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Utilization of Basil Leaf Extract as Anti-Mosquito Repellent: A Case Study of Total Mosquito Mortality (*Aedes aegypti* 3rd Instar)

Ismalia Husna^{1*}, Endah Setyaningrum², Tundjung Tripeni Handayani², Yogi Kurnia³, Endah Kinarya Palupi⁴, Rofiqul Umam⁵, Bibin Bintang Andriana⁵

¹Department of Medical Education, Faculty of Medicine, Universitas Malahayati, Lampung, Indonesia

²Department of Biology, Universitas Lampung, Bandar Lampung, Indonesia

³Department of Biology Education, Universitas Islam Negeri Raden Intan Lampung, Indonesia

⁴Faculty of Mathematics and Science, Institut Pertanian Bogor, Bogor, Indonesia

⁵School of Science and Technology, Kwansai Gakuin University, Sanda, Japan

* ismalia@malahayati.ac.id

Abstract. This study aims to determine the most effective concentration of extract that kills the larvae in the third instar *Aedes aegypti*, knowing the value of LC₅₀ and LC₉₀ anthen knowing the value of LT₅₀ and LT₉₀ of forest basil leaf extract (*Ocimum sanctum*). Research is using a completely randomized design with factorial, and the first factor is forest basil leaf extract with 5 level concentrations, there are 0.3%, 0.6%, 0.9%, 1.2%, 1.5%, and 0% as control, whereas the second factor is observation period that began after the death of the larvae. Observations were made up to 4320 minutes. The results showed the influence of forest basil leaf extract against third instar larvae mortality of *Aedes aegypti*, and the most effective concentration of basil leaf extract to kill the larvae was 1.5%. LC₅₀ values of this study was 0.97%, and for the LC₉₀value was 1.42%. Value of LT₅₀and LT₉₀of this study is 5,71 hours or 342,31 and 17,02 hours or 1021.22 minutes. The conclusion of this study is basil leaf extract forest affect mortality third instar larvae of *Aedes aegypti*, and the most effective concentration of extract was 1.5%.

1. Introduction

Dengue Hemorrhagic Fever (DHF) is a health problem, especially in countries that have a tropical climate. From 1968 to 2009, the World Health Organization (WHO) recorded Indonesia as the country with the highest DHF cases in Southeast Asia. DHF disease in Indonesia was first discovered in the city of Surabaya in 1968 total 58 people infected and 24 people died. Since then DHF has spread widely throughout Indonesia. Even until the end of 2018, there were no drugs that could effectively treat DHF [1].

Eradication of *Aedes aegypti* is the main way to eradicate DHF. Eradication can be done on adult mosquitoes by spraying (fogging), with insecticides by organophosphate, synthetic pyrethroids, and carbamates. While the eradication of the larvae can be done by using larvicides, known as abatinization. Larvaside which is often used is temephos [2].



The use of plant-based insecticides is an alternative in controlling *Aedes aegypti* larvae. Vegetable insecticide is an insecticide made from active compounds of plant secondary metabolites that is able to provide one or more biological activities [3], both influences on aspects of physiology and behavior of insects, such as inhibition of feeding and spawning activities, regulating growth and development of insects, death or mortality, etc. [4].

One of the plants that have potential as natural larvicides is the basil leaves of the forest (*Ocimum sanctum*). Based on studies on the genus *Ocimum*, this plant contains alkaloids [5], flavonoids, tannins, saponins, triterpenoids, and essential oils [6]. So, this research was held to see the ability of the extract of the basil leaves of the forest (*Ocimum sanctum*) as larvaside against third instar larvae of the *Aedes aegypti* mosquito [7].

In previous studies, which have been conducted by researchers in various parts of the world, one of them is in India [8]. Basil leaves have great potential in killing dengue mosquitoes. In addition, the results of the research prove that, some people whose intensity of consuming basil leaves are quite high, are more awake from DHF due to *Aedes aegypti* mosquitoes [9].

Instar III larvae are considered sufficient to represent the condition of larvae with a size that is not too small so it is easy to observe [10], and can be used as research material because in this phase the larvae are very active in moving and foraging on water media [11]. On this basis, larvicide was created to break the life cycle of the *Aedes aegypti* mosquito.

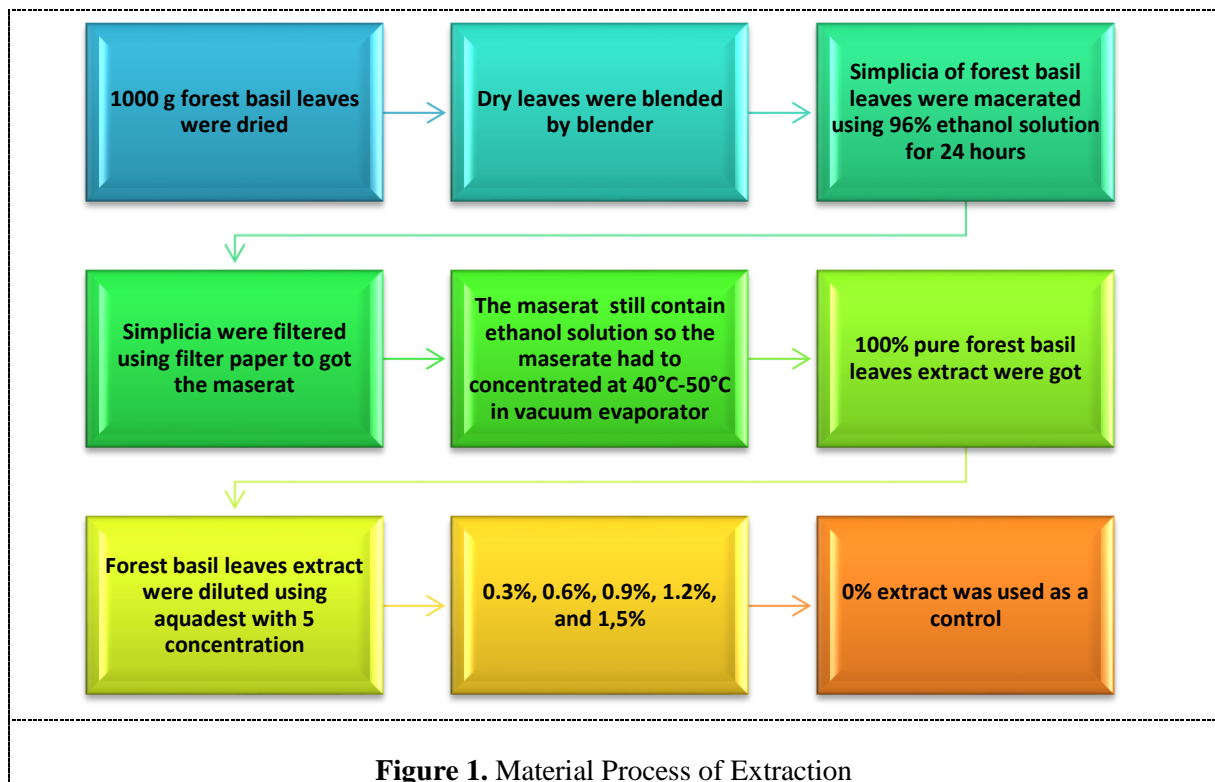
2. Method

The equipment used in this study are measuring cups, glass vessels, stirrers, filter paper, vacuum rotary evaporators, plastic trays, plastic cups, drop pipettes, and stopwatches. Meanwhile, the materials used in this study were forest basil (*Ocimum sanctum*) which had bottle green leaf and people in Sumatera, Indonesia usually call it "ruku-ruku", instar III *Aedes aegypti* larvae, ethanol as a solvent, and distilled water as extract thinner.

Manufacture of forest basil leaf extract using the method used by Harboneon [12]. Forest basil leaf extraction using the method used by Deboun and Stirckman [13]. 1000 gram fresh forest basil leaves are cleaned with water and dried by aerating for 7x24 hours. Furthermore, the simplicia of the basil leaves was macerated for 24 hours using 96% ethanol solution. After maceration, the results are filtered, then the maserat is concentrated at 400C-500C in a vacuum evaporator so that 100 grams of concentrated extract of basil leaves from the forest are produced with a concentration of 100%. Then the concentrated extract was diluted with distilled water five times with each concentration of 0.3%, 0.6%, 0.9%, 1.2%, 1.5% and 0% as a control (Figure 1).

This research was conducted on December 2017 to December 2018 (a year) at the Zoology Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, and the production of *Ocimum sanctum* leaf extracts was carried out at the Laboratory of Organic Chemistry, Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Lampung.

Then the third instar larvae of *Aedes aegypti* were put into a plastic cup containing 200 ml of a mixture of water and extracts of forest basil leaves with concentrations of 0.3%, 0.6%, 0.9%, 1.2%, 1.5% and 0% as control for 20 heads larvae with 4 repetitions. And according to [14], observations were made by counting dead larvae at each treatment at intervals of 5, 10, 20, 40, 60, 120, 240, 480, 1440, 2880, and 4320 minutes. The data obtained were analyzed using ANOVA. LC50 and LC90 values, and LT50 and LT90 values were analyzed using Probit Test.



3. Result and Discussion

3.1. Effectiveness Test

Effectiveness test was conducted to find out how much influence the treatment to the death of instar III larvae of *Aedes aegypti*. Based on experimental results, it is known that the percentage of death of instar III larvae of *Aedes aegypti* at various concentrations of extracts of forest basil (*Ocimum sanctum*) can be seen in Table 1.

Table 1. Percentage average mortality of *Aedes aegypti* larvae at various concentrations of *Ocimum sanctum* extracts.

Concentration (%)	Average larval mortality (%) at a certain minute											
	5	10	20	40	60	120	240	480	1440	2880	4320	
0	0	0	0	0	0	0	0	0	0	0	0	
0.3	0	0	0	0	0	0	0	3.75	5.00	11.25	20.00	
0.6	0	0	0	0	0	0	0	0	0	5.00	26.25	
0.9	0	0	0	0	0	0	0	0	0	2.5	42.50	
1.2	0	0	0	0	0	0	0	1.25	5.00	15.00	46.25	
1.5	0	0	0	0	0	15.00	31.25	58.75	100.00	100.00	100.00	

Death of test larvae was found in the treatment group that was given forest basil leaf extract (*Ocimum sanctum*). At a concentration of 0.3% larvae, mortality begins in the 480th minute. Whereas at concentrations of 0.6% and 0.9% larval death begins in the 2880th minute. Then at a concentration

of 1.2% larval death begins in the 480th minute, and at a concentration of 1.5%, larvae mortality begins at the earliest, 120th minutes.

At a concentration of 0.3%, the extract of the forest basil leaves is still watery and the odor is not too pungent, therefore the third instar larvae of *Aedes aegypti* continue to eat without being disturbed by a good concentration of the extract. This caused death due to the nature of the forest basil leaf extract as a stomach poison. According to Corradini et al, [15], the poison in the extract of the basil leaves which is eaten by the larvae, enters the digestive organs and then absorbed by the intestinal wall, then released with blood that will interfere with the larvae, so these larvae. While at concentrations of 0.6% and 0.9% forest basil leaves extract is more concentrated and tends to be quite pungent odor, this causes difficulties when the larvae do not use forest basil leaf extract for a while, at this time the larvae remain ready to extracting basil leaves from the forest, therefore the death of larvae was encountered a little longer at 2880th minutes.

At a concentration of 1.2% of 87 forest basil leaf extract, larval death had begun in the 480th minute. This shows that forest basil leaf extract as a contact poison. According to [16], contact poison is an insecticide that enters the body of the insect through the skin, natural holes in the body (trachea) or directly on the mouth of the insect and most contact poison is stomach poison.

Whereas at the 1.5% concentration of forest basil leaf extract, the fastest larval mortality was found at the 120th minute and the highest total larval mortality was 100%. This shows the extract of forest basil leaves as respiratory poisons (fumigants). The higher the concentration of the extract, the more concentrated and stinging the odor it causes. [17] said that the extract of the basil leaves of the forest (*Ocimum sanctum*) apart from being a stomach poison and contact poison, indirectly functions as a fumigant, because the odor caused is thought to be due to bioactive compounds which evaporate as gases. According to [12], fumigants are volatile insecticides that become gas and enter the insect's body through the respiratory system or trachea system which are then circulated throughout the body. Insecticides that affect the respiratory system of insects play a role in inhibiting respiratory enzymes, which can cause death to these insects[3]. The average number of instar larvae III *Aedes aegypti* mortality after extracting forest basil leaves can be seen in Table 2. Furthermore, the observational data were analyzed using ANOVA test followed by LSD test.

Table 2. Average number of deaths of instar iii larvae of *Aedes aegypti* after extracting forest basil basil (*Ocimum sanctum*)

Concentration (%)	Duration of contact time (minutes)					
	120	240	480	1440	2880	4320
0.3	0.00 a	0.00 a	0.75 a	1.00 a	2.25 ab	4.00 bc
0.6	0.00 a	0.00 a	0.00 a	0.00 a	1.00 a	5.25 ce
0.9	0.00 a	0.00 a	0.00 a	0.00 a	0.50 a	8.50 df
1.2	0.00 a	0.00 a	0.25 a	1.00 a	3.00 e	9.25 f
1.5	3.00 e	6.25 d	11.75 g	20.00 h	20.00 h	20.00 h

Note: the numbers in the columns that are not followed by the same lowercase letters are significantly different at the 5% (2.2) and 1% (3.0) odds in the LSD test

Based on Table 2 it can be seen that the 1.5% extract concentration in the 1440th minute is the highest extract concentration and the fastest duration of contact time in causing death to instar larvae III *Aedes aegypti*. Forest basil extract (*Ocimum sanctum*) which has an influence on the death of instar III larvae of *Aedes aegypti* is suspected due to the presence of chemical compounds contained in forest basil leaves which function as larvasides. As revealed by Gnankiné O and Bassolé, forest basil leaves (*Ocimum sanctum*) contain chemical compounds that have the potential as larvicides and are toxic to larvae, namely flavonoids, saponins, tannins, and essential oils with eugenol as their main components [18].

Flavonoids are plant defense compounds that can inhibit the appetite of insects. Saponins can inhibit proteolytic action which causes a decrease in digestive enzyme activity and protein use. Tannin can reduce the ability to digest food in insects by reducing the activity of digestive enzymes. Eugenol acts as a stomach poison and inhibits taste receptors in the mouth of larvae [19].

3.2. Lethal Concentration 50% (LC50) and 90% (LC90)

LC50 and LC90 values are concentrations of forest basil leaves extract that can kill 50% and 90% of the larvae of the tested larvae. LC₅₀ and LC₉₀ values were obtained from probit analysis. More can be seen in Table 3.

Table 3. Value of LC₅₀ and LC₉₀ Instar III *Aedes aegypti* Larvae at Various Observations Times

No	Time (minutes)	LC ₅₀ (%)	LC ₉₀ (%)
1	120	1,79	2,13
2	240	1,64	1,94
3	480	1,49	1,77
4	1440	1,37	1,48
5	2880	1,28	1,45
6	4320	0,97	1,42

Based on the data obtained, the LC50 value of this study was 0.97% at 4320th minutes. It shows that the concentration of 0.97% has been able to cause the death of instar III larvae of *Aedes aegypti* by 50% of the total larvae tested. The LC50 value is in accordance with previous studies on the potential effectiveness of combined larvae of forest basil (*Ocimum sanctum*) and castor bean seeds (*Ricinus communis*) (Gunawan, 2011), the LC50 value obtained for forest basil leaves (*Ocimum sanctum*) was 0.971%. Whereas in the fastest time, the 120th minute in the research that has been done, it takes an extract concentration of 1.79% to reach LC50. While the LC90 value of this study was 1.42% at 4320th minutes. It means, to kill larvae as much as 90% of the total larvae tested need a concentration of 1.42%. And in the 120th minute, it takes an extract concentration of 2.13% to reach LC90.

LC50 and LC90 values obtained from probit analysis showed that the longer the concentration of *Ocimum sanctum* extract was given to the third instar larvae of *Aedes aegypti*, the smaller the concentration needed to kill 50% and 90% of the larvae [20].

3.3. Lethal Time 50% (LT50) and 90% (LT90)

The LT50 and LT90 values are the time needed to kill 50% and 90% of larvae at a given solution concentration. LT50 and LT90 values calculated using probit analysis can be seen in Table 4.

Table 4. Values of LT50 and LT90 mortality of larvae instar iii *Aedes aegypti* at various concentrations

No	Concentration	LT ₅₀ (minutes)	LT ₉₀ (minutes)
1	0.3%	407.59	4620.44
2	0.6%	262.59	1649.69
3	0.9%	129.98	587.12
4	1.2%	112.86	516.15
5	1.5%	5.71	17.02

The LT50 and LT90 values respectively indicate that the concentration of 0.3% to the highest concentration used is the 1.5% concentration indicating a decrease. The LT50 value obtained was 5.71 hours or 342.31 minutes and the LT90 value of 17.02 hours or 1021.22 minutes was at a concentration of 1.5%. And it appears that at other concentrations of 0.3%, 0.6%, 0.9%, and 1.2% do not have an effective time in causing the death of instar larvae III *Aedes aegypti* because it exceeds the observation time limit of 4320 minutes[21].

This shows that of the various concentrations used, 0.3%, 0.6%, 0.9%, 1.2%, and 1.5%, it is known that the extract of the forest basil (*Ocimum sanctum*) which can quickly cause death for larvae instar III *Aedes aegypti* is the concentration 1.5% and this concentration is the most effective concentration[22].

LT50 and LT90 values obtained from probit analysis showed that the greater the concentration of the extract of the basil leaves of the forest, the less time needed to cause the death of larvae 50% and 90% of the total larvae tested[23].

4. Conclusion

Based on experimental results, it can be concluded that the extract of the forest basil (*Ocimum sanctum*) with a concentration of 1.5% is effective in killing the larvae of instar III *Aedes aegypti*. LC50 value of forest basil (*Ocimum sanctum*) extract as larvaside against *Aedes aegypti* III instar larvae was 0.97% at 4320 minutes, while the LC90 value of forest basil extract (*Ocimum sanctum*) was 1.42%. The value of LT50 and LT90 extracts of forest basil (*Ocimum sanctum*) as larvaside against *Aedes aegypti* III instar larvae were 5.71 hours 342.31 minutes and 17.02 hours or 1021.222 minutes at a concentration of 1.5%.

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