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Test the Effectiveness of Soursop Leaf Extract and Tapak Dara Leaf Extract as Mosquito Larvicide

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Abstract: *In this rainy season the risk of contracting Dengue Fever increases. Many people control dengue fever chemically. The use of chemical larvicides has many disadvantages such as environmental pollution, resistance, and negative impacts on human health. This research aims to develop a natural larvicide from extracts of soursop leaves (Annona muricata) and tapak dara leaves (Catharanthus roseus). The study used a completely randomized design, with three replications. Soursop leaf and tapak dara leaf extracts were extracted using a maceration process using 70% ethanol solvent. III-IV instar Aedes aegypti mosquito larvae were used during the experiment, to assess the effect of formula (3 levels), dose (3 levels), and exposure time for 12 hours. All data were analyzed using Two-way ANOVA. The research results showed that the F1 formula and D3 dose were effective in eradicating mosquito larvae. Where the higher the concentration of soursop leaf extract and the higher the dose, the higher the mortality of mosquito larvae.*

Keywords: *Larvicide; Soursop Leaf Extract; Tapak Dara Leaf Extract*

1. INTRODUCTION

The presence of mosquitoes is a serious problem for public health. This is because mosquitoes act as disease vectors, some of which can be caused by dengue fever, filariasis, Zica and malaria. Dengue Hemorrhagic Fever (DHF) is still a health problem in Indonesia and is experiencing an increase in cases every year. The rainy season that will occur until early 2024 will cause an increase in dengue fever cases in several regions in Indonesia. After 17 weeks of 2024, to be precise until April 28, 88,593 cases of dengue fever have been recorded in Indonesia, 621 people have died. Meanwhile, in the same period in week 17 of 2023, the number of dengue fever cases was 28,579 cases with 209 deaths. This data was presented by Ministry of Health spokesperson Siti Nadia Tarmizi on Thursday, May 2 2024 (Nordiansyah, 2024).

The increase in dengue fever cases is due to smooth facilities and population mobility, the opening of new residential areas, people's behavior that is less concerned about environmental cleanliness, the presence of four types of virus cells that circulate throughout the year and the presence of a vector that transmits the disease, namely *Aedes aegypti* (Sukmawati, 2022). The *Aedes aegypti* generally breeds around human settlements, such as in bathtubs, buckets and other objects that can collect rainwater. This has led to the emergence of many places that the *Aedes aegypti* likes to breed (Sulistyawati, 2023).

The life phase of the *Aedes aegypti* is relatively fast. So, it is necessary to eradicate mosquito nests. This activity aims to control disease vectors in the larval phase. Vector control can be carried out through physical, chemical and biological control (Putri & Jana, 2018). Currently, dengue fever prevention still relies on vector control, where community activity is very necessary. Various national movements have been started since 1980, from larvicide, focused

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fogging, mosquito nets and 3M (covering, draining, and recycling used goods), larva monitoring, eradicating mosquito nests, communication for behavioral impact (COMBI) to the 1 House 1 Movement. *Jumantik* or what is known as G1R1J (Kementerian Kesehatan RI, 2021). Even though many vector control efforts have been carried out, the number of dengue fever cases continues to increase from year to year.

In reality, Indonesian people more often control mosquito vectors using chemicals such as using larvicide because it is easier and cheaper. This method is known as abatization. The levaside used is temephos. Temephos is an insecticide that belongs to the organophosphate group. On the other hand, the use of chemical insecticides has been found to be responsible for several new problems, including insect resistance, water pollution, and adverse impacts on livestock and human health. Therefore, it is necessary to look for safer alternatives (Kolo *et al*., 2018).

Using natural, plant-based ingredients is a safer alternative. This is because natural materials are easily decomposed (biodegradable) in nature. As a result, they do not pollute the environment and are relatively safe for humans and pets (Syarif & Amansyah, 2019). Natural larvicides are natural products originating from plants which have a collection of secondary metabolites and contain bioactive compounds such as alkaloids, terpenoids, phenolics and other auxiliary compounds (Kusumawati & Istiqomah, 2022).

The soursop plant is one of the plants that can kill mosquito larvae. The leaves of the soursop plant are undoubtedly safe for humans and other organisms. In addition, the ingredients are expected to be beneficial for human health and easy to obtain. Harfriani's research (2012) states that the fruit, seeds, roots, and leaves of plants all contain annonain, saponins, flavonoids and tannins which are the active ingredients of the plant. Apart from that, there are 42% -45% essential oils contained in the seeds. The seeds and leaves have the potential to repel insects and larvae. With the results of this research, soursop leaves can be used as a natural larvicide.

Apart from that, the periwinkle plant also has the potential to be a larvicide. Tapak dara is known as an ornamental plant because of its beautiful flowers, so it is widely planted in home gardens and is easy to grow in all places. According to research by Ningrum *et al*. (2021), insect pest test results on long bean plants treated with tapak dara leaf extract decreased compared to plants not treated with the herbal pesticide tapak dara leaves. This shows that tapak dara leaves contain active substances that can be used as vegetable pesticides in the form of alkaloids, terpenoids, phenols, tannins, saponins, quinine and sterols.

Based on this explanation, researchers were interested in testing the active substance content of a combination of soursop leaf extract and tapak dara leaves to eradicate mosquito larvae. By combining the two ingredients, it is hoped that one ingredient can strengthen each other and trigger an effective reaction in eliminating mosquito larvae. According to Utami & Haneda (2010), stated that an insecticide can be said to be effective if it kills at least 80% of the test insects. It is hoped that this method will be a solution to eradicate the vector of dengue fever.

2. RESEARCH METHODOLOGY

This research was carried out at the Biology Laboratory, Faculty of Teacher Training and Education, Universitas Muhammadiyah Surakarta, from January 2024 to March 2024. The experimental plan used in the research was a completely randomized design with two factors, namely the soursop leaf and tapak dara leaf extract formula and the dose in production of mosquito larva larvicide. The method used in this research was an experimental method carried out to determine the effectiveness of soursop leaf extract and tapak dara leaf extract on mosquito larvae mortality. The tools used are grinder, blender, digital scale, beaker glass, Bunsen lamp, dropper pipette, 10 ml measuring cup, filter, stirrer, filter paper, plastic cup, aluminum foil, glass bottle and glass jar. The materials used are *Aedes aegypti* larvae, soursop leaves, tapak dara leaves, 70% ethanol and distilled water.

The samples taken were 300 *Aedes aegypti* instar III-IV mosquito larvae. And the leaf samples used were old soursop leaves and old tapak dara leaves. According to Gultom *et al.* (2020) the formation of bioactive compounds is greatly influenced by the age of plant organs. The extract is made using the maceration technique.

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Both types of leaves are dried by traditional drying (sunlight). Then the dry leaves are crushed using a grinder and filtered to obtain siplicia. Simplicia was soaked in 70% ethanol in a ratio of 1:10 for two days. After that, filtering is carried out and an extract solution is obtained which is then carried out by a thickening process. Until you get a paste of soursop leaf extract and tapak dara leaf extract.

Application to mosquito larvae, namely by making three formulas with a ratio of soursop leaf extract and tapak dara leaf extract, 75%:25%, 50%:50%, and 25%:75% (Ahyanti & Yushananta, 2022). Then dissolve it with 100 ml of distilled water. And made with three doses of 1%, 1.5% and 2.5%. Where all treatments were repeated three times. The number of test larvae was 10 per treatment container (Rumengan, 2010).

Data was collected by observing and counting the number of mosquito larvae that died in each treatment. The results obtained were analyzed using SPSS 23. The data obtained were tested using the Kruskal Wallis test because the data distribution was not normal (Kundalini *et al*., 2022). And then the Dunn's test is carried out.

3. RESULTS AND DISCUSSION

In this research, the mosquito larvae used were *Aedes aegypti* larvae obtained from the water reservoirs of Baturan Village residents' homes. Newly obtained mosquito larvae are identified based on their characteristics, namely, the shape of the larva resembles the letter S, the shape of the large and short siphon found on the last abdomen, the shape of the teeth of a comb or comb is regular like a trident, the resting position of the *Aedes aegypti* larva forms an angle on the surface of the water (Suwito, 2018).

After obtaining a population of *Aedes aeegypti* larvae, the mosquito larvae were immediately tested with a larvicide preparation based on the formula and dosage of soursop leaf extract and tapak dara leaf extract. The time for adding mosquito larvae to the preparation was recorded and left for 12 hours. This also applies to 0% dose preparations (control). Observation of mosquito larvae mortality is carried out by counting the number of mosquito larvae that sink and do not move. After making observations, results were obtained for each formula preparation and dose with three repetitions. The following is a table of the results of calculating the number of larval deaths after 12 hours of treatment.

Table 1. Effectiveness of soursop leaf extract and tapak dara leaf extract on the death of Aedes aegypti larvae

Combination	Cumulative Number of Larval Deaths			Mean Mortality
	Repetition			
	I	II	III	
Control	0	0	0	0
F1D1	9	5	6	6,67
F1D2	9	7	4	6,67
F1D3	10	8	8	8,67
F2D1	9	1	0	3,33
F2D2	10	3	2	5
F2D3	6	4	6	5,33
F3D1	2	1	5	2,67
F3D2	2	2	10	4,67
F3D3	3	1	10	4,67

Information:
F1 = soursop leaf extract 75% and tapak dara leaf extract 25%
F2 = soursop leaf extract 50% and tapak dara leaf extract 50%
F3 = soursop leaf extract 25% and tapak dara leaf extract 75%
D1 = 1% dose
D2 = 1.5% dose
D3 = 2% dose

Based on data on mosquito larvae deaths in table 1, the natural larvicide combination with the lowest average in killing mosquito larvae after 12 hours is F3D1. And the larvicide combination with the highest average in killing mosquito larvae is F1D3. From the data listed in table 1, increasing the dose will increase the number of larval deaths. However, if we look at the repetition results. The combination of F1D1, F1D2, F1D3, F2D1, F2D2 and F2D3 decreased the number of larval deaths in the third repetition. Meanwhile, the combination of F3D1, F3D2 and F3D3 experienced an increase in the number of larval deaths in the third repetition. These results can be depicted in the following graph.

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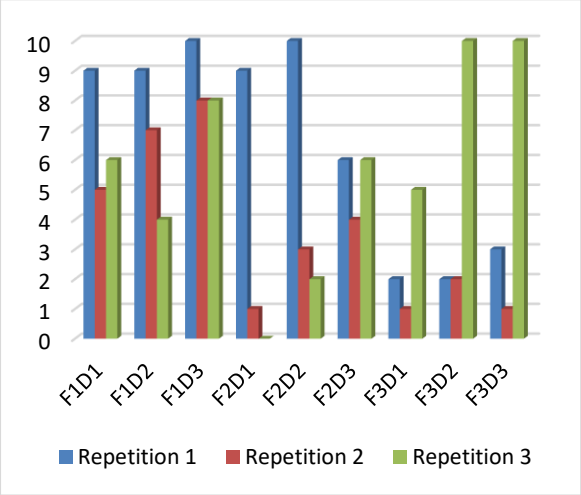

Figure 1. Comparison graph of the number of deaths of mosquito larvae in each repetition

Then the data was processed using SPSS 23 for normality and homogeneity tests. The results obtained in the normality test of the formula were not normal and the homogeneity test of the formula data was normal. And the results of the normality test and homogeneity test, the dose is normal, and the data is homogeneous. So, data processing continues with non-parametric tests. The Kruskal-Wallis's test is used to determine whether the analysis results are significantly different. Based on this test, the sig P value is <0.010 for the formula and 0.029 for the dose, meaning that H0 is rejected, and Ha is accepted, because the sig P value is <0.05. Based on these findings, there is a significant difference between the ability of each formula and test dose to kill mosquito larvae. (Putri & Jana, 2018).

An Independent Samples Test was also carried out to see whether there was a significant effect or not from the use of the formula and dose given. The following table shows the average mortality rate for mosquito larvae.

Table 2. Results of Independent Samples Test of extract formula on larval mortality

Soursop Leaf and Tapak Dara Leaf Extract Formula	Mean	N	Std. Deviation
F0	.00	3	.000
F1	7.33	9	2.000
F2	4.56	9	3.468
F3	4.00	9	3.606
Total	4.77	30	3.549

Table 3. Independent Samples Test results of extract dose on larval mortality

Dose	Mean	N	Std. Deviation
D0	.00	3	.000
D1	4.22	9	3.420
D2	5.44	9	3.539
D3	6.22	9	3.114
Total	4.77	30	3.549

The results showed that the average mortality of mosquito larvae was highest in F1 and D1. When compared with F0, F2, F3 and D0, D2, D3. After obtaining these results, a Dunn's test was carried out to find out which groups were statistically significantly different at a certain level. The following is a picture of the analysis results using Dunn's test.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
F0-F3	-11.556	5.839	-1.979	.048	.287
F0-F2	-12.667	5.839	-2.169	.030	.180
F0-F1	-19.111	5.839	-3.273	.001	.006
F3-F2	1.111	4.129	.269	.788	1.000
F3-F1	7.556	4.129	1.830	.067	.403
F2-F1	6.444	4.129	1.561	.119	.711

Figure 2. Results of formula group analysis using Dunn's test

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
D0-D1	-11.500	5.839	-1.970	.049	.293
D0-D2	-15.111	5.839	-2.588	.010	.058
D0-D3	-16.722	5.839	-2.864	.004	.025
D1-D2	-3.611	4.129	-.875	.382	1.000
D1-D3	-5.222	4.129	-1.265	.206	1.000
D2-D3	-1.611	4.129	-.390	.696	1.000

Figure 3. Results of dose group analysis using Dunn's test

The results obtained from the formula used were significant mortality rates occurring at F0-F1 and D0-D3. And there is no significant difference between F0-F3, F0-F2, F3-F2, F3-F1,

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F2-F1 and D0-D1, D0-D2, D1-D2, D1-D3, D2-D3. F1 is a formula with a concentration of soursop leaf and tapak dara leaf extract of 75%:25% and D3 is the highest dose of 2.5%. If a large dose is given and the exposure time is long, the greater the amount of poison that enters the larva's body. So, the mosquito mortality rate continues to increase. The larger the dose given, the faster the larvae die. In treatment D3 the larvae died more quickly than D1 and D2. These results indicate that significant differences occur between formulas with high concentrations of soursop leaf extract and high doses. Meanwhile, the comparison of other formulas and doses is not significant.

This shows that soursop leaf extract is more effective in eradicating mosquito larvae than tapak dara leaf extract. The cause that can occur is due to the difference in the active substance content and the concentration of the ingredients in soursop leaves and tapak dara leaves. Soursop leaves contain active compounds, namely acetogenin, anomuricin A, goniothalamine, flavonoids, alkaloids, tannins, saponins, steroids, annohexocin, annonacin, annomuricin, niacin, anti-aging balm, and reticuline (Ummah & Badrus, 2022). Meanwhile, tapak dara contains alkaloids, terpenoids, phenols, tannins, saponins, quinine and sterols (Ningrum *et al*., 2021).

Table 4. Phytochemical Screening Results of Sisak (*Annona muricata*) Leaf Extract (Rumiyanti et al., 2019)

Test Type	Result
Saponin	+
Steroid	-
Terpenoid	+
Tanin	+
Alakloid	+
Favonoid	+

Table 5. Phytochemical Screening Results of Tapak Dara (Catharantus roseus) Leaf Extract (Putri & Nasution, 2022)

Test Type	Result
Saponin	+
Steroid	+
Terpenoid	+
Tanin	+
Alakloid	+
Favonoid	+

However, in tables 4 and 5 the results of phytochemical screening of soursop leaf extract and tapak dara leaf extract show results that are not much different. Both contain active compounds that play a role in killing insect larvae. The combination of soursop leaf extract and tapak dara leaf extract should mutually strengthen the toxic effects of natural larvicide. Results that do not match this indicate that there are external factors that influence the effectiveness of the two extracts. According to Darmanto *et al*. (2019) Natural larvicides usually do not have the ability to kill insects directly, but can act as attractants, neurotoxins, disrupt the hormonal system in the insect's body, prevent insects from laying eggs, and stop the egg hatching process. Chemical compounds contained in natural larvicides, such as alkaloids, eugenol, saponins, flavonoids and tannins, provide the killing power of these larvicides (Putri & Jana, 2018).

Flavonoids are compounds that work by entering the larva's respiratory system, which then causes the larvae's nerves to be damaged so that the larvae cannot breathe and die (Syazana & Porusia, 2022). Alkaloids are substances that in large quantities inhibit the work of the enzyme acetylcholinesterase (AChE), which results in a buildup of acetylcholine, causing chaos in the impulse system to muscle cells (Nur *et al*., 2020). Saponins can cause hemolysis of blood cells so that breathing becomes obstructed and leads to death (Ningrum *et al*., 2021). Saponins can also reduce the productivity of digestive enzymes and food absorption. Tannin can reduce the ability to react with proteins and precipitate them, causing problems in the preparation of enzymes or other proteins from some plants (Kristiana *et al*., 2018).

Apart from that, the age of mosquito larvae also has a big influence on the resistance of mosquito larvae to exposure to chemicals. Adult mosquito larvae have complete bodies and high resistance. Chemical resistance by mosquito larvae is another factor that causes fluctuations in the number of mosquito larvae deaths. Because the best lifespan for mosquito larvae whose immune system is strong is between 2-5 days. The weak physical condition of the larvae in less than two days makes it easier for them to die (Syarif & Amansyah, 2019).

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Another factor that can occur is that the substance content in natural larvicides changes due to improper storage. Changes in temperature, pH and light intensity can change the chemical composition of natural larvicides to make them ineffective or increase their toxification levels. This can be seen in Figure 1, where the graph decreases from repetition one to repetition three in the formula with high levels of soursop leaf extract. And the graph increased from repetition one to repetition three in the formula with high levels of tapak dara leaf extract.

In this research, there are several shortcomings. When natural larvicide is used, the color changes to brown and the smell is unpleasant. The higher the dose used, the more visible the changes in color and smell. This will be very detrimental if residents' water reservoirs, which are sources of clean water, become unfit for use. So, it is better to use this natural larvicide in low doses.

4. CONCLUSION

From the results of research that has been carried out, the most effective larvicides in eradicating mosquito larvae are F1 (75% soursop leaf extract and 25% tapak dara leaf extract) and D1 (2.5%). The formula with a high concentration of soursop leaf extract and a high dose is effective in eradicating mosquito larvae. Meanwhile, the difference in the number of mosquito larvae deaths between the formula and other doses was not significant. There are drawbacks to this natural larvicide, namely that it still causes changes in the color and smell of the water.

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