The effect of orange water of lemon (Citrus Limon (L.) Osbeck) as a larvasid of Aedes Aegypti in third instar

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Abstract. The objectives of this study was to determine the effect of lemon juice (Citrus Limon (L.) Osbeck) as larvae of Aedes aegypti in third instar, and to determine the difference in mortality of Aedes aegypti larvae in third instar by giving different concentration of lemon juice (Citrus Limon (L.) Osbeck). This study is belonging to experimental research. The sample in this study was lemon juice with 5 times treatment, namely concentration 5%, 10%, 15%, 20%, and 25% which calculated with the replication formula. According to the finding of the research shows that in the of 10% concentration was effective in killing all Aedes aegypti larvae due to the average larval mortality of 100%, and the 15%, 20%, and 25% concentration of obtained larval mortality results of 100%. The data analyzed by using One Way ANOVA showed that probability/sig value was <0.05, therefore, H0 is rejected, while the result of f count 103.143> f table. 2.866 was obtained. Result f count> f table then H0 is rejected and H1 is accepted. It can be concluded that there was an effect of lemon juice (Citrus Limon (L.) Osbeck) as larvae of Aedes aegypti in third instar.

1. Introduction
When the rainy season needs to be watched out for puddles, as a place for reproduction or breeding of mosquitoes. Commonly caused by mosquitoes is Dengue Hemorrhagic Fever (DHF) where acute infectious disease can cause death with symptoms of high fever continuously 2-7 days, followed by red spots (petechiae) in all parts of the body. This disease can be caused by dengue virus which is transmitted from the bite of Aedes aegypti mosquitoes infected with dengue virus. In addition to being a vector of DHF, the Aedes aegypti mosquito also became a vector for yellow fever, chikungunya, and a new disease, zika disease caused by the zika virus with the Aedes aegypti mosquito vector [1].

Preventive control in the development of disease-causing vectors is done by eradicating mosquito nests, such as draining, closing, and burying places which are commonly used as mosquito breeding grounds, fogging with the use of chemicals that function as insecticides called malathion. With the risk arising from fogging such as damage to the kidneys, cancer and nervous system disorders that occur in humans. One case allegedly due to fumigation was carried out, dozens of students in Jombang Regency, East Java, were poisoned after inhaling smoke after fogging or fogging by the Village Government, on Sunday 20 November 2016 (Okezone.com). Fogging is done to kill mosquitoes at the imago stage (adult). In its development, mosquitoes have 4 stages namely egg stage, larval stage, pupa stage, and adult stage. Frying can make mosquitoes become more resistant (resistant), resistant or resistant mosquitoes will become more dangerous mosquitoes. Third instar of larvae compared to first and second instar have a larger body size and more adaptive to the environment while instar 4 in approximately 48
hours will turn into a pupa. Third instar of larvae is easily identified and is a research sample that is the WHO standard [2].

Based on Laily and Deby, that any different concentrations of papaya (Carica papaya Linn) leaves extract have effect on Aedes aegypti larvae mortality (p < 0.05), the average number of Aedes aegypti larvae mortality for 24-hours in each [3]. Based on Ratih and Riyon, Data were obtained from the number of larval deaths in the Syzygium polyanthum extract in each control group and treatment group for 24 hours [4].

Another alternative to prevent dengue virus transmitted by Aedes aegypti mosquitoes, by using natural ingredients that are safe for health. The use of natural ingredients can be found in the environment where plants or plants thrive. Like using citronella, and lemon juice. Based on Permata that Citrus Lemon containing saponins and alkaloid [5]. Saponin are glycosides which can reduce the activity of protease and amylase enzyme that play a role in digestion of larvae. Based on Haqkiki that soursop leaf extract is effective in killing larvae of Aedes aegypti [6]. Based on Siska and Maya that methanol extract of lime leaves is effective as larvicide against Aedes aegypti [7].

Based on the description above, it is necessary to examine the effect of lemon juice (Citrus Limon (L.) Osbeck) as larvae of third instar of Aedes aegypti mosquito and the difference in mortality of Aedes aegypti larvae in third instar by giving lemon juice (Citrus Limon (L.) Osbeck) different.

2. Method

The method used in this study is an experimental study conducted to determine the effectiveness of lemon juice as larvae of Aedes aegypti mosquitoes third instar. In this study the objects used were lemon and third instar Aedes aegypti mosquito larvae. The sample in this study was juice of lemon juice with a treatment of 5 times, namely the concentration of 5%, 10%, 15%, 20%, and 25%. Previous research’s conducted a preliminary test to determine the concentration of lemon juice used in the study. The preliminary test using lemon juice with a concentration of 25%, 50%, 75% and 100%. 25% concentration effectively kills larvae in the first 6 hours by 80% while 50%, 75% and 100% effective at killing 100% larvae in the first 6 hours.

Based on [8] the number of treatments, this study uses the replication formula:

\[
(t - 1) (r - 1) > 15 \\
(t - 1) (r - 1) > 15 \\
(5 - 1) (r - 1) > 15 \\
4r - 4 > 15 \\
4r > 15 + 4 \\
r > 19/4 r > 4.75 \\
r \approx 5
\]

Description:
- \( t = \) Number of treatment groups
- \( r = \) Number of replications

Table 1. Making larvicide test. Observe the death of 10 Aedes aegypti larvae for 48 hours with an interval of 6 hours. Perform this test carefully and calculate the number of dead larvae. Existing data were analyzed by SPSS 16 One Way Anova method.

| Concentration (%) | Number of Larvae | Faucet Water | Lemon Juice(ml) |
|------------------|-----------------|--------------|-----------------|
| 5                | 10              | 95           | 5               |
| 10               | 10              | 90           | 10              |
| 15               | 10              | 85           | 15              |
| 20               | 10              | 80           | 20              |
| 25               | 10              | 75           | 25              |
3. Results and discussion

After conducting research on the effectiveness of lemon juice (Citrus Limon (L.) Osbeck) as larvicide of Aedes aegypti in third instar mosquito carried out for 48 hours, the results were:

Table 2. The test results of the effectiveness of lemon juice as larvicidal mosquitoes Aedes aegypti third instar. Repetition n Number of initial larvae Number of dead larvae (/ hour) Total dead larvae Larval mortality (%) Average.

| Concentration (%) | Repetition n | Number of initial larvae | Number of dead larvae (/ hour) | Total dead larvae | Larval mortality (%) | Average (%) |
|-------------------|-------------|--------------------------|-------------------------------|------------------|---------------------|-------------|
| 0                 | 1           | 10                       | 0 0 0 0 0 0 0 0             | 0                | 0                   | 0           |
|                   | 2           | 10                       | 0 0 0 0 0 0 0 0             | 0                | 0                   | 0           |
|                   |             |                          | 1 1 1 2 1 1 0              | 7                | 70                  | 70          |
|                   |             |                          | 0 1 1 0 2 1 0              | 5                | 50                  | 50          |
|                   |             |                          | 0 1 2 0 1 2 1 0           | 7                | 70                  | 70          |
|                   |             |                          | 0 2 1 0 1 2 0             | 6                | 60                  | 60          |
|                   |             |                          | 1 0 2 0 1 1 1 1           | 6                | 60                  | 60          |
| 5                 | 1           | 10                       | 3 1 2 2 2 0 0 0           | 10               | 100                 | 100         |
|                   | 2           | 10                       | 3 2 3 1 1 0 0 0           | 10               | 100                 | 100         |
|                   | 3           | 10                       | 3 3 2 0 2 0 0 0           | 10               | 100                 | 100         |
|                   | 4           | 10                       | 3 1 2 2 2 0 0 0           | 10               | 100                 | 100         |
|                   | 5           | 10                       | 3 2 3 0 2 0 0 0           | 10               | 100                 | 100         |
| 10                | 1           | 10                       | 5 2 3 0 0 0 0 0           | 10               | 100                 | 100         |
|                   | 2           | 10                       | 4 2 3 1 0 0 0 0           | 10               | 100                 | 100         |
|                   | 3           | 10                       | 5 2 3 0 0 0 0 0           | 10               | 100                 | 100         |
|                   | 4           | 10                       | 5 2 3 0 0 0 0 0           | 10               | 100                 | 100         |
|                   | 5           | 10                       | 4 3 3 0 0 0 0 0           | 10               | 100                 | 100         |
| 15                | 1           | 10                       | 6 3 1 0 0 0 0 0           | 10               | 100                 | 100         |
|                   | 2           | 10                       | 6 2 2 0 0 0 0 0           | 10               | 100                 | 100         |
|                   | 3           | 10                       | 6 4 0 0 0 0 0 0           | 10               | 100                 | 100         |
|                   | 4           | 10                       | 5 3 2 0 0 0 0 0           | 10               | 100                 | 100         |
|                   | 5           | 10                       | 6 2 2 0 0 0 0 0           | 10               | 100                 | 100         |
| 20                | 1           | 10                       | 7 3 0 0 0 0 0 0           | 10               | 100                 | 100         |
|                   | 2           | 10                       | 9 1 0 0 0 0 0 0           | 10               | 100                 | 100         |
|                   | 3           | 10                       | 8 2 0 0 0 0 0 0           | 10               | 100                 | 100         |
|                   | 4           | 10                       | 8 2 0 0 0 0 0 0           | 10               | 100                 | 100         |
|                   | 5           | 10                       | 8 2 0 0 0 0 0 0           | 10               | 100                 | 100         |
| 25                | 1           | 10                       | 8 2 0 0 0 0 0 0           | 10               | 100                 | 100         |
|                   | 2           | 10                       | 8 2 0 0 0 0 0 0           | 10               | 100                 | 100         |
|                   | 3           | 10                       | 8 2 0 0 0 0 0 0           | 10               | 100                 | 100         |
|                   | 4           | 10                       | 8 2 0 0 0 0 0 0           | 10               | 100                 | 100         |
|                   | 5           | 10                       | 8 2 0 0 0 0 0 0           | 10               | 100                 | 100         |

Source: Personal documentation Result of Effectiveness of Lemon Juice Extract, 2018

Based on the research that has been done, it is obtained the average mortality of third instar Aedes aegypti mosquito larvae, which is at a concentration of 5% with an average mortality of 62% for 42 hours, at a concentration of 10% the mortality of larvae reaches 100% for 30 hours, 15% concentration obtained 100% larval mortality for 24 hours, while for concentrations of 20% and 25%, the results of the number of larvae deaths by 100% in each concentration for 18 and 12 hours.
The results of the study tested the effectiveness of lemon juice as a larvae of Aedes aegypti mosquito in third instar, squeezing lemon juice into several concentrations, namely concentrations of 5%, 10%, 15%, 20% and 25%. The results obtained are as in Table 4.1 described above, namely at a concentration of 5%, the average mortality results were 62% and at concentrations of 10%, 15%, 20%, and 25% obtained larval mortality results of 100%.

These results illustrate that the large amount of concentration given as a test medium in the implementation of this study can affect the amount of larval mortality. Because of the many concentrations of lemon juice that is given, there are also more toxic substances in lemon juice in this test medium which results in more larval mortality. The content in lemons such as flavonoid, limonen, and tannin is toxic so it can be used as a larvacide of Aedes aegypti mosquitoes. Flavonoid compounds are a group of phenol compounds which are red, purple and blue dyes, and as a yellow dye found in plants. Flavonoids which are a group of phenols can cause protein clotting. Protein denaturation causes the permeability of the cell wall in the digestive tract to decrease. This will cause nutrient transport to be disrupted so that growth is inhibited and eventually mosquito larvae will die.

Flavonoids are a type of poisonous compound, flavonoids are used as active ingredients in the manufacture of vegetable insecticides. Flavonoids enter the mouth of insects / natural holes in the body of insects and cause immersion in the nerves [9].

Limonoid works to inhibit skin changes in larvae as limonoid stomach poisons, limonoid can enter the body Aedes Aegypti larvae third instar then enter the digestive larvaside then enter the digestive organs which will disrupt the body’s metabolism larva. Resulting in a lack of energy for life activities which causes the larvae to spasm and eventually die [10].

Lemon fruit also contains tannin compounds which are said to be sour sources of fruit. Tannins can reduce the activity of digestive enzymes and absorption of food so that tannins are stomach poisons. The study of the effectiveness of lemon juice as larvicides of Aedes aegypti mosquito in instar III is experimental research using simple experimental and data analysis processing, statistical test results of research using One Way Anova method which obtained the probability / sig value of 0.000 because probability / sig < 0.05 then H0 is rejected, besides the results of statistical tests obtained f count 103.143 > ftable 2.866. Thus the results of the probability / sig value of 0.000 < 0.05 and fcount > ftab then H0
is rejected and H1 is accepted. So it can be concluded that there is an effect of lemon juice (Citrus Limon (L.) Osbeck) as larvae of Aedes aegypti mosquitoes third instar.

4. Conclusion

Based on the results and discussion that has been described, it can be concluded that lemon juice (Citrus Limon (L.) Osbeck) has larvicidal effects of third instar Aedes aegypti mosquitoes and has a third instar larvae mortality based on statistical tests using One Way Anova, which is obtained by the probability / sig value of $0.000 < 0.05$ and the Fcount value $>$ Ftable.

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