THE BIOLARVICIDAL EFFECTIVENESS OF AVOCADO SEED ETHANOL EXTRACT IN GRANULE FORM ON HIGHEST MORTALITY PERCENTAGE WITH LETHAL TIME

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ABSTRAK

Demam berdarah dengue (DBD) mengalami peningkatan kasus seiring terjadinya perubahan iklim. Hal ini menjadi ancaman bagi masyarakat, tingkat kematian mencapai 1.237 dari kasus 143.266 kasus DBD pada tahun 2022. Pengendalian larva penting demi mencegah perkembangannya menjadi vektor. Penggunaan larvasida kimia telah diteliti menyebabkan resistensi pada larva. Permasalahan tersebut mendorong adanya usaha kami untuk memberantas larva *Aedes aegypti* dengan mengidentifikasi potensi senyawa pada biji alpukat (*Persea americana* Mill.) sebagai salah satu tanaman komoditi di Aceh. Penelitian ini merupakan penelitian eksperimental yang bertujuan untuk mengetahui potensi biolarvasida ekstrak etanol tanaman dalam sediaan granul terhadap larva Ae. aegypti dengan konsentrasi ekstrak bertingkat 12,5%, 25% dan 50%. Penelitian ini menggunakan desain Rancangan Acak Lengkap dan dianalisis secara regresi linear serta probit LT 50 dan LT 95. Hasil penelitian menunjukkan persentase kematian tertinggi pada konsentrasi 25% yaitu sebesar 50,6% kematian larva. Nilai estimasi *lethal time* pada konsentrasi 25 % adalah LT_{50} = 311.112801 dan LT_{95} = 500.379746 yang berarti memerlukan waktu ±12 jam untuk membunuh 50% dari 75 larva dan ±20 jam untuk membunuh 95% larva.

Kata Kunci: Demam Berdarah, *Lethal Time*, Aedes aegypti, Biolarvasida, *Persea americana* Mill.

ABSTRACT

Dengue Hemorrhagic Fever (DHF) cases have increased along with climate change. This can be a threat to society, the death rate has reached 1.237 out of 143.266 cases of dengue fever in 2022. Control of larvae is important to prevent their growth into vectors. The use of chemical larvicides has been studied and shows resistance in larvae. This problem encourages us to eradicate Aedes aegypti larvae by identifying potential compounds in avocado (*Persea americana* Mill.) seed as one of most potential commodities in Aceh. This study is an experimental study that aims to determine the biolarvicide potential of ethanol extract in granule form against Ae. Aegypti larvae with multigrade 12.5%, 25% and 50% concentrations of extract. This study used a Completely Randomized Design that analyzed by linear regression and probit LT 50 and LT 95. The results showed the highest percentage of mortality at a concentration of 25% which resulted in 50,6% larval mortality. The estimated lethal time value at 25% concentration is LT_{50} = 311.112801 and LT_{95} = 500.379746 which means it takes ±12 hours to kill 50% of 75 larvae and ±20 hours to kill 95% of larvae.

Keyword **:** Dengue Hemorrhagic Fever, Aedes aegypti, Biolarvicide, Lethal Time, *Persea americana* Mill.

A. INTRODUCTION

Dengue is an infectious disease caused by dengue virus (DENV) and transmitted through mosquito bites (arboviral disease). Two arbovirus vectors in dengue virus infection cases are Aedes aegypti and Aedes albopictus (World Health Organization, 2011; Zulfa et al., 2022). Aedes aegypti is the primary vector of dengue cases due to the tendency of female Aedes aegypti mosquitoes to suck human blood rather than animal blood (anthropophilic) for their ovulation cycle (gonotrophic cycle) (Rezza, 2012).

Dengue virus infection causes plasma leakage resulting in massive blood loss and even death due to profound shock(World Health Organization, 2011). WHO reported 8 times of increasing cases in the last 2 decades following the increase in population movement, environmental changes and climate change. According to the Ministry of Health, there were 73.518 cases of dengue fever in 2021 with 705 deaths. Cases increased in 2022 with a total of 143.266 cases and 1.237 deaths. (Kemenkes RI, 2022)

One of the methods that have been implemented to reduce the mortality rate of DHF is vector control using chemical insecticides in order to reduce transmission cases by inhibiting the growth and development of vectors. The use of chemicals insecticides such as pyrethroids, organophosphates, organochlorines, and carbamates has been around for decades, and several recent studies have shown the level of resistance to these insecticides, especially in various regions of Indonesia. Aceh province is one of the provinces with the highest level of resistance to deltamethrin (Silalahi et al., 2022). The development of new types of insecticides may offer a solution to the increasing resistance to these insecticides.

Indonesia is also faced with environmental problems in addition to climate change, which is high levels of organic waste. Organic waste comes in the form of parts of fruits or vegetables that are not consumed such as stems, leaves, and seeds. Avocado fruit as one of the most commodities in Aceh that is often consumed by the community contributes to increasing its organic waste because part of the avocado fruit is generally only the flesh and skin of the fruit that is consumed while the avocado seed is wasted.

The problems of insecticide resistance and organic waste are being considered in this study to find alternative solutions. The development of new types insecticides can be done by utilizing avocado seeds because each part of the plant contains phytochemicals that can be used to kill larvae so that it is called a biolarvicide.

Exposure to biolarvicides on vector larvae is considered effective because it can suppress the growth and development of larvae so that they do not develop into adult mosquitoes, the larval stage has also reached the phase of complete body parts so that it can receive stimulation from these phytochemical compounds (Utami & Porusia, 2023).

In this research experiment, biolarvicides were made by utilizing ethanol extract of avocado seeds and then formed into granules in 3 different concentrations. The granule form will simplify its use when it becomes a product. This biolarvicide will then be tested to calculate its potential in killing larvae at $LT_{50,95}$.

B. RESEARCH METHOD

Time and Place of the Research

This research was conducted from August 2023 to May 2024. Samples of Aedes aegypti larvae for larvicide efficacy tests were taken from clean water containers in community residences in Banda Aceh City. Species identification and efficacy tests will be carried out at the Parasitology Laboratory of the Faculty of Medicine, Abulyatama University..

Avocado Seed Extraction

Extraction begins with making simplisia, avocado seeds are sliced and dried in an incubator at 20°C for 24 hours to reduce water content. Then the maceration process is carried out by soaking the simplisia in 96% ethanol for 72 hours and storing it at temperatures below -10°C. The results of the soak were filtered and then evaporated with a certain method in the Chemistry Laboratory of Syiah Kuala University until a thick avocado seed extract was obtained.

Phytochemical Compound Testing of Avocado Seeds

Phytochemical content tests were carried out at the Chemistry Laboratory of Syiah Kuala University by mixing reagents and using certain methods to see positive results from testing the compounds contained.

Avocado Seed Efficacy Testing

The efficacy test was conducted to obtain the $LT_{50,95}$ value. The test used a completely randomized design (CRD) with each concentration tested in stages, namely 12.5%, 25%, and 50%. The test was conducted by placing 25 third instar test larvae in a

50ml solution of extract concentration for each repetition. Observations of larval mortality began at the 10th, 20th, 30th, 40th, 50th, 60th, 2nd hour, 4th hour, 6th hour, 8th hour, and 24th hour after contact. If the control mortality is more than 10%, then it will be corrected by Abbot's formula (Susanti & Boesri, 2012).

Preparation of Avocado Seed Granulation

The method of making biolarvicidal wet granulation by mixing the results of the thick extract of evaporated avocado seeds with chemicals ingredients, such as aqua bidestillate, lactose, magnesium stearate, polyvinyl piropidol / PVP to make it stick together and form lumps, then molded using a 20 mesh sieve. Then the mixture was oven with the aim of evaporating the water content and organic solvents so that dry granulation was formed. The drying temperature was 40^0C for 8 hours.

Data Analysis

Analysis of $LT_{50,95}$ was analyzed by probit regression and linear regression using SPSS application. Assumption tests were also carried out so that the probit data generated in this study could be continued with a simple linear regression test. Granule analysis was analyzed univariately and interpreted in table form.

C. RESULTS AND DISCUSSION

Phytochemical Test Results

After conducting phytochemical testing on avocado seed extract, positive results were obtained for alkaloid compounds (dragendrof, mayer and wagner), saponins, polyphenols, quinones and triterpenoids. Alkaloids work by disrupting major cellular and physiological functions by affecting AChE receptors in the nervous system, regulating hormone activity, and causing toxicity (Balachandran et al., 2021). Saponins work by damaging the cell structure on the surface of the mucous membrane of the larval digestive tract, so that the walls of the larval digestive tract become easily damaged (Rohmah et al., 2020). Triterpenoids have a similar structural form to animal steroids and other steroid hormones. This structural form found in some subclasses of triterpenoids such as phytoecdysones works by mimicking molting hormones so that it can disrupt larval development and cause death (Abubakar et al., 2019).

Mortality Percentage from Efficacy Test Results

Tabel 1. Average results and Percentage Mortality of Aedes Aegypti Larvae at Various Concentrations of Ethanol Extract of Avocado Seed

Concentration (%)	Treatment	Sample	Mean And Percentage of Larval After Exposure with Avocado Seed Extract in Minute												Mean Mortality at 1440th Minute ± Standard Deviation	Percentage of Death at 1440th Minute
			10	20	30	40	50	60	120	180	240	360	720	1440		
12.5	1	25	0	0	0	0	0	0	0	0	0	1	1	2	2 ± 0	8%
	2	25	0	0	0	0	0	0	0	0	0	1	1	2		
	3	25	0	0	0	0	0	0	0	0	0	0	1	2		
Total			0	0	0	0	0	0	0	0	0	2	3	6		
Average Death per Time			0	0	0	0	0	0	0	0	0	0,67	1	2		
25	1	25	0	0	0	0	0	0	1	1	2	4	7	13	12,67 ± 1,53	50,6%
	2	25	0	0	0	0	0	0	1	1	3	3	5	11		
	3	25	0	0	0	0	0	0	0	0	2	4	7	14		
Total			0	0	0	0	0	0	2	2	7	11	19	38		
Average Death per Time			0	0	0	0	0	0	0,67	0,67	2,33	3,67	6,33	12,67		
50	1	25	0	0	0	0	0	0	1	1	1	2	6	8	10,33 ± 2,08	41,3%
	2	25	0	0	0	0	0	0	1	1	1	1	5	11		
	3	25	0	0	0	0	0	0	1	1	1	1	6	12		
Total			0	0	0	0	0	0	3	3	3	4	17	31		
Average Death per Time			0	0	0	0	0	0	1	1	1	1,33	5,67	10,33		

The table explains that this study has 3 treatment groups, each group is repeated 3 times with concentration groups of 12.5%, 25% and 50% in each group. Each repetition was observed in minutes for 24 hours. The results showed that at 25% concentration, the highest percentage of larval mortality occurred at 50.6%. While at 50% concentration the percentage of mortality was 41.3%, and the percentage of mortality was 8% at 12.5% concentration.

Lethal Time Probit Analysis Results

Tabel 2. Probit Analysis Results on Lethal Time $(LT)_{50,95}$ of Ethanol Extract of Avocado Seeds

Concentration (%)	Treatment	Sample	Mean And Percentage of Larval After Exposure with Avocado Seed Extract in Minute												Lethal Time (LT)
			10	20	30	40	50	60	120	180	240	360	720	1440	
12.5	1	25	0	0	0	0	0	0	0	0	0	1	1	2	LT 50: 1630.302285 LT 95: 2714.507034
	2	25	0	0	0	0	0	0	0	0	0	1	1	2	
	3	25	0	0	0	0	0	0	0	0	0	0	1	2	
Total			0	0	0	0	0	0	0	0	0	2	3	6	
Average Death per Time			0	0	0	0	0	0	0	0	0	0,67	1	2	
25	1	25	0	0	0	0	0	0	1	1	2	4	7	13	LT 50: 311.112801 LT 95: 500.379746
	2	25	0	0	0	0	0	0	1	1	3	3	5	11	
	3	25	0	0	0	0	0	0	0	0	2	4	7	14	
Total			0	0	0	0	0	0	2	2	7	11	19	38	
Average Death per Time			0	0	0	0	0	0	0,67	0,67	2,33	3,67	6,33	12,67	
50	1	25	0	0	0	0	0	0	1	1	1	2	6	8	LT 50: 457.208665 LT 95: 788.436442
	2	25	0	0	0	0	0	0	1	1	1	1	5	11	
	3	25	0	0	0	0	0	0	1	1	1	1	6	12	
Total			0	0	0	0	0	0	3	3	3	4	17	31	
Average Death per Time			0	0	0	0	0	0	1	1	1	1,33	5,67	10,33	
P-value															P1 = 0,000 P2 = 0,000 P3 = 0,000

The table shows that the results obtained from the three treatment groups during the 24-hour experimental period, the value of the estimated lethal time in treatment group 1 with a concentration of 12.5% is LT_{50} = 1630.302285 and LT_{95} = 2714.507034, meaning that it takes ±67 hours to kill 50% of 75 larvae, and ±113 hours to kill 95% of larvae. Furthermore, in treatment group 2 with a concentration of 25%, the LT_{50} = 311.112801 and LT_{95} = 500.379746 values were obtained, which means it takes ±12 hours to kill 50% of 75 larvae, and ±20 hours to kill 95% of larvae. In treatment group 3 with a concentration of 50%, the LT_{50} = 457.208665 and LT_{95} = 788.436442 values were obtained, which means it took ±19 hours to kill 50% of 75 larvae, and ±32 hours to kill 95% of the larvae.

Thus, it can be concluded that the high and low percentage of concentration used will affect the death of larvae. Statistical test results obtained based on time and concentration are significant with a confidence level of p (0.05), seen from the p value <0.05.

Linear Regression Analysis Results

Table 3. Regression test results of the effect of time on probit

Time	Probit		
	Concentration 1 (12,5%)	Concentration 2 (25%)	Concentration 3 (50%)
10	0	0	0
20	0	0	0
30	0	0	0
40	0	0	0
50	0	0	0
60	0	0	0
120	0	3,0732	3,2493
180	0	3,0732	3,2493
240	0	3,6775	3,2493
360	3,0732	3,9506	3,3836
720	3,2493	4,3349	4,2512
1440	3,5949	5,0175	4,7802
P-Value	0,000	0,004	0,005
Regression Equation	Y= -0,003+0,003x	Y = 0,908+0,004x	Y = 0,883+0,004x
R	0,854	0,757	0,751
R^2	0,729	0,574	0,564

Based on the table above, avocado seed ethanol extract is effective as a larvicide in controlling dengue vectors with a sig value <0.05 and the rate of influence in treatment group 1 is 0.854 (72.9%), treatment group 2 is 0.757 (57.4%), and treatment group 3 is 0.751 (56.4%). This means that the ethanol extract of avocado seeds has a strong influence on larval mortality at any time, so that the longer it is used, the higher the larvicide mortality rate.

The model obtained is Y = -0.003 + 0.003x, Y = 0.908 + 0.004x, Y = 0.883 + 0.004x, which means that every additional unit of time, the possibility of larval mortality will also be greater. Based on this, it appears that avocado seed ethanol extract has acute toxicity and is included in the highly toxic criteria in the strong category.

D. CONCLUSION

Avocado seed ethanol extract is effective as a larvicide with the highest percentage of mortality at the second concentration (25%) resulting in 50.6% larval mortality. The effect of avocado seed ethanol extract on larval mortality is in the strong category at each time, where the more time used, the higher the larvicide mortality rate. The regression model obtained is Y = 0.908 + 0.004x at the second concentration, indicating that if one unit of time increases, the probit value of larval mortality also increases. Based on this, it appears that avocado seed ethanol extract has high toxicity.

Suggestions for future researchers who will conduct research on the same topic are advised to increase the dose of extracts in the mixture to get more varied research results.

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