



The Effect Of Variations In The Concentration Of Granule Leaves “Mimba”(Azadirachta Indica A, Juzz) On Mortality Of Larvae *Aedesaegypti*

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Abstract

Dengue fever is one of the diseases that found in tropical climates, including Indonesia. Dengue virus is transmitted to humans through the bite of an infected female mosquito of the species *Aedesaegypti*.

One of the ingredients that can be used as vegetable pesticides is “mimba” leaves that contain active ingredients namely tannins, saponins, alkaloids, flavonoids, steroids, and triterpinoids that are able to decrease appetite, interfere with growth, reproductive processes and metamorphose processes of insects. The purpose of the study was to determine the effectiveness of the killing power of “mimba”leaf extract granules (AzadirachtaIndica A Juzz) against *Aedesaegypti* larvae with various concentrations. The method uses quasi

experimentation with sampling techniques in this study Posttest only control group design. The sample size is as many as 800 larvae with 4 replications at each concentration. Observations were made for 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 24 hours, 48 hours. The concentration of “mimba”leaf extract granules (AzadirachtaIndica A Juzz) include 0.25%, 0.50%, 0.75%, 1%, 1.5%, 2%, *temephos* as positive control and aqua DM as negative control.

Kruskallwallis test results showed there was a significant difference between the concentration of 0.25% granules of “mimba”leaf extract with a significance value of 0.047 ($p < 0.05$). At a concentration of 0.25% effectively killed the larvae of *Aedesaegypti* as much as 92% after observation for 48 hours.

Keywords: *Aedesaegypti*, granule leaves “mimba”(AzadirachtaIndicaA,Juzz)

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Introduction

Dengue fever is one of the diseases that found in tropical climates, including Indonesia. In addition to being affected by the climate, tropical diseases are also triggered by environmental factors such as poor hygiene and sanitation. The nature of the *Aedes aegypti* mosquito has a tendency as a mosquito that is active in the house and the *Aedes albopictus* mosquito is active outside the home. *Aedes aegypti* mosquitoes lay eggs in clear water, such as bathtubs, jars, drums, flower vases, and used goods that can hold rainwater.

Indonesia Incidence Rate (IR) in Indonesia has increased from 2017 - 2019. Of the 33 provinces of Central Java is ranked 30th for the number of dengue pain per 100,000 populations. Especially Banyumas district experienced fluctuation up and down from 2015 to 2019. Incidence Rate in 2015 by 13.6%, in 2016 by 49%, in 2017 by 3.3%, in 2018 by 3.1%, in 2019 by 11% per 100,000 populations. While the death rate / Case Fatality Rate (CFR) in 2015 amounted to 0.4%, in 2016 by 1.6%, in 2017 by 1.5%, in 2018 by 3.6%, in 2019 by 5.9% per 100,000 populations (KabBanyumas Profile, 2019). Larvae of *Aedes aegypti* are found in many used goods located around the house.

Control of vectors and disease-carrying animals by physical, biological, chemical and environmental management methods. Control vectors by physical methods by changing salinity, trapping installation. Biological methods by utilizing predators and organisms that produce toxic, examples of betta fish, tilapia, tadpole frogs and utilizing repellent or mosquito repellent plants. Chemical methods by using chemicals to reduce vector populations such as *temephos*. Chemical control can lead to widespread environmental health disturbances and target vector resistance. Methods of environmental management with environmental modification by closing cracks, and environmental manipulation by draining water storage regularly and periodically. (Decree No. 50 of 2017).

Based on research conducted in three areas in Jakarta municipalities showed that the larvae of *Aedes aegypti* against *temephos* 0.02ppm the highest larval deaths on 24-hour observations were South Jakarta (72%) followed by East Jakarta (53%) and West Jakarta (33%). Test the vulnerability then all three regions are already resistant to *temephos*. (HeniPrasetyowati)

The research that has been done in several places regarding the use of chemical pesticides over a long period of time and certain doses can cause resistance. Natural ingredients that can be processed into vegetable pesticides are able to replace synthetic pesticides. The results of previous research on plants that can be used as ingredients in the manufacture of vegetable pesticides include "mimba" plants. With the active content contained in the mimba plant include salanin, tannin, *saponin* and *triterpinoid* that are able to reduce appetite, interfere with growth, reproductive processes and metamorphose processes of insects Control of *Aedes aegypti* larvae in addition to *temephos* among others utilizing kasiat contained in plants that are able to kill larvae. One of the plants that becomes an alternative is the "mimba" plant. In research on phytochemical tests and antioxidant activity "mimba" leaf extract (*Azadirachta Indica* Juss) obtained the results of methanol extract 80% has the highest antioxidant activity with ic50 value of 83.28. (Supriyanto et al, 2017).



The other study on the diversity of plant species as natural pesticides in Savana BakolBaluran National Park said there are seven species that serve as natural pesticides from 38 plant species found in savanna Bakol, one of which is “mimba”. Processed “mimba” plants are alternative chemical pesticide substances that serve as insecticides (Octavia D, 2008). Azadirachtin substances contained in “mimba” seeds and leaves are effective as insecticides. Azadirachtin does not directly kill insects but decreases appetite, disrupts the growth, reproductive process and metamorphose process of insects.

The active ingredient contained in “mimba” leaves is able to inhibit the growth of *Aedes aegypti* larvae so researchers want to examine the granule preparations of “mimba” leaves (*Azadirachta indica* A, Juzz) which are used as *Aedes aegypti* larvae to see the influence of concentration variations on mortality of *Aedes aegypti* larvae.

Methods

1. Research time

“Mimba” leaves (*Azadirachta indica* A, Juzz) are obtained in the courtyard around the campus of the Department of Environmental Health located in Baturadean, Banyumas, Central Java. “Maserasi” was carried out in the Laboratory of the Department of Environmental Health, extraction and manufacture of “mimba” leaf granules (*Azadirachta indica* A, Juzz) at the Laboratory of Pharmaceutical Biology of the University of Muhammadiyah Purwokerto. The granule test was conducted at the Technology Laboratory of Muhammadiyah Purwokerto University. Rearing is carried out in the laboratory of animal installations Balitbang Class I Banjarnegara, laboratory research is carried out in Balitbang Entomology Laboratory Class I Banjarnegara.

This research was conducted in Balitbang Class I Banjarnegara, in April-June 2021 stage conducted rearing, preliminary stage, experiment

2. Tools and materials used to make granules

Thick extract of “mimba” leaves, 96% ethanol, lactose, PVP (Poly vinyl pyrrolidone) mortar, sieve mesh 20, oven.

3. Materials used

Granules from “mimba” leaf extract (*Azadirachta indica* A, Juzz), larvae of *Aedes aegypti* and aqua-DM

4. Research methods

In each treatment and variation in the concentration of granule methods in this quasi experimental study. The sampling technique in this study posttest only control group design. This design can measure the effect of treatment by comparing multiple treatment groups and control groups. Sampling technique using purposive sampling where the subject to be examined with the criteria of larvae *Aedes aegypti* instar III and larvae in a living or actively moving state. The total number of *Aedes aegypti* needed in the study amounted to: 25 larvae x 6 treatment groups x 4 repetitions x 1 test = 600 larvae of *Aedes aegypti* instar III. The type of hypothesis used is comparative to the scale of numerical variables. The number of research groups as many as 6 groups and not paired, so the test used is One Way Anova (*parametric test*). The One Way



Anova test is used to determine a meaningful influence on the treatment group. If it does not qualify, data transformation or Kruskal Wallis test. If there is a meaningful difference, a Post Hoc test is performed to see the meaning of each group when compared to other groups.

5. Conduct of research

a. Extract mimba leaves

“Mimba” leaves are dried, then “mimba” leaves (*Azadirachta Indica* A, Juzz) are mashed using a blender until they become an even powder. The blended “mimba” leaves are then put in a sealed jar and soaked with 96% ethanol with a 1:10 for 5 - 6 days and stirred daily for 30 minutes. The soaking results are filtered using a flannel cloth, then inserted into the rotary evaporator until it evaporates, the last stage of evaporation using a water bath.

b. Making granules extracted “mimba” leaves (*Azadirachta Indica* A, Juzz)

“Mimba” leaf extract (*Azadirachta Indica* A, Juzz) is put in a mortar, then added lactose stirred until homogeneous, add PVP (Poly Vinyl Pyrrolidone) that has been dissolved with aquadest little by little until a homogeneous period is formed, added ethanol 96% a few drops to add homogeneous, then sing using the number 20, the result will be dried using an oven with a temperature of 600, After drying it is re-used using the number 20.

c. Granule formulation

Granules are made by wet granulation method with the materials used are extracts of “mimba” leaves, *laktsa* and PVP. The composition of 50,000ppm granules in 250 grams requires 12.5 grams of extract plus fillers in the form of 250grams of lactose and binders of 2% PVP Treatment using concentrations of 0.25%, 0.50%, 0.75%, 1%, 1.5% and 2% as larvacida *Aedes aegypti* granules from “mimba” leaf extract (*Azadirachta Indica* A, Juzz). In making 100ml solution with a concentration of 0.25% it takes granule concentration of 50,000ppm with a dose of 5 grams, 0.50% concentration required granule concentration of 50,000ppm with a dose of 10 grams, a concentration of 0.75% required granule concentration of 50,000ppm with a dose of 15 grams, a concentration of 1% required granule concentration of 50,000ppm with a dose of 20 grams, a concentration of 1.50% required granule concentration of 50,000ppm with a dose of 30 grams and a concentration of 2% required granule concentration of 50,000ppm with dosi 40 grams.

Results and Discussions

The results of research that has been conducted in Balitbang class I Banjarnegara regarding the average mortality of *Aedes aegypti* larvae based on exposure time.

Table 1. The average mortality of larvae *Aedes Aegypti* based on exposure time of granule extract “mimba”.

Concentration	Number of larvae of <i>aedes aegypti</i> instar III	Average Mortality Larva <i>Aedes aegypti</i>								
		1	2	3	4	5	6	12	24	48
0.25%	25	0	0	0	0.25	1.5	2.25	4.5	6.75	23



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0.5%	25								12.7	
		0	0	0	1.25	1.75	3	6	5	25
0.75%	25							12.2		
		0	1.75	2.75	6.75	9	5	14.5	21	25
1%	25							13.7	15.7	
		0	2.5	3.75	7.5	10	5	5	22	25
1.5%	25	0.2							20.7	
		5	3	5	8	10	13.5	15	5	25
2%	25								24.2	
		0	2.75	7	10.5	15	17.5	21	5	25
Positive Control	25	12.5	18.3	21.6	24.1					
		5	3	7	7	25	25	25	25	25
Negative Control	25	0	0	0	0	0	0	0	0	0

Table 1 in the implementation of the study was replicated 4 times at each concentration. Obtained the results of early mortality of larvae vary from the length of exposure time of other antra larval mortality at 1 hour at a concentration of 1.5% with an average mortality of 0.25 then 2 hours, namely a concentration of 0.75% as much as 1.75, 1% as much as 2.5 and 2% as much as 2.75 and 4 hours at concentrations of 0.25% as much as 0.25 and 0.5% as much as 1.25. At the 48th hour observation there was a significant increase in larval death compared to the 24th hour observation stated that the longer the exposure of “mimba” leaf granules (*Azadirachta Indica* A, Juzz) mortality of *Aedes aegypti* larvae is increasing.

Table 2 Percentage mortality of larvae of *Aedes aegypti* after exposure to granule extract “mimba” leaves (*Azadirachta Indica* A, Juzz.)

Concentration	Number of test larvae	Average Percentage – Average Mortality of Larvae <i>Aedes aegypti</i> Jam- (%)								
		1	2	3	4	5	6	12	24	48
Concentration 0.25%	25	0	0	0	1	6	9	18	27	92
Concentration 0.50%	25	0	0	0	5	7	12	24	51	100
Concentration 0.75%	25	0	7	11	27	36	49	58	84	100
Concentration 1%	25	0	10	15	30	40	55	63	88	100
Concentration 1.5%	25	1	12	20	32	40	54	60	83	100
Concentration 2%	25	0	11	28	42	60	70	84	97	100



Positive Control	25	5	73.3	86.6	96.6	10	10	10	100	10
		0	3	7	7	0	0	0		0
Negative Control	25	0	0	0	0	0	0	0	0	0

Table 2 in the implementation of the study was replicated 4 times at each concentration. The average percentage of mortality of *Aedes aegypti* larvae after exposure to “mimba” leaf extract granules (*Azadirachta indica* A. Juss) for 48 hours showed a concentration of 0.25% of larval mortality as much as 92% (23 tails) and concentration of 0.5%, concentration of 75%, concentration of 1%, concentration of 1.5%, concentration of 2% of larval mortality as much as 100% (25 tails). In the implementation of the study was replicated 4 times. From the results stated that the longer the larvae of *Aedes aegypti* are exposed to “mimba” leaf granules (*Azadirachta indica* A. Juss) the more mortality of *Aedes aegypti* larvae.

Table 3 normality test results

	Concentration code	Kolmogoriv-Smirnov			Shapiro -Wilk		
		Statistic	df	Significance	Statistic	df	Significance
Larval mortality after 48 hours of exposure	Concentration 0,25	.208	4	.	.950	4	.714

Larval mortality after 48 hours of exposure Concentration 0.25 .208 4. 950 4 714

Normality test results using saphiro-wilk because the sample is less than 50. A significant value at saphiro-wilk 0.714 ($0.714 > 0.05$) means normal distributed data. Because the data is normally distributed, homogeneity tests are carried out.

Table 4 results of the larval mortality homogeneity test after 48 hours of exposure.

Levene Statistics	df1	df2	sig
27.000	5	18	.000

Homogenous tests are performed to determine the next test. From the test result of homogeneity of variances the value of significance is 0.000 ($p < 0.05$) means the data is not homogeneous. The requirement of the “Anova test” is normal distributed data and homogeneous data because one of the conditions is not met so the “Anova test” cannot be implemented and used non-parametric test, namely Kruskal-Wallis.

Table 5 kruskallwallis test results

	Larval mortality after 48 hours of exposure
Chi-Square	16.342



df	5
Asymp. Sig	.006

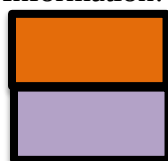
Based on table 4 states that there is a difference in mortality of *Aedes aegypti* larvae between groups of meaningful treatment statistically with a significance value of 0.006 ($p < 0.05$). Therefore $p < 0.05$, it can be concluded that there is a difference in the concentration of “mimba” leaf granules (*Azadirachta indica* A, Juzz) and the time of application to the mortality of *Aedes aegypti* larvae.

Table 6 Mann-Whitney test results

Concentration of <i>Granul Ektrak Daun Mimba</i> (<i>Azadirachta indica</i> A, Juzz)								
	0,25	0,50	0,75	1	1,5	2	+	-
0,25		0,047	0,047	0,047	0,047	0,047	0,047	0,014
0,50			1	1	1	1	1	0,008
0,75				1	1	1	1	0,008
1					1	1	1	0,008
1,5						1	1	0,008
2							1	0,008
+								0,008
-								

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Information:



Insignificance ($p > 0,05$)

Significance ($p < 0,05$)

Based on the table above it can be seen that the comparison between granule leaves “mimba” concentration 0.25% with concentration 0.50%, concentration 0.75%, concentration 1%, concentration 1.5%, concentration 2% and positive control with significance value 0.047 ($p < 0.05$) and negative control with significance value 0.014 ($p < 0.05$) means there is a significant difference. Comparison between granule extract leaves “mimba” concentration of 0.50%, concentration 0.75%, concentration 1%, concentration 1.5%, concentration 2%, positive control with negative control significance value ,008 ($p < 0.05$) means there is a significant difference. Comparison between granule ekstrak leaves “mimba” concentration 0.50%, concentration 0.75%, with concentration 1%, concentration 1.5%, concentration 2%, positive control with significance value 1 ($p > 0.05$) has no difference meaning significant.



DISCUSSION

The average mortality of larvae after being observed for some time (table 1) showed that the highest average mortality of “mimba” leaf extract granules (*Azadirachta Indica* A, Juss) averages the lowest mortality at 0.25 concentrations of 23 (92%) and average mortality at concentrations of 0.50%, 0.75%, 1%, 1.5% 2% as much as 100% (25 tails). The longer the larvae of *Aedes aegypti* are exposed to granules of “mimba” leaves the more mortality increases. A significant increase in mortality of *Aedes aegypti* larvae occurred on observations from 24 hours to 48 hours. The highest percentage increase in average mortality at a concentration of 0.25% by 65% (from 27% to 92%) and an increase in mortality of the lowest concentration of 2% by 3% (from 97% to 100%).

The normality test result stated normal with a significance value on Saphiro-Wilk of 0.714 ($p > 0.05$) meaning that the normal distributed data and homogeneous results state not to be homogeneous with a significance value of 0.000 ($p < 0.05$) means the data is not homogeneous. The Anova test cannot be performed because the requirements of the Anova test are normal distributed data and homogeneous data. Follow-up tests with non-parametric kruskallwallis tests to see the differences between treatments. The kruskallwallis test obtained a result of 0.006 ($p < 0.05$) meaning there is a difference in the variation in the concentration of “mimba” leaf granules (*Azadirachta Indica* A, Juss) and the time of application to mortality of *Aedes aegypti* larvae. Mann Whitney's Post Hoc test results showed a significant difference in concentration 0.25 with a significance value of 0.047 ($p < 0.05$). It is assumed that the death of *Aedes aegypti* larvae is due to exposure to “mimba” leaf extract granules containing the active ingredients alkaloids, *flavonoids*, steroids, *saponins*, tannins, *terpenoids*. These active substances have benefits as stomach toxins, respiratory toxins, inhibition of skin turnover process or *moulting*, decreased appetite so that it can cause death in the larvae of *Aedes aegypti*.

Based on the results of observations, the larvae over time movement is limited, when given a touch will bend the body. Larvae that have not really died if given a little touch will move like convulsions at the base of the test site, namely *beker* glass. In contrast to control larvae where resting on the surface of the water and when given a little touch will move actively assumed due to contact with alkaloid compounds. The larvae will move to the surface of the water. The larval body over time turns white how flavonoids work by entering the larva's body through the respiratory system which will then cause weakness in the nerves and damage to the respiratory system and result in the larvae unable to breathe and eventually die (Kumara CJ, 2021).

In line with previous studies on the effectiveness of ethanol extract of “mimba” leaves (*Azadirachta Indica* A, Juss) as a *larvalide* against *Aedes aegypti* larvae with concentrations of 0.25%, 0.5%, 1%, 1.5%, 2%. With 25 test animals, the observation time is 48 hours, repetition as much as 3 times. Lethal Concentration (LC50) repeat I 1,561%, repeat II 1,686%, repeat III 1,501% obtained an LC average value of 1.52%. Lt50 values on repeat I 36,359 hours, repeat II 37,788 hours, repeat III 36,879 hours and average LT50 37,009 hours. Based on the normality test with Saphiro Wilk obtained a significance value of $0.004 < 0.05$ meaning that the data is not normally distributed. Continued with the Kruskal-Wallis test obtained a value of $0.002 < 0.05$ meaning that there is an average difference in the cumulative number of *Aedes aegypti* larva deaths



between the concentration of ethanol extract of “mimba” leaves against positive control and negative control. (NuraeniDwisusanti).

Other research on the formulation and activity of granules of black cumin flour (*Nigella sativa* L) This formulation of granules of black cumin flour granules is made in 3 concentrations namely concentrations of 1%, 5% and 10%. Black cumin flour can be formulated into the preparation form of granule biolarvasida where all formulas qualify for a good physical quality test, while for the biolarvasida activity test a concentration of 10% shows the results of larvae that die on average within 24 hours which is as many as 9 larvae. This suggests that black cumin flour (*Nigella sativa* L.) is very potentially used as a granule preparation of biolarvasida (10% concentration) with the same larval mortality result with positive abate control ($P > 0.05$). (DwiRachmawatyDaswi) also on research on the potential of granule preparations from makasar fruit standardized extract (*Brucea Javanica* L Merr) as biolarvasida in *Culex quinquefasciatus* say. Concentrations of 15ppm, 22.5ppm, 30ppm, 37.5ppm 4 grams abate with a sample of 600 larvae of *Culex quinquefasciatus* say. The weight of the fruit granumkasar 4 grams in a crock container containing 50 liters of water with observations of 24 hours and 48 hours. The results of the study 30ppm concentration can kill larvae 95% larvae *Culex quinquefasciatus* say. The 24-hour observation time received a value of LC50 25.6ppm and LC90 30.9 ppm. The 48-hour observation time received LC50 values of 25.2ppm and LC90 29 ppmper 50 liters of water in jars. Mann Whitney tests at each granule concentration averaging $p = 0.001$ ($p < 0.05$) (Yogi Chess son)

In line with RizkiKhalalia's research on the killing power test of tobacco waste extract (*Nicotiana glauca* L) against *Aedes aegypti* larvae obtained results there is a difference in the average pair of *aedes aegypti* larvae deaths significantly ($p < 0.05$). Concentration values that are not different are granule concentrations of 10% with granules of 15%, granules of 20% and dextrin of 10% of granule concentrations of 15% with granules of 20% and concentrations of dextrin of 10% with water.

Conclusion

Based on the results of the study, it can be concluded that

1. “Mimba”leaf granules (*Azadirachta Indica* A, Juzz) is able to inhibit the organization of larvae of *Aedes aegypti* instar III.
2. “Mimba”leaf granules (*Azadirachta Indica* A, Juzz) effective as a biolarvasida with a larval mortality rate of over 80% at a concentration of 0.25% for 48 hours.



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