



TOXICITY ASSESSMENT OF AVOVO LEAF EXTRACT (*Persea Americana* Miller) ON MORTALITY OF *Aedes Aegypti* LARVA

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Abstract

Aedes aegypti mosquitoes are mosquitoes that can spread various diseases such as dengue hemorrhagic fever (DHF), Zika, chikungunya, and other viruses. An alternative to being able to eradicate mosquitoes apart from the use of chemicals is with vegetable insecticides, vegetable insecticides are more economical, easy to obtain and do not cause effects such as resistance and polluting the

environment, Plants that have the potential as natural larvicides are avocado leaves which contain steroids, flavonoids, phenolics and tannins. The purpose of this study was to determine the ability of Avocado (*Persea Americana* Miller) leaf extract to reduce the mortality of *Aedes aegypti* larvae within 24 hours. This research is an experimental study, using a completely randomized design (CRD) for grouping and treatment of third instar *Aedes aegypti* larvae. The sample size used was 800 larvae with 4 repetitions in each treatment group. The data measured was the treatment of papaya leaf extract on *Aedes aegypti* larvae with concentrations of 1%, 2%, 3%, 4%, 5% and 6%. The results showed that there was a statistically significant difference in the mean mortality of *Aedes aegypti* larvae between groups with a p value of 0.000 (0.05). Concentration of avocado leaf extract (*Persea Americana* Mill) of 3.979% can kill 50% of *Aedes aegypti* larvae for 24 hours while 90% of *Aedes aegypti* larvae kill for 24 hours requires a concentration of 5.985%.

Keywords: *Aedes aegypti*, larvicides, avocado leaf extract (*Persea Americana* Mill)

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INTRODUCTION

The *Aedes aegypti* mosquito is a mosquito that can spread various diseases such as dengue hemorrhagic fever (DHF), Zika, chikungunya, and other viruses. (Najmah, 2006) Dengue fever is caused by the dengue virus (DENV), which belongs to the Flavivirus genus of the family Flaviviridae (Louis, 2010) Cases of dengue fever were reported Globally the largest in 2019 and in 2020 there will be an increase in the number of cases including Bangladesh, Brazil, Ecuador, India, Indonesia, Maldives, Mauritania, Nepal, Singapore, Sri Lanka, Sudan, Thailand, Timor-Leste and Yemen. (WHO, 2020) Trends Cases in Indonesia with dengue fever are stated in the Incidence Rate (IR) of DHF in 2019 of 51.48 per 100,000 population and the CFR (case fatality rate) of DHF in 2019 of 0.71% and the larvae-free rate has increased in 2019 which is 79.2%. (Kemenkes, 2020) The Central Java Province CFR exceeded the national target of 1.5 percent with the national target below 1 percent, while the Incident Rate in 2019 was 25.9 per 100,000 population. The district or city in Central Java Province with the highest morbidity rate is Karanganyar district of 94.5 per 100,000 population. (Health Profile of Central Java Province, 2019)

The integrated vector control system recommended by WHO is Vector Integration Management (IVM), one of which is chemical and non-chemical control methods. Chemical control can cause resistance to mosquito insecticides. The results of the research in North Minahasa showed that the DHF vector was resistant to malathion, while the results of two trials of *Aedes* mosquito resistance were still tolerant to Cypermethrin in Manado 7-8. An alternative to being able to eradicate mosquitoes apart from the use of chemicals is with vegetable insecticides, vegetable insecticides are more economical, easy to obtain and do not cause effects such as resistance and polluting the environment. avocado leaves, galangal, mint leaves, *babadotan* leaves, zodia leaves, jackfruit leaves *sambiloto* leaves, and red shoots. 10 Based on a preliminary study of phytochemical tests, avocado leaf extract contains positive compounds, namely alkaloids, steroids, flavonoids, phenolics and tannins, compounds which have moderate positive are alkaloids, flavonoids, phenolics and tannins, these compounds have the potential as larvicides. Therefore, it is necessary to conduct further research on the mortality of *Aedes aegypti* larvae in the larvicidal test of avocado leaf extract (*Persea Americana* Miller).

METHOD

The purpose of this study was to determine the ability of avocado leaf extract (*Persea Americana* Miller) on mortality of *aedes aegypti* larvae within 24 hours by looking at the LC50 and LC90 (Lethal Concentration) values. This study was carried out from March to April 2021. The data measured were avocado leaf extract treatment with concentrations of 1%, 2%, 3%, 4%,



5% and 6%. and abate as a positive control and aquades as a negative control. The population in this study was the third instar *Aedes aegypti* larvae obtained from the Laboratory of the Banjarnegara Health Research and Development Institute, Central Java. The sample size used was 800 larvae with 4 repetitions in each treatment group. This research was conducted at the Laboratory of the Banjarnegara Health Research and Development Institute

This research is recorded in the data collection sheet, then data processing is carried out by editing, coding, data entry and tabulating. Bivariate analysis if normally distributed uses the Anova test while if it is not normally distributed using the Kruskal-Wallis test, if the results of the Kruskal Wallis test have a p value of <0.05 , then continue with the Post Hoc Mann Whitney test to prove there are differences in each pair of groups. Probit analysis was used to test the effectiveness of avocado leaf extract on 50% and 90% killing power on *Aedes Aegypti* larvae mortality.

RESEARCH RESULTS

Table 1 Phytochemical Test Results of Avocado Leaf Extract

No	Sample	Parameter	Discoloration	Results
1	Extract Leave Avocado	Alkaloids	- A white precipitate is formed in Mayer's reagent - An orange precipitate is formed in the Dragendrophyte reagent	(++)
2		Steroid	Green	(+)
3		Terp	Red or Purple	(-)
4		Flavonoid	Yellow orange to red	(++)
5		Fenolik	Strong green, red, purple, blue or black	(++)
6		Saponin	Foam or froth is formed	(-)
7		Tanin	Greenish brown or dark blue	(++)

Information :

- (-) : means that it does not contain certain compounds according to the tested parameters
- (+) : it means that the parameter tested positive is weak
- (++) : it means that the parameter being tested is moderately positive
- (+++): it means that the parameter tested is strong positive



Based on the table above, it shows that avocado leaf extract contains alkaloids, steroids, flavonoids, phenolics and tannins. Compounds that have a moderate positive are alkaloids, flavonoids, phenolics and tannins. Positive results of alkaloids were indicated by the presence of Mayer reagent which formed a white precipitate while an orange precipitate was formed in Dragendrof's reagent. A positive result of flavonoids formed an orange precipitate. A positive result of phenolic formed a green precipitate. A positive result of tannin formed blackish color. The phytochemical results of avocado leaf extract were negative, indicated by the absence of color changes, for example in the terpenoid and saponin parameter tests.



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Table 2 Observation of Aedes aegypti larvae mortality (24 hours)

Avocado leaf extract concentration (%)	Number of Aedes aegypti Larvae Instar III (tail)	Cumulative Number of Deaths of Aedes aegypti Instar III Larvae (tail)				The number of dead Aedes aegypti larvae	Morta lity Rate	Larvae Mortality Percentage (%)
		Replication						
		1	2	3	4			
Concentration 1	25	10	12	14	10	46	11.50	46
Concentration 2	25	12	14	16	15	57	14.25	57
Concentration 3	25	13	18	17	17	65	16.25	65
Concentration 4	25	17	20	19	18	74	18.50	74
Concentration 5	25	21	19	22	23	85	21.25	85
Concentration 6	25	25	24	25	24	98	24.50	98
Positive control (abate)	25	25	25	25	25	100	25.00	100
Negative control (aquades)	25	0	1	0	1	2	0.50	2

Based on the table above, the mortality results of Aedes aegypti larvae in the larvicidal test of Avocado Leaf (Persea Americana Miller) extract, the lowest at a concentration of 1%, caused the death of Aedes aegypti larvae of 46%, while the concentration of 6% caused the death of the highest Aedes aegypti larvae of 98%.



Table 3. Normality Test

Avocado extract concentration	leaf	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig
24 hour	0.50	.283	4	.	.863	4	.272
larval	1.00	.192	4	.	.971	4	.850
mortalit	1.50	.382	4	.	.801	4	.103
y	2.00	.151	4	.	.993	4	.972
	2.50	.192	4	.	.971	4	.850
	3.00	.307	4	.	.729	4	.024

Based on the table above, it shows that the Shapiro-Wilk test has a sig value of 0.001 ($p < 0.05$), it can be interpreted that the data used for the normality distribution test is not normal because the significant value is < 0.05 .



Table 4. Kruskal-Wallis Test (Avocado Leaf Extract

24 hour larval mortality	
Chi-Square	20.817
df	5
Asymp. Sig.	.001*

Based on the Kruskal-Wallis test, the p-value of 0.001 (0.05 means that there is a difference in the mean mortality of *Aedes aegypti* larvae in the larvicidal test of Avocado Leaf Extract (*Persea Americana* Miller)

Table 5. Mann-Whitney . test

Avocado Leaf Extract Concentration								
	(+)	(-)	1%	2%	3%	4%	5%	6%
(+)		0.029	0.029	0.029	0.029	0.029	0.029	0.343
(-)			0.029	0.029	0.029	0.029	0.029	0.029
1%				0.114	0.57	0.029	0.029	0.029



2%					0.20	0.029	0.029	0.029
3%						0.114	0.029	0.029
4%							0.029	0.029
5%								0.029
6%								

Information :

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

Based on the table above, the comparison between positive control and negative control has a value of sig0.029 ($p < 0.05$), which means that there is a significant difference between positive control and negative control. The comparison between positive control and avocado leaf extract concentration of 1%, 2%, 3%, 4%, 5% had a sig0.029 value ($p < 0.05$) meaning that there was a significant difference. However, the comparison of positive control with 6% papaya leaf extract concentration had a sig0.343 value ($p > 0.05$), which means there was no significant difference.

Table 6. Probit Test Results (LC50 and LC90 Avocado Leaf Extract)

No.	Probit Test	Results	Upper and Lower Limits
1	LC ₅₀	3.976%	2.679-4.482
2	LC ₉₀	5.985%	5.405-8.010

Based on the table above, the results of the Probit Test the LC50 value of Avocado Leaves (*Persea Americana* Miller) is 3.976% while the LC90 value is 5.985%.

DISCUSSION

The results of phytochemical screening on avocado leaves contain alkaloids, steroids, flavonoids, phenolics and tannins. The content of alkaloids that enter the body of the larvae through absorption and degradation of skin cell membranes. In addition, alkaloids can also interfere with the nervous system of the larvae. Alkaloids are anticholinesterases that function to inhibit the action of the acetylcholinesterase enzyme which affects the transmission of nerve impulses. This anticholinesterase is the mechanism of action of Organophosphate and Carbamate compounds as insecticides. This causes the cholinesterase enzyme to be phosphorylated and become inactive. The inactivity of cholinesterase causes inhibition of the acetylcholine degradation process, resulting in the accumulation of acetylcholine in the synaptic cleft. This will cause a disturbance in the transmission of stimuli that can reduce muscle coordination, convulsions, respiratory failure and death (Wibawa, 2012). Kill the larvae. (Ry M, 2017) Flavonoids are plant defense compounds that inhibit insect appetite. Saponins can inhibit the work of proteolytic enzymes which cause a



decrease in the activity of digestive enzymes and the use of protein. Tannins can reduce the ability to digest food in insects by reducing the activity of digestive enzymes. (Safirah, 2016)

The results of the larvicidal test in the laboratory experimental stage are directly proportional to the increase in the percentage of *Aedes aegypti* larvae mortality for 24 hours along with the increase in concentration. Based on the observations, the concentration that caused the highest mortality was at a concentration of 6% which caused 98% of larval mortality.

Based on the Kruskal-Wallis test, a p-value of 0.001 (0.05) was obtained, statistically it showed that there was a difference in the mean mortality of *Aedes aegypti* larvae in the larvicidal test of avocado leaf extract (*Persea Americana* Mill.). This proves that the avocado leaf extract (*Persea Americana* Mill) was able to cause larval mortality in laboratory larvicide tests.

Probit test is an analysis to determine the effective dose of concentration on the mortality of *Aedes aegypti* larvae. Based on observations, the LC50 value of avocado leaf (*Persea Americana* Mill) was 3.979% with a lower limit value of 2.679% and an upper limit of 4.482%, meaning that the concentration of avocado leaf extract (*Persea Americana* Mill) of 3.979% could kill 50% of *Aedes aegypti* larvae for 24 hours. The value of LC90 which is able to kill 90% of *Aedes aegypti* larvae for 24 hours requires a concentration of avocado leaf extract (*Persea Americana* Mill) of 5.985%. with a lower limit value of 5.405% and an upper limit of 8.010%

This is in line with other studies with 24-hour exposure which gave the highest mortality impact, namely 100% in the treatment with a concentration of 1000 ppm, while the 48-hour exposure which gave the highest impact occurred at a concentration of 800 ppm. LC50 and LC90 at exposure time of 24 hours were 314.614 ppm and 602.073 ppm while LC50 and LC90 at exposure time of 48 hours were 255.444 ppm and 484.542 ppm, respectively. (Fajri, 2010) The results of other research tests showed that avocado leaf juice was effective in killing *Aedes aegypti* larvae at LC50. In addition, avocado leaf extract has the potential as a larvicide and at a concentration of 0.0314% of the extract was able to produce a lethal concentration (LC90).

Avocado leaf extract also has an effect on the type of mosquito larvae, including the Effect of Avocado leaf ethanol extract (*Persea Americana* Mill) has an effect on the mortality of *Anopheles aconitus* L larvae. At a concentration of 0.20 mg/100 ml its killing power in 24 hours reaches 100%. The results of Probit analysis showed LC50 at 0.058 mg/100 ml and LC90 at 0.110 mg/100 ml. The higher the dose of avocado leaf ethanol (*Persea Americana* Mill) the greater the killing power. This is supported by several active compounds contained in the avocado leaf extract. This was also explained in another study about the high content of secondary metabolites in avocado leaves, namely 0.03% flavonoids, 0.12% tannins, 0.08% saponins, and 0.06% alkaloids. (Krissanti, 2018) According to WHO 2015 the concentration of larvicides is considered effective if it can kill 10-95% of the tested larvae. (WHO, 1999)



CONCLUSION

Based on the research that has been done, it can be concluded that the concentration of avocado leaf extract (*Persea Americana* Mill) of 3.979% can kill 50% of *Aedes aegypti* larvae for 24 hours while the killing power of 90% of *Aedes aegypti* larvae for 24 hours requires a concentration of 5.985% .

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