

ORIGINAL ARTICLE

Efficacy of Garlic Solution (*Allium sativum*) in Killing of Aedes SP Larva

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ABSTRACT

Introduction: Aedes sp can transmit various diseases including dengue fever, chikungunya whose symptoms are almost similar to dengue fever. In the recent times a new case is the virus outbreak of Zika. Many efforts have been made to suppress the density of the vector populations with the control of Aedes sp mosquitoes. One of them is by using vegetable larvicides namely garlic solution (*Allium sativum*). **Methods:** This research is a pseudo experiment with posttest design with a control group. The aim of this research study is to know the effectiveness of garlic solution in killing the larva Aedes sp. This research sample is 484 tails that are bred by the researchers themselves. The concentrations used in this study were 0%, 60%, 70%, 80% and 90% with 5 repetitions. The free variable in this study is the concentration of garlic solution and the bound variable is the larva Aedes sp. **Results:** The results of the study using one-way ANOVA test ($p = 0.001$) indicate that there are differences in the larvae deaths between each concentration. Observation by using the Abbot formula at a concentration of 60% it is found to be susceptible in killing of Aedes sp larvae due to the death of larvae at 100%. **Conclusion:** This means there is a direct relation between the increasing concentration of garlic solution and the number of dead larvae of Aedes sp. So, garlic can be one of the alternatives in vector control that is safe for the environment.

Keywords: Garlic Solution, Aedes sp, Larvicide

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INTRODUCTION

Mosquitoes belong to the family Culicidae, the order Diptera and the class Insecta class (1). Female mosquito bites humans and animals with their fascicles and act as vector of diseases in humans and animals. Aedes sp can transmit a variety of diseases including dengue fever and chikungunya disease with symptoms similar to dengue fever. A new case of Zika virus has been discovered with the outbreak in the early 2015 and the vector is again Aedes sp. Although no definite cases have been found in Indonesia but awareness regarding this disease is necessary (2). Aedes sp is now better known as a vector for the transmission of dengue fever (3).

The World Health Organization (4) estimates 50 million people infected with dengue fever every year. Dengue fever (DBD) is a problem of public health in Indonesia because the numbers of sufferers continue to grow, and the spread is wider. Dengue disease was first reported in 1968 in Jakarta and Surabaya. In 2010 dengue disease has spread in 33 provinces. Since it was first discovered the case of the DBD increased steadily with 2004

cases presently. The rise of the DBD case is inversely proportional to the death rate (CFR) due to DBD. In the beginning dengue was found in Surabaya and Jakarta CFR with 40% cases, then it continued to decline and in the year 2010 reached 0.87%. The most cases of DBD are reported in areas with high levels of density, such as provinces like Java, Bali and Sumatra. The Incidences Rate (IR) in 2010 has reached 65,62/100,000 inhabitants with Case Fatality rate 0.87 (5).

North Sumatera Province is a DBD endemic area. In 2012, the number of DBD cases was recorded as 4,367 with IRS amounting to 33 per 100,000 inhabitants. Compared to 2010 and 2011, this figure suffered a sharp decline from 72 per 100,000 population of 2010 and 45/100,000 inhabitants, but still higher when compared with the year 2006 and previous years (6).

Other than that chikungunya disease is highly potential considering the vector that causes the disease is both Aedes aegypti and Aedes albopictus mosquitoes (7). Chikungunya disease also need attention as the disease causes joint pain such as hand, wrist, and ankles and the larger joints such as knee and shoulder, resulting in difficulty in walking (8).

Zika virus (ZIKV) is transmitted by Aedes aegypti and Aedes albopictus mosquitoes (9). Recent study in 2018

shows Indonesia ranked as the third country at risk for ZIKV exposure due to the monthly volume of airline travelers (10). Resistant variety of *Aedes aegypti* against commonly used insecticides has been reported in Jakarta, Indonesia (11,12).

Many efforts have been made to suppress the density of vector populations of *Aedes* sp mosquito, namely chemical control by using insecticide. Chemical control is one of the very highly recommended ways to control mosquitoes in a short time, for a large population. But during the process it should be remembered that these controls have considerable side effects and can damage the environment. To minimize the side effects of the use of chemical insecticide in vector control, utilization of natural insecticide that can eradicate the vectors is suggested to reduce the disease cases caused by *Aedes* sp. In addition, as these substances are made from natural materials, so it is expected that this type of insecticide will be easier to decompose (biodegradable) in nature. So, it will not pollute the environment and is relatively safe for humans and livestock (13).

Presently eradication of infectious mosquitoes *Aedes* sp is done by spraying insecticide against adult mosquitoes. *Aedes* sp transmit diseases like bloody dengue, chikungunya or Zika viruses (7). But in this process, some mosquitoes are left alive and from where the population is going to increase again. Therefore, mosquito repellent must be also used to eradicate or to minimize the vector. Genetic eradication of *Aedes* sp, with vegetable larvicides using traditional plants is also done. Traditional plants, such as garlic can be an alternative substitute for insecticide. Garlic is chosen as an alternative because this plant is already known to the community and can be easily acquired throughout Indonesia (14).

Garlic plants can be one natural alternative option of vector control disease caused by *Aedes* sp mosquitoes. The compounds present in garlic include Allicin and Sulfur Ammonia acid Allin. The Allicin Lyase Enzyme converts sulfuric ammonia acid Alin to Pyruvic Acid, Ammonia, and Allicin Anti Microbes. Next, Allicin changes to Diallyl disulfide. Allicin and Diallyl Sulphide compounds have many benefits and are effective as drugs. Allicin and its derivatives also have a larvicidal effect (15).

Results of previous studies (10) shows that at concentrations of 10%, 15%, 20%, garlic solution (*Allium sativum*) can kill the larva *Aedes* sp effectively as it kills more than 50% of the larvae. Garlic oil has also been used in other countries to eradicate mosquitoes. The life cycle of mosquitoes consists larval stage which is found in the puddle. In such cases Garlic oil can used which causes clotting of certain protein structures resulting in larval death before maturity. The effectiveness of garlic as an insecticide in domestic gardens is by the same

process (16).

In connection with the above descriptions, researchers are interested in conducting study on the effectiveness of garlic solution (*Allium sativum*) in killing the larva *Aedes* sp.

MATERIALS AND METHODS

Design Research

This research is a pseudo-experimentation (experimental Quasi) to determine the effectiveness of garlic solution (*Allium sativum*) in killing *Aedes* sp larva taking into account factors that affect the life of the larva *Aedes* sp, namely temperature, humidity, and duration of contact. This research was designed by using the Post Test Control Group only (draft post test with control group). This study was conducted at Jalan Bakti Luhur No. 27 B Medan from February – July 2016.

The *Aedes* sp larva was distributed in 4 containers each with 22 larvae for treatment Group and one container of control group. This is in accordance with consideration for the WHO experimentation WHO (2016) where 20-25 larvae sample size was used. Then Deuteronomy is performed on each treatment by 5 times repetition and the amount of treatment is 4. So, after repeated treatment for 20 times the larvae needed to be obtained was done by calculation:

Total treatment x number of repetitions = $22 \times 4 \times 5 = 440$ tails, plus 22 tails for control group, and 22 tails for supply if mosquito larva as test material is dead (17). Thus the total larvae of *Aedes* sp required as much as 484 tails.

Data processing

Data processing is computerized and presented in tabular form to see the percentage of larvae deaths.

With the interpretation of the results of this vulnerability test, when death of larvae:

98%-100%: Vulnerable

80%-98%: doubtful

Below 80%: resistance (18).

Data Analysis

Univariate Analysis

Analysis of the data of each variable was done by using frequency distribution table, average (mean), median and standard deviation. This analysis only generates the frequency distribution and percentage of each variable (19) has been conducted.

Bivariate analysis

Bivariate analysis is conducted to see if there are significant differences between the control groups and the treatment group by:

1. Independent T-test was used to see the difference in larvae deaths after 1 hour of treatment. For the 24

treatments One Way ANOVA test was used to see the difference in larvae deaths at a concentration of 90%,80%,70%,60% and 0% to see if it fulfills the assumption:

2. Normal distribution of data was tested using the Kolmogor of-Smirnov test.
3. Normal and homogeneous distribution data was tested with homogeneity test
4. Samples/groups Independent was done
5. The type of linked data is numeric with category (for categories more than two groups)
6. If it does not meet the above assumptions, then crucial test was used (20).

RESULTS

Effectiveness of garlic solution (*Allium sativum*) in killing the larva *Aedes sp*

The garlic solution was given in various concentrations of 90%, 80%, 70%, 60% and 1 control group then mixed with experimental aquades with 5 times repetition. The sample is used for 484 tail larvae with each container filled with 22 tails larvae. Observation is done with 1 hour with contact of garlic solution, and if there is a living larva then it is transferred to another container without garlic solution which is further observed for 24 hours. The results are presented in Table I.

Based on Table I it is known that the average temperature before treatment is 27°C, the average temperature of 1 hour during treatment is 27°C and the average temperature 24 hours after treatment is 29°C and the average humidity before treatment is 63%, the average humidity of 1 hour during treatment is 63% and the humidity rate is 24 hours after the treatment is 64%.

Table I: Temperature and Moisture in Test and Treatment Groups

Measured variables	Measurement time			
	Replication	Before treatment	1 hour during treatment	24 hours After treatment
Temperature	I	27°C	27°C	29°C
	II	27°C	27°C	29°C
	III	27°C	27°C	29°C
	IV	27°C	27°C	29°C
	V	27°C	27°C	29°C
Moisture	I	63%	63%	64%
	II	63%	63%	64%
	III	63%	63%	64%
	IV	63%	63%	64%
	V	63%	63%	64%

According to Table II It can be noted that the average death of larvae at a concentration of 90% as much as 21 larvae, at a concentration of 80% the average death of larvae by 19 larvae, at a concentration of 70% of larvae there was deaths by 18 larvae, and in concentrations of 60% average death of larvae as much as 17 larvae, while in the control there is no dead larvae.

The average death of larvae after 24 hours at a concentration of 90% was as much as 1 larvae, at a concentration of 80% of the average larval deaths was 3 larvae, at a concentration of 70% on average larvae deaths by 4 and at concentrations of 60% the average death of the larva as many as 5 larvae die, while in the control no dead larvae (Table II).

Table II: Distribution of Larva *Aedes sp* death amount during 1 and 24 hour treatment with garlic solution

Time	Replication	Number of test larvae	The total death of the Larva <i>Aedes SP</i>									
			Any concentration (%) / 100 ml									
			90%		80%		70%		60%		Control	
			+	-	+	-	+	-	+	-	+	-
After 1 hour	1	22	19	3	17	5	16	6	15	7	0	22
	2	22	20	2	18	4	17	5	16	6	0	22
	3	22	21	1	19	3	18	4	17	5	0	22
	4	22	22	0	20	2	18	4	18	4	0	22
	5	22	22	0	20	2	19	3	18	4	0	22
Amount			104	6	94	16	88	22	84	26	0	110
Average			21	1	19	3	18	4	17	5	0	22
After 24 hour	1	22	3	0	5	0	6	0	7	0	0	22
	2	22	2	0	4	0	5	0	6	0	0	22
	3	22	1	0	3	0	4	0	5	0	0	22
	4	22	0	0	2	0	4	0	4	0	0	22
	5	22	0	0	2	0	3	0	4	0	0	22
Amount			6	0	16	0	22	0	26	0	0	110
Average			1	0	3	0	4	0	5	0	0	22

Description:
 +: Number of dead larvae
 -: Number of live larvae

According to table III It can be noted that the death of the larva *Aedes* sp within 24 hours at a concentration of 90% can kill the larva *Aedes* sp as much as 5%, the concentration of 80% can kill the larva *Aedes* sp by 15%, at the concentration of 70% can kill *Aedes* sp larvae as much as 20%, the concentration of 60% can kill the larva of *Aedes* sp as much as 24% and no death of the larva of *Aedes* sp in the control.

Table III: Number and death percentage of *Aedes* sp Larva after moving to a container without garlic solution from various concentrations and observed after 24 hours

Repetition results	The total death of the Larva <i>Aedessp.</i>									
	Any concentration (%) / 100 ml									
	90%		80 %		70%		60%		Control	
	F	%	F	%	F	%	F	%	F	%
1	3	14	5	23	6	27	7	32	0	0
2	2	9	4	18	5	23	6	27	0	0
3	1	4	3	14	4	18	5	23	0	0
4	0	0	2	9	4	18	4	18	0	0
5	0	0	2	9	3	14	4	18	0	0
Amount	6	27	16	73	22	100	26	118	0	0
Average	1	5	3	15	4	20	5	24	0	0

Table IV shows that the 100% of *Aedes* larvae was killed in 1 hour and 24 hours in the concentration of 90%, and 80% concentration can kill the *Aedessp* larva as much as 100%, concentration 70% can kill the larva *Aedes* sp as much as 100%, the concentration of 60% can kill the larva *Aedes* sp as much as 100% and no death of the larva *Aedes* sp. in 0% control.

Table IV: Recapitulation of summation of the *Aedessp* Larva for 1 hour treatment and after 24 hours of treatment

Repetition results	The total death of the Larva <i>Aedes</i> SP									
	Any concentration (%) / 100 ml									
	90%		80 %		70%		60%		Control	
	F	%	F	%	F	%	F	%	F	%
1	22	100	22	100	22	100	22	100	0	0
2	22	100	22	100	22	100	22	100	0	0
3	22	100	22	100	22	100	22	100	0	0
4	22	100	22	100	22	100	22	100	0	0
5	22	100	22	100	22	100	22	100	0	0
Amount	110	500	110	500	110	500	110	500	0	0
Average	22	100	22	22	22	100	22	100	0	0

To see the vulnerability of the death of *Aedessp* larvae calculation was done using the formula from WHO standard susceptibility test and calculated with the formula abbot:

$$\% \text{ of deaths are in the test} = \frac{\% \text{ mortality in control} \times 100}{100 - \% \text{ Death control}}$$

The following results are obtained:

1. For 90% concentration

$$\frac{(100\% - 0\%)}{(100\% - 0\%)} \times 100 = 100\%$$

2. For 80% concentration

$$\frac{(100\% - 0\%)}{(100\% - 0\%)} \times 100 = 100\%$$

3. For 70% concentration

$$\frac{(100\% - 0\%)}{(100\% - 0\%)} \times 100 = 100\%$$

4. For 60% concentration

$$\frac{(100\% - 0\%)}{(100\% - 0\%)} \times 100 = 100\%$$

Independent T test result

Independent T-Test is done to see the difference in larvae deaths after 1 hour and 24 hours, it is determined by looking at the probability value, when the probability of > 0.05, then H0 is accepted, but if the probability of < 0.05 is H0 rejected.

Table V revealed that the probability P value is 0.001 (P<0.005), this indicates there is a difference in the average death of the larva *Aedes* sp after 1 hour of treatment and 24 hours of treatment.

Table V: The average difference in death of larvae after 1 hour of treatment and 24 hours

	Group	Mean	SD	SE	P-Value	N
The average death of larvae	1 hour	18.75	1.708	0.854	0.001	4
	After 24 hours	3.25	1.708	0.854		4

Test result One-way ANOVA

The retrieval of ANOVA test results is determined by looking at the probability value, when the probability >0.05, then H0 is accepted. When the probability of <0.05 is H0 rejected.

From Table IV, it is known that the F count on the above test is 9.458 and the table F value is 3.24 (F count >F table) with value p=0.001 (P<0.005). This indicates that there is an average difference in the death of larvae in the various concentrations of garlic solution in the killing of *Aedessp* larvae.

Table VII: Number of larvae after the solution of garlic with various concentrations

Various concentrations	Mean	SD	SE	Df	F	Sig.
60%	16.80	1.304	0.583	3	9.458	0.001
70%	17.60	1.140	0.510	16		
80%	18.80	1.304	0.583			
90%	20.80	1.304	0.583			

DISCUSSION

The research has been done by using 4 treatment, with concentrations of 90%, 80%, 70%, 60% and 1 control with five times repetition for 1 hour left in contact with garlic (*Allium sativum*) solution and after that the surviving larvae are removed to other containers without contact with the solution of garlic (*Allium sativum*) and observed for 24 hours. From the results of research, we

found that the death of the larvae of *Aedes* sp at different concentrations and different exposure times is different. In the life cycles of *Aedes* sp mosquitoes, the insects undergo complete metamorphosis passing through four stages, namely eggs, stadium larvae, stadium pupa, and adults.

The eggs will become larvae after 2-4 days, then larvae changes to pupa which takes a long time that is 5-9 days because at this stage larvae undergo through four stages of development. Starting from the development of instars I to II occurs within 2-3 days, until it turns into a pupa and adult mosquitoes (21). According to the table 1 it can be seen that before and after the average temperature was 27°C and humidity 64% which is average optimum condition for the normal life of larvae. This condition is not ignored as the disruptor variable in the study gained. This corresponds to the criterion that good moisture for larva life is 60%-80% and it also avoids the death of larvae due to inappropriate moisture. Then in the result it can be noted that in the control containing aquades without a solution of garlic does not have the presence of the dead larvae. It proves that Aquades has no effect on the death of the larva *Aedes* sp. At the lowest concentration of 60% in 1 hour it was found to be effective because it meets the standard of LD50, which is able to kill larvae 84% of the larva (22).

Once the dose effective in killing the larvae *Aedes* sp is obtained independent T test was done to see the median difference of death of the larva *Aedes* sp after 1 hour of garlic solution and 24 hours of observation without garlic solution. The result is $P=0.000$ ($P < 0.05$). So according to the result it shows that there is a variation in the average death of larvae at 1 hour of treatment and 24 hours of observation. In addition there is indeed a difference in the average concentration which was seen from One way Anova which obtained the result of $P=0.001$ ($P < 0.05$), from this result it can be mentioned that there is a significant difference of each concentration used for killing *Aedes* sp larva effectively.

In addition to the effectiveness of garlic solution in the killing of *Aedes* sp larvae was also tested using the Abbot formula to see the vulnerability of the *Aedes* sp larvae death obtained from 100% concentrations of 60%, 70%, 80%, and 90%. When adjusted to the criteria then the larva can be said to be vulnerable to the concentration. In previous research conducted by Simone et al., (2014) with a concentration of 1%, 5%, 10% and 20% and using 10 larvae, indicating that at 1% and 5% concentrations cannot be said to be effective because the number of dead larvae is less than 50%. While at concentrations of 10% and 20% larvae were killed effectively as larval death was more than or equal to 50%. The difference in this research with previous research is to use higher concentrations of 60%, 70%, 80% and 90% and the total number of larvae used by

more than 22 tails for each treatment and control. Thus, both research results obtained shows that the results are directly proportional, that is the higher the concentration level of garlic solution used, higher the power of the solution to kill larva more effectively.

The killing power of garlic solution is presence of allicin and diallyl sulphide which plays an important role as anti-microbial and anti-parasitic. Allicin disrupts the synthesis of parasitic cell membranes so that parasites cannot develop further. Allicin is also toxic to parasitic or bacterial cells. Allicin works by damaging the Sulfhydryl (SH) found in proteins. Allicin will damage the cell membrane of the larva so that the lysis occurs; as a result, the larvae cannot develop further. Oil content in garlic solution change water surface tension so that larvae have difficulty to take air from water surface. So, the larvae do not get enough oxygen for growth, causing death of larvae (23).

In addition, other content in garlic allegedly plays an active role in larval deaths is flavonoids. This substance works as a respiratory inhibitor. Flavonoids are thought to interfere with energy metabolism within the mitochondria by inhibiting the electron transport system. Garlic, onions, etc. are rich in phytochemicals with their antimicrobial properties(24). The dietary phytochemicals blocking the ATP synthesis in tumor and bacterial cells and thereby affecting ETP and beneficial (25,26).

Thus, this research has been able to prove that the provision of garlic solution affects the death of the larva of *Aedes* sp mosquito.

Further research on the method of processing garlic is more applicative so that the results can be applied more extensively to the community and can be used for daily to prevent the existence of the larva of *Aedes* sp. The use of natural substances such as garlic in the killing of *Aedes* sp larva is beneficial for the public. So further research and awareness is necessary for more widespread use such natural larvicides. So, it is recommended for health workers to spread the use traditional plants that can be used as substitutes to eliminate *Aedes* sp larvae, like garlic solution.

CONCLUSION

From the research results efficacy of garlic solution in various concentrations of 90%,80%,70%, and 60% to kill the larva *Aedes* sp. Garlic (*Allium sativum*) can be used as an alternative ingredient to kill *Aedes* sp. larva in concentrations of 60% because it able to kill 100% of larvae. There was statistical dissimilarity in the death of larvae between each concentration and the results of independent t test indicates there is a difference in the average death of larvae at 1 hour of treatment and after

24 hours.

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