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Application of papaya leaf extract *(Carica papaya L.)* as a natural insecticide on the larvae of the Aedes aegypti mosquito vector of dengue fever

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Abstract— *The toxicity of the ethanol extract of papaya leaves (Carica papaya L.) on the mortality of the Aedes aegypti mosquito as a vector of dengue hemorrhagic fever. The aim of the study was to determine differences in the concentration of ethanol extract of papaya leaves (Carica papaya L.) on the mortality of Aedes aegypti mosquitoes and the most effective concentration (LC50) for 24 hours of treatment. The study used a completely randomized design with 5 extract treatments, namely: 10 ppm; 50 ppm; 100 ppm; 500 ppm and 1000 ppm and 1 control group with 3 replications. The results of the study were analyzed using one-way analysis of variance. and continued with the BNT test at a significance level of 0.05. To determine the most effective concentration of papaya leaf extract in killing the larvae of Ae. aegypti used Probit Analysis (Finney Method) with Minitab 17 software.The results showed that increasing the concentration of ethanol extract of papaya leaves (Carica papaya L.) would increase the number of test larvae mortality. Based on the results of the probit analysis test, the most effective concentration to kill 50% of Aedes aegipti mosquito larvae was 413.906 ppm (LC50 = 413.906 ppm). Ethanol extract of papaya leaves (Carica papaya L.) has the potential to be developed as a natural insecticide for the Aedes aegypti mosquito vector of dengue hemorrhagic fever.*

***Keywords*— Extract, Papaya leaf, natural insecticide, Aedes aegypti.**

I. INTRODUCTION

North Sulawesi in January 2015 was categorized as an Extraordinary Event (KLB), the five-year cycle of the Dengue Hemorrhagic Fever (DHF) outbreak that hit eight districts/cities in North Sulawesi, killing eight people who were positive for the virus transmitted by the Aides aegypti mosquito.

Until now, no vaccine has been found to kill the virus that causes dengue fever. One way to prevent the spread of dengue hemorrhagic fever (DHF) is to prevent transmission of the dengue virus, namely by controlling and eradicating vectors to cut off disease transmission. (WHO, 2005). The World Health Organization (WHO) estimates that every year there are 50-100 million cases of dengue virus infection worldwide.

Fogging is one of the mechanical control methods. Unfortunately, smoking is considered less effective because it tends to repel mosquitoes from their nests, not kill mosquitoes. The chemical method used is the distribution of larvicides such as abate in mosquito breeding places. Indeed, the use of chemical larvicides has succeeded in controlling Aedes aegypti larvae, but the continuous use of chemical larvicides will actually cause resistance and various environmental problems. cause environmental pollution, poisoning, death of non-target organisms, and produce residues.

Due to the negative impact caused by chemical insecticides, it has prompted experts to look for alternatives to vector eradication, namely by using natural insecticides that are safer, easier, cheaper, and do not have a toxic impact on humans. Control using vegetable insecticides or biolarvicides is one of them. Vegetable insecticides made from plants that contain bioactive compounds that are toxic to insects. Organic pesticides are easily biodegradable so they are not harmful to the environment (Octavia, et al. 2008).

The LC_{50} value is a certain concentration that causes the death of the test organism as much as 50%, while the LC_{90} value causes the death of 90% (Klaassen and Watkins 2003)

Plants that have been extracted and isolated by researchers containing active compounds of vegetable insecticides on Aedes aegypti mosquito larvae are soursop seeds (Annona muricata) with LC_{50} = 117. 27 ppm (Komansilan et al. 2012), Hutun seeds (Barringtonia asiatica Kurz) with Lethal Concentration LC_{50} = 35.72 ppm (Komansilan and Suriani. 2016) and lavender leaf (Lavandula angustifolia) with Lethal Concentration LC_{50} = 87.0285 ppm (Komansilan et al. 2021).

Papaya (Carica papaya L.) is one of the plants that can be used as a natural insecticide, because it is effective in controlling insects (mosquitoes). Chemical constituents in papaya leaves include papain enzymes, karpain alkaloids, pseudo-karpains, glycosides, carposids, saponins, saccharose, dextrose, and levulose. (Dalimartha, 2005). Robinson (1998) stated that alkaloids can interfere with the formation of the constituent components of peptidoglycan in bacterial cells, so that the cell wall layer is not fully formed and causes bacterial death.

Flavonoids are polyphenol compounds. Phenol compounds are able to denature protein bonds in cell membranes, so that the cell membrane becomes lysed and the possibility of phenol penetrates into the cell nucleus resulting in changes in cell permeability which can result in inhibition of cell growth or cell death (Peleczar and Chan, 1986).

According to Alboneh (2012), in his research, the substances in papaya leaves which are thought to have potential as insecticides for Aedes aegypti mosquitoes are papain enzymes, saponins, flavonoids, and tannins. The use of natural larvicides can be done to reduce chemical larvicide resistance which is still widely used by the community (Astuti 2009).

II. RESEARCH METHODS

Research Location and Time

This research was conducted at the Integrated Science Laboratory, Faculty of Mathematics and Natural Sciences, Manado State University from May to September 2022.

Tools and Materials

The tools used are: test tube, mosquito cage, blender, filter, rotary evaporator, desiccator, digital scale, measuring cup and micro pipette. Research materials: papaya leaves, ethanol, aquades, Aedes aegypti mosquito larvae, fish feed, paper strain.

Research design

The design used was a completely randomized design (CRD), with 6 treatments and 3 replications, namely: K1 = Control, K2 = .10ppm, K3 = .50ppm, K4 =100ppm, K5 = 500ppm and K6 = 1000ppm

Observation

The parameter observed was the mortality percentage of the Aedes aegypti mosquito, which was calculated using the formula proposed by Kundra (1981):

$M = a/b \times 100\%$

Where: M = percentage of mosquito mortality

Ae. aegypti

a = number of mosquitoes Ae. Dead Aegypti

b = number of mosquitoes Ae. aegypti who used.

Work procedures

1. Lava propagation of Aedes aegypti mosquitoes

a) Aedes aegypti mosquito larvae media is made by filling a plastic container with water and the inner wall is lined with filter paper. Filter paper serves as a place for female mosquitoes to attach their eggs.

b) Eggs attached to filter paper are then dried at room temperature and stored in a closed container. For hatching eggs, filter paper is dipped in a plastic tray filled with water and after 24 hours the eggs will hatch and grow into first instar larvae.

c) First instar larvae will develop into second, third (4 days) and IV instar (2 days) instar larvae. Once every 2 days, the larvae were fed 1-2 grams of fish pellets. III/IV instar larvae used in the test.

2. Extract Making

Making papaya leaf extract is as follows:

a) Papaya leaves are separated from the stems, washed, and allowed to dry in the air to avoid sunlight.

b) Dried papaya leaves are mashed using a blender.

c) The mashed leaves were extracted by maceration using technical ethanol until all the components had been extracted.

d) The ethanol extract obtained was evaporated with a vacuum rotary evaporator until thick.

3. Toxicity Test.

a) Provide a solution of papaya leaf ethanol extract in a test tube with concentrations: 10ppm, 50ppm, 100ppm, 500ppm and 1000ppm.

b) In each test tube, 10 larvae of Aedes aegypti mosquitoes are inserted

c) The calculation of mortality was carried out after 24 hours of treatment.

Data analysis

To distinguish the toxicity between treatments of several concentrations of papaya leaf extract against Aedes aegypti mosquito larvae, it was analyzed using one-way ANOVA analysis at a 95% confidence level ($\alpha = 0.05$), followed by the BNT test. Analysis to determine Lethal Concentration (LC_{50}) can be determined through Probit analysis, which aims to determine the concentration of papaya leaf extract (Carica papaya linn) which can kill Aedes aegypti mosquito larvae by 50% of the population tested.

III. RESULTS AND DISCUSSION

1. Comparative Test of Mortality Rate of Ae. Aegepty in Giving Ethanol Extract of Papaya Leaves *(Carica papaya linn).*

The data presents the number of deaths and the mortality rate of Ae. aegepty at five levels of concentration, namely 1000 ppm, 500 ppm, 100 ppm, 50 ppm, 10 ppm, and 0 ppm (control). The data used is mortality rate data (mortality) in the form of percentage scores from 0% to 100%. The 0% figure states that out of 10 mosquito larvae, none have died, while the 100% indicates that all 10 mosquito larvae have died.

The first step before the analysis is carried out is to describe the research variables (descriptive statistics) which includes the presentation of the average value and variation (standard deviation) of each concentration of the ethanol extract of papaya leaves.

Table 1. Description of the Average Value and Variation of Each Concentration of Ethanol Extract of Papaya Leaf Ethanol (Carica papaya linn).

Concentration	The average	Variation
0 ppm (Control)	0.00	0.00
10 ppm	0.00	0.00
50 ppm	1.00	1.00
100 ppm	3.00	0.00
500 ppm	6.67	0.58
1000 ppm	9.33	0.58

Source: Primary Data Processed, 2022

Graphically the data contained in table 1 can be presented as follows:

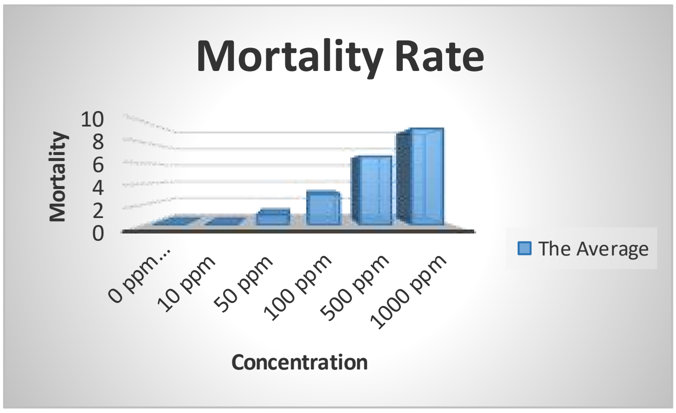

Fig.1. Description of the Average Value and Variation of Each Concentration of Giving Ethanol Extract of Papaya Leaves (Carica papaya linn).

In Figure 1, the height of the bar graph represents the average mortality rate of mosquito larvae for each concentration. From the table and figure above, it appears that there are differences in the mortality rate of Ae. Aegypti at various concentration levels from 0 ppm (control), 10 ppm to 1000 ppm. To find out whether there are differences in the mortality rate of Ae. Aegypti which were significant (significant) at the five concentrations were tested with One Way ANOVA or equivalent to a Completely Randomized Design.

Then, One-way ANOVA was tested. Treatment or concentration of Papaya Leaf Ethanol Extract (Carica papaya linn). declared significant (significantly different) if the value of Fcount > Ftable or the value of Sig F (P-value) < 0.05 (error rate 5%).

Table 2. Results of One-Way ANOVA Concentration Data on Papaya Leaf Ethanol Extract (Carica papaya linn).

Mortality rate					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	224.667	5	44.933	161.760	,000
Within Groups	3.333	12	,278		
Total	228.000	17			

The test results in table 2 show that the Fcount value is 161,760, and Sig F is 0.000. From the F-Statistics table, it is obtained Ftable of 2.90 Because the value of Fcount > Ftable and Sig F < 0.05 indicates that there are significant differences in the mortality rate of mosquito larvae at various concentrations.

To find out which concentration gives the highest mortality rate, a post hoc test is used, namely the smallest significant difference test (honestly significance difference). given a different notation (different subset), indicating that there is a difference between concentrations.The following are the complete test results:

Table 3. Follow-up Test Using Test (BNT) Concentration Data Giving Ethanol Extract of Papaya Leaves (Carica papaya linn).

Concentration	The average	Notation
0 ppm (Control)	0.00	a
10 ppm	0.00	a
50 ppm	1.00	b
100 ppm	3.00	c
500 ppm	6.67	d
1000 ppm	9.33	e

Note: The same notation shows insignificant differences, while different notations show significant differences.

In table 3 above, it can be seen that given a concentration of 0 ppm (control, or without ethanol extract), the mortality rate of Ae. aegypti at 0.00% or no test larvae will die. With an increase in concentration to 10 ppm, it gives the same test larvae mortality rate (same notation) when compared to a concentration of 0 ppm, which is 0.00%. On the other hand, by increasing the 50 ppm ethanol extract, it gave a higher mortality rate of mosquito larvae (different notations) when compared to concentrations of 0 ppm and 10 ppm, which was 10.%. Meanwhile, by increasing the concentration to 100 ppm in the administration of ethanol extract, it gave the mortality rate of Ae. aegypti which is bigger (different notation) when compared to 50 ppm concentration, with a mortality rate of 30.00%. With an even higher concentration, namely 500 mortality rate of 66.70% and concentration of 1000 ppm, the mortality rate of aedes aegypti mosquito larvae reached 93.30% mortality rate. So it was concluded that the concentration of 10 ppm, 50 ppm, 100 ppm, 500 ppm and 1000 ppm would give the mortality rate of Ae mosquito larvae. aegypti different.

The increase in mosquito mortality was caused by an increase in the concentration of the extract. This indicates that each extract concentration has a different toxic level. It was proved that the low concentration of the extract had a low level of toxicity, causing low larval mortality. On the other hand, with a high concentration of extract, it will have a high level of toxicity, causing high mortality. This is in line with what was expressed by Watuguly (2003), that the factor that most determines the potential danger or safety of a compound is the relationship between the levels of chemical substances and their effects. In addition, the interaction of a toxic substance with biological systems is directly related to the amount of toxic material.

2. Biolarvicide Toxicity Test from papaya leaf extract *(Carica papaya L.)* against Mosquito Larvae Aedes aegypti

The results of the toxicity test of papaya leaf extract against Aedes aegypti mosquito larvae are presented in table 4.

Table 4. The results of the toxicity test of the ethanol extract of papaya leaves (Carica papaya L.) against the larvae of Ae. aegypti after 24 hours of treatment

Extract Type	Concent ration (ppm)	Number of dead larvae / 10 head			Percent mortality
		D 1	D2	D	
control	0	0	0	0	0
Papaya leaf	1000	9	10	9	93.33
	500	7	7	6	66.66
	100	3	3	3	30
	50	1	2	0	10
	10	0	0	0	0

To find out at what concentration papaya leaf extract was most effective in killing the larvae of Ae. aegypti requires a more in-depth analysis tool, namely Probit Analysis (Finney Method) using Minitab 17 software.

The data used as a whole were obtained from 10 larvae of Ae. aegypti in each replication (there were 3 replications) so that 30 larvae of Ae. aegypti as a whole. Table 5 below presents the estimation parameters of the probit analysis model:

Table 5. Parameter estimation model of papaya leaf extract probit analysis on larvae Ae. aegypti

Parameter Estimates		Standard Error	95,0% Normal CI	
Parameter	Estimate		Lower	Upper
Mean	413,906	45,2901	325,139	502,673
StDev	326,504	44,2593	250,325	425,866

Graphically, the probit analysis curve is presented as follows:

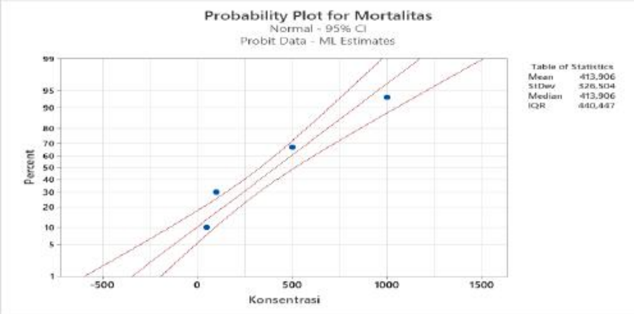

Fig.2. LC_{50} value of papaya leaf extract on Ae. aegypti larvae after 24 hours treatment.

Table 5 and Figure 2 present the LC_{50} or Mean Lethal Concentration values of papaya leaf extract based on the results of Probit Analysis. The test results show the value of the LC_{50} mortality concentration of Ae. aegypti is by giving a concentration of 413.906 ppm. Thus, the concentration figure of 413.906 ppm is the concentration of the most effective papaya leaf ethanol extract to kill Ae. aegypti as much as 50% for 24 hours of treatment. According to the toxicity criteria based on the Australian Petroleum Energy Association (1994) a concentration of 413.906 from papaya leaf extract or (LC_{50} = 413.906 ppm) at 24 hours of observation was included in the criteria for Moderately Toxic

Dyah (2011) explained that the flavonoid compounds contained in papaya extract have various pharmacological activities and have chemical structures that are toxic to pests, this if given in sufficient concentrations it is able to poison pests properly through the digestive process so that plants become healthy in carrying out their activities. absorption of nutrients for vegetative growth processes such as plant height.

Juliantara (2010), reported that papaya leaves contain secondary metabolites such as alkaloids, flavonoids, terpenoids, saponins and other compounds such as papain enzymes which are used as vegetable pesticides.

Pesticide residues cause insect feeding activity to decrease and even stop. In addition, insects also showed a decrease in movement activity. In addition, papaya leaves contain flavonoid substances that work as neurotoxins which are thought to cause aphids to experience a decrease in movement activity. (Dyah, (2011) This is in accordance with Rosyidah (2007), who explained that flavonoid compounds can cause weakness in nerves. as well as damage to the spiracles that result in insects unable to breathe and eventually die.

IV. CONCLUSION

Ethanol extract of papaya leaves (*Carica papaya L.)* has toxicity to the mortality of Aedes aegypti mosquitoes. Based on the test results, the extract concentration of 413.906 ppm was able to kill 50% of Aedes aegypti mosquitoes. The most effective concentration used to kill 50% of Aedes aegypti mosquitoes was 413,906 ppm.

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