



**PAPAYA LEAVES EXTRACT EFFECTIVENESS TEST (Carica papaya L)
AS A LARVICIDINE Aedes aegypti INSTAR III**

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Abstract

The *Aedes aegypti* mosquito is a vector of dengue hemorrhagic fever which has a major role in the transmission of the disease in Indonesia. Mosquito vector control is expected to have an impact on decreasing the vector population of the *Aedes aegypti* mosquito so that it is no longer significant as a disease transmitter. Therefore, natural larvicides are used, one of which is papaya leaf (*Carica papaya* L) which contains alkaloid compounds, tannins and flavonoids which have a larvicidal effect. This research is experimental to see the killing power of papaya leaf extract (*Carica papaya* L) against *Aedes aegypti* larvae and to determine the LC50 and LC90 of papaya leaf

extract against *Aedes aegypti* larvae. The sample size used was 800 larvae with 4 repetitions in each treatment group. Observation time was carried out 24 hours. The measured data was the treatment of papaya leaf extract against *Aedes aegypti* larvae with concentrations of 0.5%, 1%, 1.5%, 2%, 2.5% and 3% and abate as a positive control or distilled water as a negative control. that there is a difference in the mean mortality of *Aedes aegypti* larvae between groups which is statistically significant with a value of $p0.000 \leq \alpha (0.05)$. Probit analysis results found that papaya leaf extract (*Carica papaya* L) with a concentration of 0.591% was able to kill 50% of *Aedes aegypti* larvae for 24 hours. Papaya leaf extract (*Carica papaya* L) with a concentration of 2,873% was able to kill 90% of *Aedes aegypti* larvae for 24 hours .

Key words: Aedes aegypti, larvicides, papaya leaf extract (Carica papaya L)

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INTRODUCTION

The *Aedes aegypti* mosquito is a mosquito that acts as a vector for various diseases, one of which is Dengue Hemorrhagic Fever (DHF). Although several species of *Aedes* sp can also act as vectors, *Aedes aegypti* is still the main vector in the spread of Dengue Hemorrhagic Fever (DHF). (Soedarto, 2012) In 2013 there were 2.35 million cases of DHF in America, of which 37,687 were reported. the case is a severe DHF. The development of dengue cases at the global level is increasing, as reported by the World Health Organization (WHO), from 980 cases in nearly 100 countries in 1954-1959 to 1,016,612 cases in nearly 60 countries in 2000-2012 (WHO, 2014).

The Incidence Rate (IR) in Indonesia in 2015 was 50.75% and Case Fatality Rate (CFR) 0.83%. (Ministry of Health, 2017) The number of deaths due to dengue fever in 2015 was 1,071 people with a total reported number of cases of 129,650 people. . The 2016 Incidence Rate in Indonesia is 78.85% and the 2017 Incidence Rate has decreased, namely at 26.10%. %) decreased compared to the previous year as many as 507 cases and at the age of 10-14 years as many as 87 cases (29%). Whereas in 2018 the Incidence Rate in Indonesia was 24.75% and an increase in the Incidence Rate in 2019 showed 51.48%, while the Case Fatality Rate was 0.71%. (Kemenkes, 2020)

This is still a health problem in various regions. Vector behavior and its relationship with the environment, such as climate, vector control, urbanization and so on, affect the occurrence of dengue fever outbreaks in urban areas. Control of mosquito vectors can be carried out by physical control, biological control and chemical control. Chemical control can be done using abate or temefos. However, the use of chemical-based insecticides still has various disadvantages such as the continuous use of synthetic insecticides that will pollute the environment, increase larval resistance and can cause gene mutations. (Nisa, 2015) Control using vegetable insecticides or biolarvicides is an alternative way to vector control of the spreader. Vegetable insecticides made from plants contain bioactive compounds that are toxic to insects. Organic larvicides are easily biodegradable so they are not harmful to the environment (Supono, 2015)



One of the many plants found in Indonesia and has the potential as a natural larvicide because it can kill mosquito larvae, namely papaya leaves (*Carica papaya* L). Papaya leaves contain active ingredients such as papain enzymes, karpain alkaloids, pseudocarpaine, glycosides, carposids, saponins, flavonoids, saccharose, dextrose and levulose. (Enis, 2016) Of these ingredients, those that have potential as insecticides are the enzymes papain, saponins, flavonoids, alkaloids and tannins. These compounds cause various reactions in the larva's body so that they can interfere with the growth and development of the larvae. (Kalimuthu, 2011) This is what underlies the research on papaya leaf extract (*Carica papaya* L) as a potential larvicide against *Aedes aegypti* instar III larvae. .

METHOD

This research is experimental to see the killing power of papaya leaf extract (*Carica papaya* L) against *Aedes aegypti* mosquito larvae and to determine the LC50 and LC90 papaya leaf extract against *Aedes aegypti* larvae. This research was conducted from March to April 2021. papaya leaf extract against *Aedes aegypti* larvae with concentrations of 0.5%, 1%, 1.5%, 2%, 2.5% and 3% and abate as a positive control or distilled water as a negative control. The population in this study were 3rd instar *Aedes aegypti* larvae obtained from Laboratory of the Research and Development Center for Health Banjarnegara, Central Java.

The sample size used was 800 larvae with 4 repetitions in each treatment group. Observation time was 24 hours. This research was conducted at the Laboratory of the Banjarnegara Health Research and Development Center. The inclusion criteria were active third instar *Aedes aegypti* larvae, while the exclusion criteria were *Aedes aegypti* larvae damaged or died before treatment.

This research was recorded in a data collection sheet, then data processing was carried out by means of editing, coding, data entry and tabulating. Bivariate analysis was carried out in the larvicide test at the laboratory experimental stage to determine the effect of variation in treatment concentrations in the test group on the number of *Aedes aegypti* larvae mortality in each group on exposure time. A prerequisite for performing a difference test is a normality test using the Shapiro-Wilk test. The Shapiro-Wilk test was used on the grounds that in this study the data on the number of sample groups was less than 50. The results of the normality test



showed that the data were not normally distributed ($p \leq 0.05$), so the Kruskal Wallis test was used to prove statistically there were differences in the mortality of *Aedes aegypti* larvae between groups. . If the Kruskal Wallis test results have a p value of <0.05 , then continue with the Post Hoc Mann Whitney test to prove that there are differences in each pair of groups. To determine the effect of the killing power of papaya leaf extract (*Carica papaya* L) on *Aedes aegypti* larvae, it was stated by the values of LC50 and LC90 (Lethal Concentration).

RESEARCH RESULT

Table 1.Observation of *Aedes aegypti* Larvae Mortality (24 hours)

Concentration	Number of Larvae Aedes aegypti Instar III (tail)	Cumulative Amount of Death of Instar Aedes aegypti Larvae III (tail)				Mortality Rate	Larvae Mortality Percentage (%)
		Replication					
		1	2	3	4		
Positive control (abate)	25	25	25	25	25	25	100%
Negative control (distilled water)	25	0	0	0	0	0	0%
Concentration 0.5%	25	13	15	11	16	13.7	54.8%
Concentration 1%,	25	17	18	16	16	16.7	66.8%
Concentration 1.5%	25	19	19	16	17	17.7	70%
Concentration 2%	25	20	21	20	18	19.7	78.8%
Concentration 2.5%	25	22	23	23	21	22.2	88%
Concentration 3%	25	25	24	25	25	24.7	98.8%

Papaya Leaf Extract (*Carica papaya* L)



Based on table 1, related to the observation of the larvicide test of papaya leaf extract (*Carica papaya* L) on the mortality of *Aedes aegypti* larvicide, which uses concentrations of 0.5%, 1%, 1.5%, 2%, 2.5% and 3% with 4 replications with each of them. using 25 larvae per container. It can be seen that the mortality percentage of instar III *Aedes aegypti* larvae at 24 hours exposure is at a concentration of 0.5% of 54.8%, 1% concentration of 66.8%, 1.5% concentration of 70%, 2% concentration of 78.8%, 2.5% concentration of 88 %, 3% concentration of 98.8%. While the positive control (abate) showed the percentage of larval mortality was 100% and in the negative control (aquades) there was no mortality of *Aedes aegypti* larvae.

Table 2. Normality Test

Papaya leaf extract concentration		Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig
24-hour larval mortality	0.50	.214	4	.	.963	4	.798
	1.00	.283	4	.	.863	4	.272
	1.50	.298	4	.	.849	4	.224
	2.00	.329	4	.	.895	4	.406
	2.50	.283	4	.	.863	4	.272
	3.00	.441	4	.	.630	4	.001

Based on table 2, it shows that the Shapiro-Wilk test has a sig value of 0.001 ($p < 0.05$), it means that the data carried out by the normality test are not normally distributed because the significant value is < 0.05 .

Table 3. Kruskal-Wallis test

	24-hour larval mortality
Chi-Square	29.833
df	7
Asymp. Sig.	.000

Based on table 3, there is a difference in the mean mortality of *Aedes aegypti* larvae between groups which is statistically significant with a value of $p_{0.000} \leq \alpha (0.05)$.

Table 4. Mann-whitney test



Papaya Leaf Extract Concentration								
	(+)	(-)	0.5%	1%	1.5%	2%	2.5%	3%
(+)		0.029	0.029	0.029	0.029	0.029	0.029	0.686
(-)			0.029	0.029	0.029	0.029	0.029	0.029
0.5%				0.057	0.029	0.029	0.029	0.029
1%					0.343	0.029	0.029	0.029
1.5%						0.114	0.029	0.029
2%							0.029	0.029
2.5%								0.029
3%								

Keterangan:

	Not significant ($p > 0.05$)
	Significant ($p < 0.05$)

Based on table 4, the comparison between positive and negative controls has a sig0.029 value ($p < 0.05$), which means that between positive and negative controls has a significant difference. The comparison between positive control and papaya leaf extract concentrations of 0.5%, 1%, 1.5%, 2%, 2.5% had a sig0.029 value ($p < 0.05$) which means it has a significant difference. However, the comparison between the positive control and the concentration of papaya leaf extract 3% had a sig0.686 value, which means that there was no significant difference. The comparison between negative controls had a significant difference with the concentration of papaya leaf extract 0.5%, 1%, 1.5%, 2%, 2.5 % and 3% because the sig0.029 value ($p < 0.05$).

Table 5 Probit Test Results (LC50 and LC90)

No.	Probit Test	Result	Upper and Lower Boundaries
1	LC ₅₀	0.519%	0.186-0.777
2	LC ₉₀	2.873%	1.976-7.175

Based on table 5, the results of the Probit Test, the LC50 value of papaya leaf extract is 0.591% and the LC90 value of papaya leaf extract is 2.873%.



DISCUSSION

Based on the results (Table 1) larvicide test of papaya leaf extract (*Carica papaya* L) against larvicidal mortality of *Aedes aegypti*, which uses concentrations of 0.5%, 1%, 1.5%, 2%, 2.5% and 3% with 4 replications with each using 25 larvae per container. It can be seen that the mortality percentage of instar III *Aedes aegypti* larvae at 24 hours exposure is at a concentration of 0.5% of 54.8%, 1% concentration of 66.8%, 1.5% concentration of 70%, 2% concentration of 78.8%, 2.5% concentration of 88 %, 3% concentration of 98.8%. The results of these studies indicate that the higher the concentration of papaya leaf extract (*Carica papaya* L), the higher the mortality rate of *Aedes aegypti* larvae.

The results of the Kruskal Wallis test showed that there were differences in the mean mortality of *Aedes aegypti* larvae between groups which were statistically significant with a value of $p_{0.000} \leq \alpha (0.05)$ (Table 3). On the other hand, the results of the Post Hoc Mann Whitney test showed that there was a difference in the mean mortality of larvae between pairs of groups (Table 4). The comparison between negative controls had a significant difference with the papaya leaf extract concentrations of 0.5%, 1%, 1.5%, 2%, 2.5% and 3% due to the sig0.029 value ($p < 0.05$). This proves that papaya leaf extract (*Carica papaya* L) is able to cause mortality of *Aedes aegypti* larvae.

In the Probit Test results, there are LC50 and LC90 values. The LC50 value is the concentration of certain papaya leaf extract (*Carica papaya* L) which can cause mortality of *Aedes aegypti* larvae as much as 50% of tested animals within 1x24 hours. Meanwhile, the LC90 value is the concentration of certain papaya leaf extract (*Carica papaya* L) which can cause mortality of *Aedes aegypti* larvae as much as 90% of tested animals within 1x24 hours. Papaya leaf extract (*Carica papaya* L) with a concentration of 0.591% was able to kill 50% of *Aedes aegypti* larvae for 24 hours, and papaya leaf extract (*Carica papaya* L) with a concentration of 2,873% was able to kill 90% of *Aedes aegypti* larvae for 24 hours (Table 5) .

This is in line with the results of research by Kurniawan et al, regarding the effectiveness of papaya leaf extract (*Carica papaya* L) as larvicide instar III *Aedes aegypti* using the treatment group, namely 0% (negative control), 0.25%, 0.75%, 1% and abate 1% (positive control). Each group contains 25 larvae with 4 repetitions. The data obtained were analyzed using the Kruskal wallis test, Post Hoc Mann Whitney test and Simple Linear Regression Test. The results



showed that the mortality rate of larvae was 67%, 71%, 85%, 95% at a concentration of 0.25%, 0.50%, 0.75% and a concentration of 1%. Papaya leaf ethanol extract (*Carica papaya* L) is effective as a larvicide against third instar *Aedes aegypti* larvae. The LC₅₀ value is 0.37%. (Kurniawan, 2015)

This larvicidal effect is caused by the active components contained in papaya leaves, namely alkaloids, flavonoids and tannins. Alkaloids are a class of compounds that are widespread in almost all types of plants. All alkaloids contain at least one nitrogen atom which is usually alkaline. Alkaloids can be found in the seeds, leaves, twigs and bark of plants. Alkaloid levels from plants can reach 10-15%. (Wahyuni, 2018) The content of these alkaloids acts as stomach poison and contact poison. Alkaloids in the form of salts so they can degrade the cell membranes of the digestive tract to enter and damage cells and can also interfere with the nervous system of larvae by inhibiting the action of the acetylcholinesterase enzyme. Where this enzyme cannot carry out its duties in the body, especially by continuing to send commands to the digestive tract of the larvae, so that their movements cannot be controlled. The occurrence of color changes in the larva's body becomes more transparent and the larva's body movements slow down when stimulated by touch and always bend the body which is also caused by alkaloid compounds (Ahdiyah, 2015).

Based on the observations, the test larvae showed one of the symptoms of poisoning due to alkaloid compounds. This compound causes the larva's body movement to slow down when stimulated by touch, and always bends the body. Another symptom is in the form of up and down movements in the medium. Symptoms of anxiety when observed were seen when compared to the negative control group, where the control larvae showed a resting condition with the surface forming a certain angle. In addition, these alkaloid compounds cause color changes in the larva's body to become more transparent.

Tannins are the most abundant content after alkaloids. Tannins are plant polyphenols that dissolve in water and can coagulate protein. (Chinthia, 2015) Tannins cannot be digested by the stomach and have binding capacity with protein, carbohydrates, vitamins and minerals. Tannins can interfere with insects digesting food because tannins will bind to proteins in the digestive system that insects need for growth. (Putri, 2019) Tannins can reduce the ability to



digest food by reducing the activity of digestive enzymes (proteases and amylase) and disrupting intestinal protein activity. Larvae that eat plants with a high tannin content will get less food, as a result, there will be decreased growth and death.

Flavonoids are a type of compound that is toxic or alleopathic, which is a compound from sugar that is bound to flavones. Flavonoids have specific characteristics, namely a very sharp odor, bitter taste, can dissolve in water and organic solvents, and easily decompose at high temperatures. Flavonoids attack the nerves in several vital insect organs, resulting in a weakening of the nerves, such as breathing and causing death. (Bintang, 2019) Flavonoids work as respiratory inhibitors. Inhibitors are substances that inhibit or decrease the rate of chemical reactions. Flavonoids are thought to interfere with energy metabolism in the mitochondria by inhibiting the electron transport system (Amirullah, 2019).

There is a slight difference between the results of this study and some studies that have been conducted by other researchers. This can occur due to several factors, including biological factors such as the location of the original plant, species, papaya varieties which are very diverse in each region, how the material is stored, the age of the plant, and the parts of the plant used. Apart from biological factors, there are also chemical factors that can influence, including the types of active compounds, as well as the quality and quantity of active compounds contained in the ingredients. In addition, the extraction method, the difference in the tools used, the size of the material, the hardness of the material, the dryness of the material, the solvent used, the heavy metal content can also affect the final result of the test. Different species of research objects can also affect because the toxicity of an insecticide generally differs from one species to another.

CONCLUSION

Based on the research that has been done, the following conclusions can be drawn:

1. Papaya leaf extract (*Carica papaya* L) has a larvicidal effect on third instar *Aedes aegypti* larvae.
2. Papaya leaf extract (*Carica papaya* L) with a concentration of 0.591% is able to kill 50% of *Aedes aegypti* larvae for 24 hours.
3. Papaya leaf extract (*Carica papaya* L) with a concentration of 2,873% was able to kill 90% of *Aedes aegypti* larvae for 24 hours.



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