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Toxicity Test of Difference in Concentration of *Aloe Vera (Aloe vera*) as a Larvicide *of Aedes aegypti*

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ABSTRACT

The dengue virus that causes dengue fever, which is very deadly, has not been resolved. The prevalence is very high. So far, the way to break the chain of transmission is to eradicate the vector. The chemical control applied causes many disadvantages, so the active ingredients of aloe vera extract can be used as a safer alternative to natural insecticides. This study aims to determine the difference in the concentration of *Aloe vera* extract as a larvicide. Experimental design with *post test only control group design,* sample of 600 instar III larvae, in 4 groups, 6 replications (negative control, 10% extract, 20% extract, 30% extract). Sample technique by *purposive sampling* method. The larvae were put in a test extract mixture of 100 ml. Observations were made 60 minutes after the treatment and the number of larval deaths was calculated. The percentage of larval mortality 60 minutes after treatment was 0% in control, 69.2% in 10% extract, 76% in 20% extract, 86.6% in 30% extract. Results were obtained with significant differences in the treatment groups. Aloe vera extract solution is a larvicide against *Aedes aegyti*.
Keywords: *Aloe Vera, larvasida, aedes aegypti*

PRELIMINARY

The dengue virus that causes dengue fever, which is very deadly, has not been resolved in Indonesia until now [(1)]. Around 390 million people are infected with Dengue Hemorrhagic Fever (DHF) every year in the world, with the incidence of dengue fever in Asia-Pacific accounting for 75% of all cases between 2004 and 2010. Indonesia has the second highest prevalence of dengue fever of any other 30 endemic countries [(2)]. The high incidence rate and the breadth of the affected area every year show the existence of this disease in the community. This continuity is due to the availability of *Aedes aegypti* vectors. As a country with a tropical climate, Indonesia is a good habitat for *Aedes aegypti* [(3).]

The unavailability of effective vaccines and drugs to prevent dengue fever is a problem in the management of this disease [(4); (5).] Until now, an effective way to prevent or control dengue fever is to control the mosquito population that causes its transmission [(6)]. *Aedes.sp* is the main vector of dengue fever. This species breeds in clean waters indoors and outdoors. The lack of public concern for environmental cleanliness has become a breeding place for *Aedes aegypti*. This situation will always be a threat to humans, because *Aedes aegypti* acts as a vector of dengue disease [(7)].

Efforts to control *Aedes aegypti* can be carried out by eradicating larvae, either biologically, namely by using natural enemies, or chemically, namely by using chemicals that are larvicide [(8)]. So far, the government's efforts have been made to control the *Aedes aegypti* vector by chemical methods in the form of *fogging* and insecticides as larvicides in breeding sites that are difficult to clean. Chemical larvicides have a new impact because they are difficult to degrade and pollute the environment such as the death of predators, toxic to populations other than the target, increased resistance to the target, the killing of pets, and even to humans [(8)]. For example, temefos is the most commonly used larvicide and has been shown to show resistance in Southeast Asia, the Caribbean, and South America [(9)]. Therefore, many studies have been conducted to find alternatives to larvicides that are more environmentally friendly and non-resistant [(10)].

Aloe vera (Aloe vera) and clove leaves (*Syzygium aromaticum*) are plants that have long been used in traditional medicine and food in different parts of the world. Plants have beneficial phytochemicals *(such as flavonoids, terpenoids*, etc.) that have shown significant larvicide activity against a variety of insects and disease vectors. However, fewer studies have been conducted to test the effectiveness of this plant as a larvicide in controlling the larvae of *Aedes aegypti* [(11)].

In the effort to control *Aedes aegypti* larvae, the use of natural larvicide methods has several advantages compared to synthetic larvicides. This method focuses on natural sources such as plants, which are not only environmentally friendly and biodegradable but also much lower cost. The availability of plants in various parts of the world also makes it easier to use them in mosquito control.

Previous research has explored various types of plants that have the potential to be natural larvicides. Among them is the test study of putri malu leaf extract [(12)], which shows that Mimosa Pudica leaf extract is effective as a larvicide against the larvae of *Aedes aegypti*. Another study by Ramkumar et al. found that an insect-reducing extract from Azadirachta indica was effective in controlling the larvae of *Anopheles, Culex* and *Aedes*. In addition, Kayode and Ashafa in their research found that Solanum aculeastrum plant extract showed good larvicide activity.

Previously, studies conducted on plant-derived larvicides have shown good efficacy in controlling insects and disease vectors. Studies on larvicide activity from aloe vera plant extract (*Aloe vera*) have shown effective control activity against the larvae of *Anopheles*, the mosquito that carries malaria. Meanwhile, clove extract (*Syzygium aromaticum*) is able to inhibit the activity of the larvae *of Culex quinquefasciatus*, a mosquito that carries filariasis.

The various methods of extracting plant extracts and fractionation techniques in the study provide a variety of possible effects of botanical larvicids on disease vectors such as *Aedes aegypti*. To optimize the effectiveness of natural larvicides, the identification of the most effective bioactive components in inhibiting the growth and development of mosquito larvae is of great importance.

The effectiveness of aloe vera plant extract (*Aloe vera)* as a natural larvicide in breaking the life cycle of *Aedes aegypti* mosquito larvae will be an important step in mosquito control efforts that are more environmentally friendly, effective, and sustainable. The results of this study will be very useful to improve the efficiency of mosquito control programs and reduce the negative impacts caused by the use of synthetic larvicides in the long term.

Research Purposes

The purpose of this study is to test the toxicity of the difference in the concentration of aloe vera extract as a larvicide aedes aegypti. The dosage of use as a larvicide is 10%, 20%, and 30% extract. The study also involved negative control (no larvicide). All of them will be used to observe the effectiveness and speed of larvicide action in disrupting the larval life cycle.

RESEARCH METHOD

The type of research used is experimental with the aim of conducting tests to determine the comparison of larvicides derived from aloe vera plant extract with concentrations of 10%, 20% and 30% in controlling the growth and development of *Aedes aegypti larvae*.

The subject of the int study is aloe vera extract. The object of this study is "*Aedes aegypti* larvae". Based on the recommendation of the *World Health Organization* (13), the sample size used for larvicide testing is 20-30 larvae for each treatment, so in this study 25 larvae are used. The determination of the number of repetitions in this study is used Federer's formula as follows:

$$(r-1)\,(t-1) \geq 15$$

r = is a lot of repetition
t = is the number of treatment groups

Based on Federer's formula, it can be calculated that the number of repetitions that can be done is:

$(r-1)\,(t-1) \geq 15$
$(4-1)\,(r-1) \geq 15$
$3\,(r-1) \geq 15$
$3r - 3 \geq 15 + 3$
$3r \geq 18$
$r \geq 6$

Based on the results of the calculation above, the number of treatment repetitions was carried out 6 times, so that the entire sample to be used in this study is:

Number of larvae x Number of replicas x Number of treatments

Therefore, based on the calculation of the formula, the number of larvae required is as follows:
25 x 6 × 4 = 600 larvae.

The concentration series used in this study were 15%, 25% and 35%. To obtain a series of papaya leaf extract concentrations, dilution is carried out using the formula:

$$V1 \cdot N_1 = V2 \cdot N_2$$

Information:
V1: volume from scratch required
N1: initial concentration
V2: desired volume of solution
N2: desired solution concentration

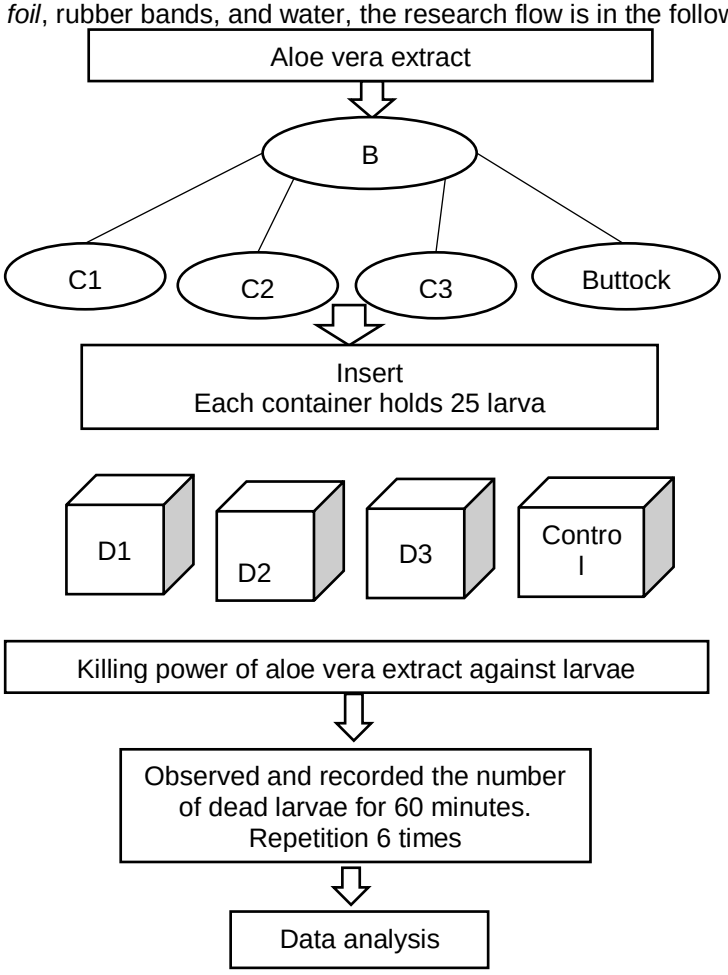
The volume of solution used in this study was as much as 100 ml on each repetition. Example for 15% of papaya leaf extract in 100 ml of aquades.

$V1 \cdot N_1 = V2 \cdot N_2$

V1 . 100% = 100 ml . 15%

V1= 15 ml means that 15 ml of papaya leaf extract is diluted in a measured flask with aquades to a volume of 100 ml.

The research instruments are blenders, knives, sieves, trays, spoons, jars, analytical balances, funnels, *rotary evaporators*, *500 ml glass beakers*, glasses, and *stirrers* (stirres). The tools used for the research are test tubes/beaker cups, counters, thermometers, hygrometers. The ingredients used in the study were aloe vera extract, aedes aegypti larvae, water aquades and 95% ethanol. filter paper, 96% ethanol, *aluminum foil*, rubber bands, and water, the research flow is in the following picture:



Information

1. B= Larvae
2. C1=Clove Extract Concentration 10%
3. C2=Clove Extract Concentration 20%
4. C3=Clove Extract Concentration 30%
5. D1=Reservoir and 25 larvae with a concentration of 10%
6. D2=Reservoir container and 25 larvae with a concentration of 20%
7. D3=Reservoir and 25 larvae with a concentration of 30%
   Control = a condition in which no treatment is given

The data will be analyzed using ANOVA one-way to see the effect of aloe vera extract on the life cycle of *Aedes aegypti larvae.* If the extract shows a significant effect on the mosquito's life cycle, it will be followed by an LSD *(Least Significant Difference) test.*

The LSD test aims to see the difference in the dosage of aloe vera extract as a larvicide. This will provide a better understanding of their benefits to *Aedes aegypti*.

RESULTS

The results of the testing of aloe vera extract (*Aloe* vera) can be seen in the following table 1.

Table 1 Number of *Aedes Aegypti Larval Deaths in Each Treatment of* Aloe Vera Extract

Dose	Number of Larvae (tails)	Number of larvae that die after 60 minutes						Sum	Average
		Repetition							
		I	II	III	IV	V	WE		
Control	25	0	0	0	0	0	0	0	0
10%	25	19	18	19	16	17	15	104	17,3
20%	25	22	16	18	19	23	16	114	19
30%	25	23	21	18	24	24	20	130	21,7

Based on Table 1, each treatment on aloe vera extract (*Aloe vera*) except for control there was a death of Aedes *aegypti mosquito larvae*, with the highest average mortality at a dose of 30%, which was 21.6 larvae. For the average percentage of deaths in the following figure:

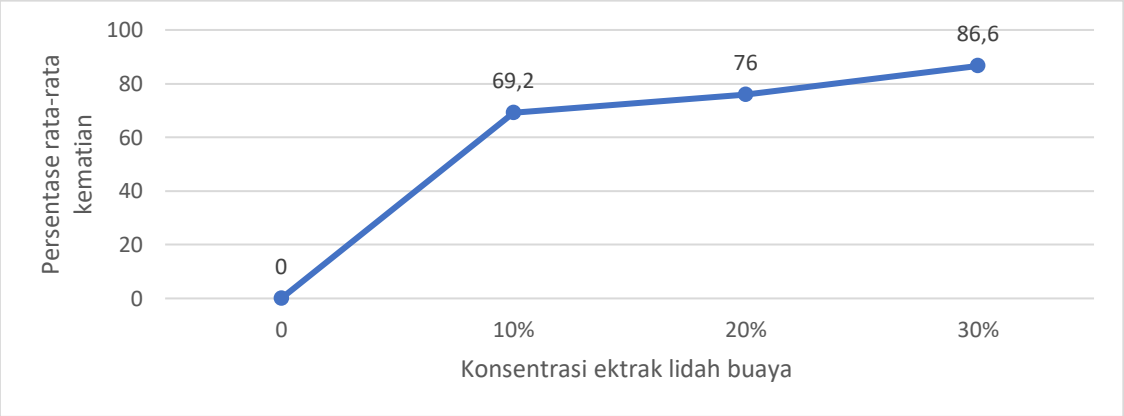


Figure 1. Average mortality of Aedes aegypti larvae with aloe vera extract (*Aloe vera*)

To determine the difference in the concentration of aloe vera extract against *the death of* Aedes aegypti *larvae*, an anova test was carried out, in the following table:

Table 2 Results of *Anova Statistical Test* on Aloe *Vera* Extract As a Larvicide *Aedes aegypti*

NO		Responds				
		Sum Squared	df	Middle Square	F	Mr
1	Treatment	1739,33	3	579,778	133,795	**0,000**
2	Galati	86,667	20	4.333		
3	Total	1826,00	23			

Based on Table 2 above, the value of sig. (p-value) < α, which is 0.000 < 0.05, then reject it, H-0., and with a confidence level of 95%, it can be concluded that there is a difference in the number of mosquito deaths based on the concentration dose of Aloe Vera Extract.

DISCUSSION

After 60 minutes of treatment, the average number of larval deaths was 0% in the negative control group, 69.2% at 10% extract concentration, 76% at 20% extract concentration, 86.6% at 30% extract concentration. In the treatment group, the death of Aedes aegypti larvae occurred, while in the control group, there was no death of Aedes aegypti larvae. Table 1 shows that Aloe vera extract has a larvicide effect, as indicated by the fact that its larvicide power varies at different concentration levels, with higher concentration levels resulting in the death of more larvae up to a certain concentration level.

The results of the anova statistical test can be known as the value of sig. (p-value) < α, which is 0.000 < 0.05, then reject, H-0., and with a confidence level of 95%, it can be concluded that there is an effect of the concentration of aloe vera extract as a larvicide of Aedes Aegypti on larval death.

The effectiveness of Aloe Vera is due to the presence of various active compounds contained in the Aloe Vera extract. Some of these active compounds, namely the content of euganol, have the potential as natural larvicides. Euganol interacts with enzymes in the digestive tract of mosquito larvae, disrupting metabolic processes and inhibiting energy production in larvae, without which larvae cannot develop and eventually die.

Aloe vera (Aloe vera) extracted using the percolation method, contains eugenols, saponins, flavonoids and tannins (14). This study explains efforts to prevent dengue fever by utilizing natural ingredients containing active metabolites in the form of flavonoids, tannins, saponins and alkaloids to control the Aedes aegypti mosquito vector. From several journals that have been read, the content of this substance can be used as a larvicide for Aedes aegypti mosquitoes which is more environmentally friendly compared to the use of chemical abatization.

Research by Indri Ramayanti and Ratika Febriani (15) entitled "Effectiveness Test of Larvicide of Papaya Leaf Extract (Carica papaya Linn) against Aedes aegypti Larvae" found that papaya leaf extract (Carica papaya Linn) had an LC50 concentration of 3.73%, which caused the death of Aedes aegypti larvae.

Flavonoid compounds act as respiratory toxins or powerful respiratory inhibitors, entering the mosquito's respiratory tract and weakening its respiratory muscles and nerves. As a result, the mosquito's breathing process is stopped or unable to breathe which eventually dies. This is supported by a study conducted (16), "Test of the Effectiveness of Bitter Melons Fruit Extract (Momordica Charantia) on the Mortality of Aedes aegypti Larvae", which found that bitter melon extract has flavonoid content and is proven to have the smallest effect on the larvacides of Aedes aegypti mosquitoes with a concentration of 0.8% (16).

Saponins irritate the mucosa of the larvae's digestive tract and cause them to lose their appetite and die. Additionally, saponins can damage the waxy coating that protects the insects' outer bodies, causing them to lose a lot of bodily fluids and die (17).

Alkaloids function as stomach toxins and inhibit the feeding ability of larvae. Allegedly, alkaloids can stop the enzyme acetylcholine, which leads to the buildup of acetylcholine, which causes the system to send impulses to muscle cells to become irregular. Aedes aegypti had seizures, became paralyzed, and eventually died (18).

Because of tannins, the activity of protease enzymes to convert amino acids will be reduced. Tannins can bind to proteins in the larvae's digestive system, which are needed by the larvae for growth, and larval cell metabolism can be disrupted, causing the larvae to be deficient in nutrients. Aedes aegypti larvae will die if this continues (19).

CONCLUSION

Based on the results that have been obtained, the author can draw the following conclusions: clove leaf extract (Syzygium aromaticum) with various effective doses as a Larvicide Aedes Aegypti. It is necessary to examine the phytochemistry of the content contained in clove leaf extract (Syzygium aromaticum), and test or trial in the field.

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