0	0	0
1000 ppm	25	12	14	11	9	46	11.5	46
2000 ppm	25	23	23	23	22	91	22.75	91
3000 ppm	25	23	23	23	24	93	23.25	93
4000 ppm	25	25	25	25	25	100	25	100
Control (+)	25	25	25	25	25	100	25	100

This research used ethanol as the extract solvent and third instar *Aedes aegypti* larvae as test animals. Ethanol is a solvent with low toxicity so it does not interfere with test results and can obtain optimal secondary metabolites. Instar III is considered to be quite representative of the condition of the larvae and the size of the third instar larvae is not too small so it is easy to observe and this larva is a form that is actively looking for food (Hitipeuw *et al*., 2022). The Probit analysis revealed that the LC_{90} value, representing the lethal concentration required to cause 90% mortality in the test population. This indicates a high level of toxicity of the tested **OPLE** towards the target organism. The narrow confidence interval suggests good precision of the estimation. Result of Probit LC_{90} Analysis was showed in the **Table 3**.

Table 3. Result of Probit LC_{90} Analysis

	Concentration (ppm)	Lower concentration	Upper concentration
LC_{90}	2190,621	112	3166

Aedes aegypti mosquito larvae in their third instar were completely destroyed with temephos (Positive Control). This is due to the fact that this class of larvicides inhibits the cholinesterase enzyme in both vertebrates and invertebrates. This causes disturbances in nervous activity because acetylcholine accumulates into choline and acetic acid; therefore, if the enzyme is inhibited, acetylcholine hydrolysis does not occur. As a bridge between nerves and muscles, acetylcholine facilitates the transfer of electrical impulses that cause muscles to contract for an extended period of time, resulting in convulsions (seizures). Temephos 1% will bind to the cholinesterase enzyme and cause it to be damaged, which will cause constant muscle contractions and spasms as well as the eventual death of the larvae (Rahmaningtyas *et al*., 2022).

The physical characteristics of water colorlessness, tastelessness, and odorlessnessare met by the condition of the water after the delivery of temephos. This indicates that the general population can safely use temephos when using it as a larvicide in water. It should be mentioned, that if temephos is used excessively and gets into the body's organs, it will cause an overstimulation of the nerves, which can make those who take it queasy, sick, and confused (Manyullei *et al*., 2015). The choice of 1000 ppm concentration was because in preliminary tests no larval deaths were found at lower concentrations. Referring to **Table 3**, the minimum LC90 concentration of OPLE is 112 ppm and the maximum is 3166 ppm. The choice of this concentration has an impact on a slight color change in the water so that it does not meet the requirements as a biolarvicide. However, the OPLE test shows that this concentration does not cause irritation. **OPLE** also has an anti-corrosion effect on steel water containers at a minimum concentration of 2% (Ea, 2018). The rate of death of *Aedes aegypti* larvae in the sample group is shown in **Table 4**.

Table 4. LT_{50} Probit Analysis Results

Concentration	LT_{50} (Hour)	Lower Time (Hour)	Upper Time (Hour)
1000 ppm	58,959	53,310	83,409
2000 ppm	40,465	35,527	47,991
3000 ppm	39,394	35,112	45,229
4000 ppm	37,183	33,115	42,457
Control (+)	15,712	12,223	18,674

There was an increase in larval mortality in all concentration groups. The highest increase in mortality at a concentration of 4000 ppm with LT_{50} was 37,183 hours, meaning the length of time taken by oil palm leaf extract (*Elaeis guineensis* Jacq.) with a concentration of 4000 ppm to kill 50% of *Aedes aegypti* larvae was 37.183 hours. This study shows that **OPLE** biolarvicide has very low potency. Based on these findings, three reasons could account for the death of the larvae: low water quality, high active chemical level, or a combination of both. So, it is hoped that future research can examine the combination with other extracts and isolate the active compound of **OPLE** as a biolarvicide. This study has several limitations that should be considered. First, the larvicidal tests were conducted under controlled laboratory conditions, which may not fully replicate the complexity of natural environments where various biotic and abiotic factors could influence the efficacy of the extract. Second, the extract used was crude and not fractionated, thus the specific active compounds responsible for the larvicidal activity remain unidentified. Third, the study focused solely on the larval stage of *Aedes aegypti*, without evaluating potential effects on pupae or adult mosquitoes, which limits the understanding of the extract's full mosquito control potential. In addition, the long-term environmental impact and toxicity to non-target organisms were not assessed.

4. CONCLUSION

Oil palm (*Elaeis guineensis* Jacq.) leaf extract has a biolarvicidal effect on third instar *Aedes aegypti* larvae at 2190 ppm (LC_{90}) for 40 hours. This research encourages the diversification of pharmaceutical formulations into environmental applications as part of vector-borne disease prevention, such as dengue fever.

5. ACKNOWLEDGMENT

In order to conduct this research, the authors would like to thank Universitas Lambung Mangkurat.

6. CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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