Biolarvicide Efficacy of Lantana Leaf (*Lantana camara* L) Essential Oil Against *Aedes aegypti* Larvae for Dengue Vector Control

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Abstract: In several regions of Indonesia, dengue hemorrhagic fever (DHF) is still a serious health problem. Controlling the vector of DHF can be done through breaking the life cycle of the *Aedes aegypti* mosquito. One of the easy and effective ways to break the life cycle of mosquitoes is in the larval phase. Lantana leaves (*Lantana camara* L.) contain active compounds lantadine, essential oils, flavonoids, alkaloids, saponins, and tannins. The use of biolarvicides is an effort to reduce the preventive occurrence of mosquito larvae resistance to temephos, and reduce the risk of decreased public health due to the use of chemical larvicides. This study aims to determine the effectiveness of lantana leaf essential oil as a biolarvicide against dengue vectors. The method used in this study is distillation, and for the process of testing the effectiveness of biolarvicides is to calculate the number of deaths of larvae that have been treated so that the percent mortality value is obtained and continued by calculating the LC50 and LC90 values using the probit test. The results showed that lantana leaf essential oil (*Lantana camara*) is effective as a biolarvicide. The LC50 was 0.562% and LC90 was 1.203% at the 12th hour. The ANOVA test showed that there were significant differences in each concentration of 0.5%, 1%, 3%, 5%, negative control and positive control because the p-value was 0.000 <0.05. From the results of this study it can be said that the compounds contained in lantana leaf essential oil (*Lantana camara*) have the potential as a biolarvicide against *Aedes aegypti* larvae.

1 INTRODUCTION

Dengue bleeding fever (DBD) is still a major health concern and threat in some areas of Indonesia. DBD instances are still rising, particularly in the wet season. As of June 2022, the Ministry of Health had registered 45.387 instances of DBD cumulatively in Indonesia. However, there have been 432 occurrences of DBD-related deaths. There have been 449 DBD cases in 34 provinces' districts and cities, while 162 districts and cities in 31 provinces have reported DBD deaths. When an *Aedes aegypti* mosquito carrying the dengue virus bites, DBD can spread (Kemenkes, 2022).

DBD can be applied to vector management by dissecting the life cycle of the *Aedes aegypti* mosquito. There are four stages in the life cycle of the *Aedes aegypti* mosquito: eggs, larvae, pupae, and adult mosquitoes (Kurniawati, *et al.,* 2020). The most widely used DBD vector controllers available today still employ chemical larvasides. Chemicals called larvasides have the potential to make *Aedes aegypti* mosquitoes resistant to their use. Because chemical larvasides contain a number of dangerous active chemicals, including dichlorvos, porpoxure, and synthetic pyrethroids, they can be exceedingly harmful to human health. It is preferable to utilize biolarvasides made from plants that include active substances such as alkaloids, saponins, flavonoids, triterpenoids, and tannins in place of chemical larvae in order to reduce hazards in the future (Raini, Isnawati, and Herman, 2017; Nandita, *et al.,* 2019).

Plants such as lantana (*Lantana camara* L.), which have a strong odor and may act as an insect repellent, can be employed as biolarvasides. Leaves: The leaves of the plant have the potential to be employed as a biolarvaside. Essential oils, flavonoids, alkaloids, tannins, lantadine, and saponins are among the active substances found in lantana leaves (Purwati, Lumowa, and Samsurianto, 2017). Lantana is a plant that grows more easily, which contributes to its abundance. Still, the amount of deterioration of what is thought to be interfering plants is low enough to be able to transform them into more beneficial

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plants when used as a biolarvaside material (Nuraini, D., & Ratnasari, 2020). This study aims to determine the effects of the biolarvaside of the essential oil of Lantana leaf (*Lantana camara* L.) on the larva *Aedes aegypti.*

2 MATERIALS AND METHODS

The study is a quantitative study with the design of an experimental study that will look at the effects of the administration of essential oils of *Lantana camara* leaves (*Lantana camara* L.) on the larva *Aedes aegypti* which is a vector of DBD disease. The population in this study is the entire larva *Aedes aegypti* that has been kept from the egg stage to the larvae.

For this experiment, the following implements were required: measuring cups, beakers, pipettes, volume pipettes, winnowers, stopwatches, analytical balances, cameras, knives, label paper, filter paper, plastic containers, rulers, pencils, stirring rods, funnels, Erlenmeyer, and spatulas. For the materials used essential oils of *Lantana camara* leaves, *Aedes aegypti* mosquito larvae, distilled water, abate powder, Na_2SO_4 made in Indonesia by Braco (2208195445), and temephose.

In this study, simple random sampling was used to sample *Aedes aegypti* larvae in stages III and IV. There is an equal chance of being chosen as a sample for every *Aedes aegypti* larva in stages III or IV.

2.1 Determination

For such a determination, the parts of the lantana plant (*Lantana camara* L.) that have leaves, stems, roots, flowers, and fruits should be collected

2.2 Steam-Water Distillation of *Lantana camara* Leaf

This study used 50 kg of *Lantana camara*. The leaves were washed until clean with running water and then dried in a windy way until the leaves were half-dry.

The half-dried Lantana (*Lantana camara* L.) leaves weigh 40 kg and are then put into a kettle, the divided into the top and bottom. The top part of the filter is used to lay the lantana leaves, while the bottom part contains water heated to a boil at 100° – 105°C. The water vapor mixed with oil from the boiler is channeled toward the separator. When it enters a separator, the condensor channels cold air, there by turning the steam phase into liquid. The essential oil produced will be separated from the water due to the difference in weight between oil and water. Then the yield will be counted. After that, screening of essential oil compounds.

2.3 Sample Treatment

The number of samples that will be used in total for this study shows in table 1.

Table 1: Details of the sample treatment used:

Treatment	Number of larvae x Replicate	Total
K (-)	25 larvae x 4	100
K (+)	25 larvae x 4	100
E_1	25 larvae x 4	100
E_2	25 larvae x 4	100
E_3	25 larvae x 4	100
E_4	25 larvae x 4	100
Total Larvae		**600**

Description :
K (-): Negative control solution aquades
K (+): Positive control solution at 1%
E1: Lantana leaf essential oil solution, 0.5% concentration
E2: 1% concentration Lantana leaf essential oil solution
E3: Lantana leaf essential oil solution of 3% concentration
E4: Lantana leaf essential oil solution, 5% concentration

2.4 Rearing *Aedes Aegypti* Larva

The eggs of the mosquitoes *Aedes aegypti* are soaked in water that is in the container. The eggs will cough in ±24 hours to become stages larva I. Fish pellets are fed to the larvae during their development until they reach stages III and IV, which happens in around 7 days. The 25 larvae in stages III or IV are placed in a container that has been set up for the treatment.

2.5 Larvaside Effectiveness Test

In this study, six groups of treatments were performed. Each group performed four repetitions. For the negative control group, Aquadest was given 100 ml of water into the container and inserted 25 larvae. For the positive control group Temephos (1%) and the essential oil group of lantana leaves with concentrations of 0.5%, 1%, 3%, and 5%, respectively, diluted in water to reach a volume of 100 ml, insert 25 larvae of *Aedes aegypti*, respectively. Then observed every hour for 24 hours, then calculated the number of dead larvae with the mortality formula as follows:

$$\% \text{ Mortality} = \frac{\text{number of dead larvae}}{\text{Total of larvae used}} \text{ x } 100\%$$

When a concentration of larvasides can kill test larvae at a rate of greater than 95%, it is considered effective (WHO, 2005).

2.6 Data Analyze

Data in this study were obtained from the results of lantana leaf extract (*Lantana camara* L.) trials against *Aedes aegypti* stages III and IV larvae. The data obtained, i.e. the number of dead larvae, are analyzed using a computer program, SPSS 25. The data analyze performed is a probit test to determine the LC50. The results of the analysis are presented in tabular form.

3 RESULT AND DISCUSSION

3.1 Result of Determination Test

Based on the results of the determination test that has been carried out on Lantana or tembelekan plants, this is indeed a species of *Lantana camara* L. This is evidenced by the characteristics of the collected plant samples such as leaves, flowers, fruits, stems and roots that have been identified in the Botany Laboratory, Department of Biology FMIPA Unila.

3.2 Result of Yield

Table 2: Calculation of Yield Value

Sample Weight (g)	Essential Oil Volume (ml)	Yield Value (%)
40000	110	0.0275

Table 2 shows the amount of essential oil obtained from steam distillation of 110 ml lantana leaves with yield value of 0.0275%.

3.3 Mortality of *Aedes aegypti* Larvae Treated with Essential Oil of Lantana Leaves (*Lantana camara*) in Stages III and IV

In the results of the biolarvaside testing of the essential oil of the lantana leaf (*Lantana camara*) against the larvae instar III and IV, *Aedes aegypti* obtained death data in Figure 1.

In Figure 1, it shows that at a concentration of 5% in the ninth hour, the mortality value has been reached at 100%; at concentrations of 3%, it has reached a mortality rate of 100% in the eleventh hour; at a concentration of 1%, it has achieved mortality rates of 100% at the 13th hour; whereas at a concentration of 0.5%, it has a mortality rate of 100 percent at the 19th hour. At concentrations of 3% and 5%, the time to kill the entire larva used in the test is faster compared to concentrations of 0.5% and 1%. This means that the higher the concentration of the essential oil of the leaf of lantana used, the faster the time it takes for the killing of the larva *Aedes aegypti*. If you look at the overall data from concentrations of 1%, 3%, and 5% in the 1st hour to the 13th hour, mortality values are obtained between 10% and 100%. Whereas for the concentration of 0.5% effective as a biolarvaside from the 2nd hour because the mortality value is >10%. It is in line with the standard established by the WHO (2005) that a larvaside is said to be effective if it can kill mosquito

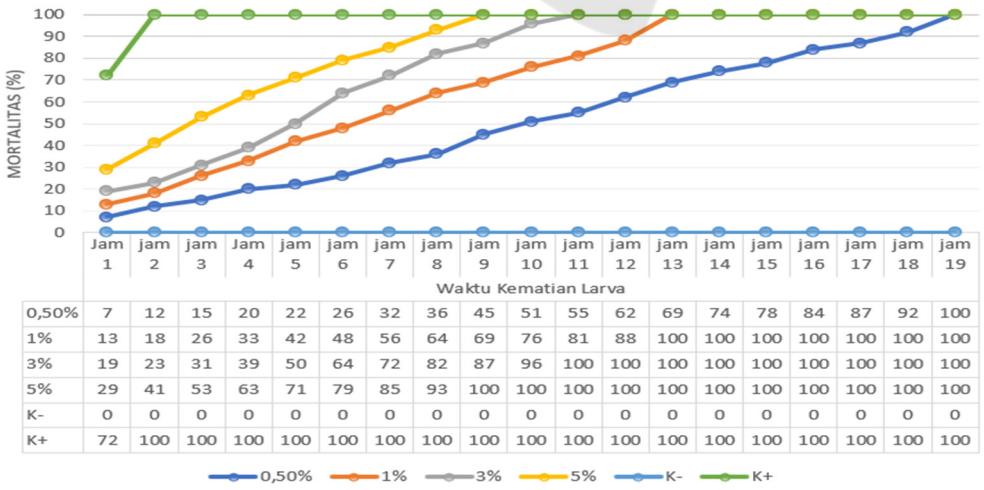

Figure 1. Mortality of instar larva III and IV *Aedes aegypti* that have been given essential oil of lantana leaves (*Lantana camara*)

larvae between 10% and 95% of the total larvae available. Observations of the larvae's deaths were only carried out until 19th hours because, at the time, all larvae at each concentration were 100% dead.

The results of this study showed that the essential oil of the leaf of lantana was effective in killing the larve of *Aedes aegypti*. In this study, the negative control group (water) did not cause the larvae to die, which means that the animal tries to be well controlled and there are no other factors causing the death of larvae.

The mechanism by which the essential oil kills larvae is by entering the body of the larva through the respiratory system, resulting in nervous disorders and damage to the breathing system, as a result of which larvae die because they can't breathe. Lantana leaf essential oils are known to have major contents such as β-caryophyllene (35.70%), caryophyllen oxide (10.04%), caryofyllene (16.37%), β-elemene (6.41%), germacrene-D (15.85%), α-humelene (9.31%), germacren (6.19%), and eucalyptol (10.75%). The compounds β-caryophylene, caryphyllene oxide, caryopyllene, germacreene-D, germcrene, and eukalyptole found in the leaf essence are potentially known as larvasides. In addition, β-caryophyllene can inhibit the growth of mosquito larvae by interfering with the synthesis of protein and DNA. Eucalyptol is capable of killing larvae by disrupting the nervous arrangement of the larva and as an abdominal poison that can interfere with larval nutrition so that larval growth becomes inhibited and eventually leads to death.

Table 3: Result of Probit Test

Observation hours	LC_{50} (%)
1st hour	24,795
2nd hour	7,460
3rd hour	6,764
4th hour	5,318
5th hour	2,047
6th hour	1,346
7th hour	0,962
8th hour	0,736
9th hour	0,734
10th hour	0,732
11th hour	0,684
12th hour	0,562

Based on Table 3, it shows that during the observation time of 12 hours, a smaller LC_{50} value was obtained for the concentration that can be used in killing larvae, as much as 50% of the total larvae existing. The LC_{50} values can only be tested until observation at 12th hours, since from 13th hours to 19th hours, each concentration of the existing mortality values has the same value. So, when performing the probit test, the LC_{50} value cannot be processed.

The maximum concentration limit for the larvaside used to kill the larva is 1% (26). The LC_{50} value at the 12th hour is 0.562, so the essential oil of the lantana leaf can be said to be effective as a biolarvaside because the concentration can kill as much as 50% of the number of existing larvae (<1%). In the results of this study, the use of biolarvase of the essence of the leaf of lantana for 7th hours resulted in LC_{50} of 0.962%. However, for the concentrations of 3% and 5% used in this study, when compared with the maximum limit of concentration according to the WHO (2005), it may be said that such concentrations exceed the limit of maximum larvacide concentration.

The biolarvacide concentrations of lantana leaf essential oils in this study used concentrations of 0.5%, 1%, 3%, and 5%. At concentrations of 3% and 5%, the lantan leaf essence oils exceeded the specified concentration limits for the use of larvacides. However, according to the WHO (2005), the standard is used for chemical larvacide. So far, there has been no further study that specifies the maximum standard for the use of biolarvacides. The use of biolarvacide has a smaller long-term effect on the environment when used over long periods because the residues of the biolarvacide will evaporate in the environment so there are no adverse effects on public health when using the biolarvacide essential oil of lantana leaves. In addition, the use of essential oil biolarvacide can reduce the impact of mosquito larvae's resistance to chemical larvicides.

4 CONCLUSIONS

Biolarvacide of Lantana leaf essential oil (*Lantana camara*) is effective in killing *Aedes aegypti* larvae with 100% mortality value at 0.5% concentration in 19th hour, 1% concentration in 13th hour, 3% concentration in 11th hour and 5% concentration in 9th hour.

The LC_{50} of Lantana leaf essential oil (*Lantana camara*) is effective as a biolarvacide from the 7th to the 12th hour at a concentration of <1%. The lower the LC_{50} value, the more toxic it is to the larvae. The smallest LC_{50} obtained was 0.562% at the 12th hour with a highly toxic category.

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