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EFFECTIVENESS OF *CITRUS AURANTIFOLIA* AND *ALLIUM SATIVUM* ON *AEDES AEGYPTI* LARVAE MORTALITY

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Abstract
Dengue Hemorrhagic Fever is an acute febrile disease caused by the dengue virus that enters the human bloodstream through a mosquito's bite from the genus Aedes. *Aedes aegypti* and *Aedes albopictus* are the main vectors of dengue transmission. DHF prevention is carried out by breaking the chain of transmission, and then biological insecticides have the potential to control vectors. This study used an experimental research design with Post Test Only Control Group Design. Data analysis used the One-Way ANOVA Test and Bonferroni Test. The study showed that the average percentage of larval mortality at a concentration of 0.10% was 27%, a concentration of 0.20% was 51%, a concentration of 0.30% was 74%, and a concentration of 0.40% was 100%. The results of the One-Way Anova test obtained a p-value= 0.000 (p=<0.05), so there was a significant difference in the number of larvae that died at various concentrations, and the results of the Bonferroni Test showed that the most effective concentration against the death of *Aedes aegypti* larvae was a concentration of 0,40%. It is expected further research finds more applicable *citrus aurantifolia* and *allium sativum* formulations to make it easier in the community.

Keywords: *Aedes aegypti*, *Citrus Aurantifolia*, *Allium Sativum*, Larvae Mortality

INTRODUCTION
Dengue Hemorrhagic Fever is an acute febrile disease caused by the dengue virus that enters the human bloodstream through the bite of a mosquito of the genus Aedes. *Aedes aegypti* and Aedes albopictus are the main vectors of dengue disease transmission (Kemenkes RI, 2014). DHF control is emphasized vector control to break the chain of transmission (Hendri, et al 2016). One way that can be done is by controlling larval habitat in water containers. The presence of larvae in water containers is a way of estimating the population density of Aedes sp mosquitoes and one of the risk factors for DHF transmission (Purnama, et al 2012). People often use fast methods to break the transmission life cycle by using larvicides (Ariyati, 2013)

Currently, there are many synthetic larvicides used by the community, namely temephos, Temephos as a larvicide whose use is very wide because it is very effective in controlling mosquito larvae but these larvae have a negative

impact on human health and the environment because they contain harmful chemical compounds, and can increase mosquito resistance (Supono, 2014). Plants that have the potential to be developed as larvicidal agents are lime (Citrus aurantifolia). Lime plants are often found in Indonesia, especially in the area around Banyuwangi because this plant can grow anywhere. *Citrus aurantifolia* also contains many chemical compounds such as citric acid, amino acids (tryptophan and lysine), flavonoids, essential oils (limonene, linalin acetate, geranil acetate, fellandren, citral, lemon camphor, kadinen, actialdehyde and anildehid), vitamins A, B1, and C (Haq, et al, 2010).

Garlic *(Allium sativum)* is also included in plant insecticides because the aroma of *Allium sativum* is very pungent and the hot and spicy taste of *Allium sativum* is thought to be able to repel worms. also repel or inhibit and even kill mosquito larvae (Sulistyoningsih, 2009). This was proven when the author tried to make initial observations by filling water into 2 containers, then, in one container, the author put *Allium sativum,* and in the second container only water without Allium sativum, then the writer placed the two containers under the table, the author puts it under the table because, as mentioned by Anggraeni (2010) that mosquitoes are abundant in dark places such as under the table, a week later the author saw that in a container containing only water there were mosquito larvae, while in a container containing only water there were mosquito larvae. which contained *Allium sativum* there were no mosquito larvae, this further made the authors believe *Allium sativum* contained chemical compounds that could be used as biological insecticides.

Based on the opinion of Muammar (2013) & Muswita (2011), it can be concluded *Allium sativum* contains active compounds such as flavonoids, atisir oil, garlic oil, and allicin. Flavonoids and essential oils act as respiratory poisons and allicin inhibits membrane synthesis, causing the death of mosquito larvae. Then, the research was conducted on natural larvicides, especially on the use of *Citrus aurantifolia* and *Allium sativum* peel against the Death of *Aedes aegypti* Mosquito Larvae.

METHOD

Research Design and Subject

This research was an experiment with a Post-test Only Control Group Design, which is a research design that does not use a pretest to the sample before doing the treatment. In this design, there are two groups, each selected at

random (randomization), the groups are considered the same before treatment. This research design measures the effect of treatment on the experimental group by comparing the group with the control group (Riyanto, 2011).

Instruments and Data Analysis Procedures

Data analysis using the One-Way ANOVA Test and Bonferroni Test.

FINDING

1. Univariate Analysis

Univariate analysis showed the average percentage of mortality of *Aedes aegypti* mosquito larvae in the treatment with various concentrations of *Citrus aurantifolia* and *Allium sativum* extracts.

Table 4.1 Frequency Distribution of Dead *aegypti* Mosquito Larvae with Variations in Concentration *Citrus aurantifolia* and *Allium sativum* Extract in 1 Hour Observation.

Repeat	Control	0,10%	0,20%	0,30%	0,40%
	∑ Dead Larvae (Tail)				
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	0	0	0	0	0
5	0	0	0	0	0
Total	0	0	0	0	0
Average	0	0	0	0	0
Percentage (%)	0%	0%	0%	0%	0 %

Table 4.1 shows that of the 25 *Aedes aegypti* larvae after contact for 1 hour with various concentrations, there were no *Aedes aegypti* larvae that died in the control and various concentrations (0.10%, 0.20%, 0.30%, and 0). ,30%).

Table 4.2 Frequency Distribution of Dead *aegypti* Mosquito Larvae with Variations in Concentration *Citrus aurantifolia* and *Allium sativum* Extract in 2 Hour Observation.

Repeat	Control	0,10%	0,20%	0,30%	0,40%
	∑ Dead Larvae (Tale)				
1	0	1	2	4	7
2	0	2	2	3	6
3	0	1	3	4	6
4	0	2	3	4	7
5	0	1	4	4	5
Total	0	7	14	19	31
Average	0	1,4	2,8	3,8	6,2
Percentage (%)	0%	5,6%	11,2%	15,2%	24,8%

Table 4.2 shows that of the 25 *Aedes aegypti* larvae after contact for 2 hours with various concentrations, there were *Aedes aegypti* larvae that died with the highest average being 6 (24.8%) at a concentration of 0.40%.

Table 4.3 Frequency Distribution of Dead *aegypti* Mosquito Larvae with Variations in Concentration *Citrus aurantifolia* and *Allium sativum* Extract in 3 Hour Observation.

Repeate	Control	0,10%	0,20%	0,30%	0,40%
	∑ Dead Larvae (Tale)				
1	0	2	4	6	9
2	0	3	4	6	9
3	0	2	5	6	8
4	0	3	5	7	9
5	0	3	5	6	8
Total	0	13	23	31	43
Rata-rata	0	2,6	4,6	6,2	8,6
Percentage (%)	0%	10,4%	18,4%	24,8%	34,4%

Table 4.3 shows that of the 25 *Aedes aegypti* larvae after contact for 3 hours with various concentrations, there were the highest *Aedes aegypti* larvae that died, namely: 8 (34.4%) at a concentration of 0.40%.

Table 4.4 Frequency Distribution of Dead *aegypti* Mosquito Larvae with Variations in Concentration *Citrus aurantifolia* and *Allium sativum* Extract in 24 Hour Observation.

Repeat	Control	0,10%	0,20%	0,30%	0,40%
	∑ Dead Larvae (Tale)				
1	0	6	12	18	25
2	0	6	12	19	25
3	0	7	13	18	25
4	0	8	13	18	25
5	0	7	14	20	25
Total	0	34	64	93	125
Average	0	6,8	12,4	18,6	25
Percentage (%)	0%	27,2%	51,2%	74%	100 %

Based on the results of the study, it was known that the effect of the peel extract of *Citrus aurantifolia* and *Allium sativum* on the mortality of *Aedes aegypti* mosquito larvae that were treated with various concentrations of control, 0.10%, 0.20%, 0.30%, and 0.40% showed an increase in the average number of dead *Aedes aegypti* mosquito larvae, at a concentration of 0.40% *Citrus aurantifolia* and *Allium sativum* effectively killed an average of 25 individuals (100%).

2. Bivariate Analysis

The One-Way Anova test is to test a design of more than one variable. The statistical test in this study was analyzed using a 95% confidence level or 0.05 with the one-way ANOVA method. This method is used to determine whether or not there is a larvicidal effect, by first conducting normality and a homogeneity test, the results of the sig value are obtained. 0.00> of 0.05.

Table 4.5 One Way Anova Test Results Number of Dead Aedes Aegpty Mosquito Larvae on Addition of *Citrus aurantifolia* and *Allium sativum* Extract with Various Concentrations and Controls Within 24 Hours Observation

Concentration Variable	Mean	SD	95 % CI	ρ *value*
Kontrol	.00	0.000	.00 – .00	0.000
0,10%	6.80	0.837	5.76 – 7.84	
0,20%	13.00	1.000	11.76 – 14.24	
0,30%	18.60	0.894	17.49 – 19.71	
0,40%	25.00	0.000	25.00–25.00	

Table 4.5 the results of the One-Way Anova test, the value= 0.000 <0.05 can be interpreted that statistically Ho is rejected and Ha is accepted, so it can be concluded that there is a difference in the number of *Aedes aegypti* mosquito larvae that die on the addition of extract Citrus aurantifolia and *Allium sativum* with various concentrations.

Furthermore, to determine the difference in the average difference in the number of *Aedes aegypti* mosquito larvae that died on the addition of *Citrus aurantifolia* and *Allium sativum* extracts with various concentrations and controls, the Bonferroni test was carried out. The results of the Bonferroni test can be seen in Table 4.6.

Table 4.6 Bonferroni Test Results Number of Dead *Aedes aegypti* Mosquito Larvae on Addition of Lime *(Citrus aurantifolia)* and Garlic *(Allium sativum)* Peel Extract with Various Concentration Variations in 24-hour Observation Time

Concentration of Lime Peel Extract (Citrus aurantifolia)		The average difference in larval mortality	ρ *value*
Control	0,10%	-6.800	0.000
	0,20%	-13.000	0.000
	0,30%	-18.600	0.000
	0,40%	-25.000	0.000
0,10%	0,20%	-6.200	0.000
	0,30%	-11.800	0.000
	0,40%	-18.200	0.000
0,20%	0,30%	-5.600	0.000
	0,40%	-12.000	0.000
0,30%	0,40%	-6.400	0.000

Table 4.6 shows the difference in the average difference of dead *Aedes aegypti* mosquito larvae between control and treatment with concentrations of lime and garlic peel extract 0.10%, 0.20%, 0.30% and 0.40% there is a difference significant with value 0.000 <0.05.

DISCUSSION

The results of univariate analysis show that the peel extracts of *Citrus aurantifolia* and *Allium sativum* have an effect as larvicides of the *Aedes aegypti* mosquito and each variation of the concentration have different levels of effectiveness. The increase in the average mortality of Aedes sp larvae occurred because the higher the concentration, the higher the average

mortality of *Aedes aegypti* larvae. This happens because the greater the concentration used, the more active substances contained in the lime peel are flavonoids, saponins, tannins, and essential oils (limonoids, linalin acetate, geranil acetate, fellandren, and anildehid). (Haq, et al 2010) and the more active substances contained in white garlic, namely allicin and garlic oil, can be more effective in killing *Aedes aegypti* mosquito larvae.

The results of the One Way Anova test in table 4.5 show that there are differences in the concentration of lime *(Citrus aurantifolia)* and garlic *(Allium sativum)* peel extracts on the mortality of *Aedes aegypti* mosquito larvae. In general, the lime peel has been used in the field of beauty and health and garlic is used for cooking spices. The content of essential oils contained in the orange peel can be used as herbal medicine (Gunawan & Mulyani. 2004). This is in accordance with the role of extract *Citrus aurantifolia* which contains limonoids, flavonoids, saponins, and tannins found in lime peels, which work to inhibit skin turnover in larvae and enter the digestive tract of *Aedes aegypti* mosquito larvae through concentration immersion. ingested extract. Then it will enter the digestive organs, be absorbed by the intestinal wall, and circulate with the blood which will interfere with the metabolism of the larvae body, causing the mosquito larvae to spasm and eventually die due to a lack of energy for their life activities (Murdani, 2014). Allicin content in garlic can inhibit the development of 3rd instar larvae into 4th instar larvae or 4th instar larvae will not turn into pupae and will eventually die because the cell membrane has been damaged (Zulaikah, 2014). This is thought to cause the larvae to not get enough oxygen for their growth, resulting in the death of the larvae (Agnetha, 2008).

Bonferroni test results showed a tendency to increase the number of dead *Aedes aegypti* mosquito larvae with variations in concentration. At a concentration of 0.40%, it has the highest effectiveness in killing 25 *Aedes aegypti* mosquito larvae within 24 hours. Therefore, extract *Citrus aurantifolia* can be used as an alternative as a larvicide against the environmentally friendly *Aedes aegypti* mosquito larvae as a substitute for Temephos. The concentration variation was used as a comparison of the effectiveness of the extract in killing *Aedes aegypti* mosquito larvae.

The main thing in suppressing the larval population is the use of various larvicides, both biologically and chemically. The chemical larvicide that has been used in Indonesian society is temephos. Temephos as a larvicide has a

very wide use because it is very effective in controlling mosquito larvae, but this larvicide has a negative impact on human health and the environment because it contains harmful chemical compounds, and can increase mosquito resistance (Supono, 2014).

Temephos contains toxic compounds, this is due to the high toxicity of xylene, one of the components found in abate products. Another characteristic of organophosphate insecticides is that temephos inhibits the action of a group of enzymes called cholinesterases. This specific type is found throughout the body including the nervous system, brain, and bloodstream. Symptoms of acute exposure also include nausea, headache, loss of muscle coordination, and difficulty breathing. Therefore, it is necessary to make an effort to obtain natural insecticides, one of which is by using of *Citrus aurantifolia* and *Allium sativum* because they are more environmentally friendly, do not pose a danger, and have other advantages, namely easy to obtain and easy to use. can reduce the amount of waste / organic waste, because so far the peel of *Citrus aurantifolia* is only thrown away without further use and garlic is only used as a kitchen spice while the use of abate (temephos) can cause resistance (resistance). *Aedes aegypti* larvae to chemicals, residues and health problems.

This research is in line with research by Evy, et al (2017) describing the Utilization of *Citrus aurantifolia* as Larvicide *Aedes aegypti* Instar III. It used the maceration method (2 x 24 hours) and showed *Citrus aurantifolia* had the ability to kill *Aedes aegypti* mosquito larvae. Aurantifolia the most effective in killing *Aedes aegypti* larva. This study is also in line with Simone PM, et al.'s (2014) study describing the effect of *Allium sativum* on Aedes larvae. The lowest concentration of 1% (2 tails) could kill Aedes spp larvae by an average of 20% within 24 hours after treatment. While the highest concentration is 20% in 24 hours after treatment can kill an average of 100% (20 tails).

CONCLUSIONS AND SUGGESTIONS

There was a significant difference in the number of larvae that died at various concentrations, and the most effective concentration against the death of *Aedes aegypti* larvae was a concentration of 0,40%. It is expected that further research finds more applicable *citrus aurantifolia* and *allium sativum* formulations to make it easier in the community.

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