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Effectiveness of Soursop Leaf Extract (*Annona muricata*) and Ylang-ylang Flowers (*Cananga odorata*) as a Larvasicide to Remove Mosquito Flars

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***Abstract**: One of the few countries with high humidity levels, it can cause poor air quality and an increase in diseases such as dengue fever. Dengue fever is a disease caused by the Aedes aegypti mosquito. Due to the large number of cases of dengue fever, various types of drugs have emerged to treat this condition. Predator, genetics, pathogen and parasite control, as well as physical, biological and chemical control are several ways to control larvae. Efforts to control mosquitoes can be made by using mosquito repellent repellents by using plants that come from nature which contain essential oils as environmentally friendly repellents. One of the plants that contain essential oils is soursop leaves and ylang-ylang flowers. The aim of this research was to determine the effectiveness of soursop leaves and ylang-ylang flowers as larvicides to eradicate mosquito larvae. This research is experimental in nature with the samples used, namely Culex type mosquito larvae. This research was conducted at the Biology Laboratory, Faculty of Teacher Training and Education, Universitas Muhammadiyah Surakarta. The samples for this study were 28 containers with an initial number of larvae of 25 individuals. The results showed that there were several mosquito larvae that experienced death with the lowest mortality in F1D1 with an average of 20 deaths and the highest mortality in F3D3 with an average of 23.67 deaths. Mosquito larvae experienced death with the highest time at 9 hours of observation and the time of death decreased at 12 hours of observation. The use of larvicidal extracts from soursop leaves and ylang ylang flowers is more effective in reducing the number of mosquito larvae with F3 having the highest average number of deaths with a dose of 2.5 g of soursop leaves: 7.5 ylang ylang flowers within 9 hours.*

***Keywords:** Larvicides, Soursop Leaf, Ylang-ylang Flower, Mosquito larvae*

1. INTRODUCTION

Indonesia is one of the few countries with high humidity levels, which can cause poor air quality and an increase in diseases such as dengue fever. Dengue fever or often called DHF, is a disease caused by the *Aedes aegypti* mosquito. Due to the large number of dengue fever cases, various types of drugs have emerged to treat this condition. Predator, genetic, pathogen and parasite control, as well as physical, biological and chemical control are some ways to control larvae.

Chemical control of dengue fever has the potential to have negative impacts. Therefore, effective and safe environmental management is needed to mitigate its negative impacts. One alternative that can be used is vegetable insecticide (Syazana *et al*., 2023). The use of natural insecticides in Indonesia can be good, because Indonesia has a variety of plants that have the potential to act as natural insecticides. One tool that can be used to detect infection. Soursop plants and ylang-ylang flowers are plants that have potential as botanical insecticides that can be used to control disease vectors (Dias *et al*., 2019).

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Soursop (*Annona muricata*) is a plant from the Annonaceae family which is usually used as a traditional medicine for various diseases such as worms, dysentery, constipation, diarrhea and scabies. Annonaceae is known to produce insecticidal activity. Plant species in this family contain a series of toxic compounds such as acytogenin, alkaloids and flavonoids which make this plant an insecticide (Syazana et al., 2022). Annonain is an alkaloid compound found in soursop. This compound is toxic, has a bitter taste, and has metabolic properties towards one of the amino acids. Annonain has more complex and effective toxic effects against insects, such as anti-larvae and anti-feeding. The saponins found in soursop can inhibit the growth hormone of the *Aedes aegypti* mosquito, causing its development to deviate (Akbar *et al*., 2023). This is in accordance with research (Ugochi *et al*., 2019), soursop leaves contain tannins of 3.4 mg/g, phenols 20.85 mg/g, flavonoids 0.24 mg/g, glycosides 86.57 mg/g, alkaloids 294, 70 mg/g, and saponin of 396, 63 mg/g.

Ylang flowers (Cananga odorata) can produce essential water that contains aroma. Essential oils are synthesized in plant cells because of several chemical reactions with air. Ylang ylang can repel mosquitoes because of its fragrant and distinctive smell which mosquitoes don't like, and because it contains geraniol, linalol and daneugenol. Kenanga flowers (Cananga odorata) can cause the death of Aedes aegypti larvae because they contain substances or compounds that can kill Aedes aegypti larvae. Several substances or compounds that play an important role in killing Aedes aegypti larvae are saponin essential oils, flavonoids (Fikri *et al*., 2020). This is in accordance with research (Maulita, 2021), giving ylang ylang flower extract with a concentration of 5% can kill 21% of mosquitoes, a concentration of 25% can kill 49% of mosquitoes, a concentration of 50% can kill 83% of mosquitoes, a concentration of 75% can kill 99% mosquito.

From the combination of soursop leaves and ylang ylang flowers, ylang ylang flower extract (Canangium odoratum) has larvicidal effectiveness on Ae larvae. aegypti with a concentration of 0.02% test larvae death reached 96% in the 24th hour, the results of the Mann-Whitney test showed that between the control group and all concentrations there was a statistically significant difference in the number of larval deaths. A concentration of 0.02% is very effective in killing Ae.aegypti mosquito larvae. The results of the Mann-Whitney test for length of contact showed that a contact time of 24 hours had a very significant effect on the death of Ae.aegypti larvae (Fikri *et al*., 2020). Meanwhile, giving 0.2% soursop leaf extract for 24 hours can kill 8% of Culex sp larvae. Giving 0.4% soursop leaf extract for one hour, two hours, 24 hours can kill 4%, 4% and 16%. Giving 0.6% soursop leaf extract for one hour, two hours, 24 hours can kill 8%, 8% and 24%. And giving 0.8% for one hour, two hours, 24 hours can kill 8%, 8% and 32% (Melliska, 2022).

This research aims to determine the level of effectiveness of soursop leaf extract and flowerylang-ylang to overcome the problem of the negative impact of chemicals and reduce the content of dangerous chemicals in lavarside. So, in the process of making lavarside using natural ingredients to reduce the excessive use of synthetic pesticides which in the long term can cause several losses such as mosquitoes becoming resistant, poisoning in humans and livestock, as well as environmental pollution, an effort is needed to find an alternative that more effective in controlling mosquito populations. One alternative is the use of natural pesticides to reduce environmental pollution problems.

2. RESEARCH METHOD

This study used a completely randomized design (CRD) with 2 factorials. The variables studied were formula (combination of soursop leaf extract and ylang-ylang flower extract), dose, and exposure time. The formula consists of three levels, namely F-1 (75%:25%), F-2 (50%:50%), and F-3 (25%:75%). The dosage consists of three levels, namely 1%, 1.5% and 2.5%. Exposure time was calculated from the time the larvae were exposed to the specified combination of formula and dose and observed for up to 12 hours. Death recording was carried out at five times, namely 30 minutes, 1 hour, 3 hours, 6 hours, 9 hours and 12 hours. The entire experiment was carried out using 3 treatments and resulted in a combination of overall factors, namely 3×3 with 3 repetitions.

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So, the total number of experiments was 10 times, and 28 data were obtained.

Place and time of research

Research was conducted at the Biology Laboratory, Faculty of Teacher Training and Education, Muhammadiyah University of Surakarta, Jl. A. Yani No.157, Pabelan, Kartasura, Sukoharjo, Central Java 57169. This research was carried out from January to March 2024. The research stages started from preparing tools and materials, making plant extracts, diluting the extract according to the specified formula, preparing the larvae. Culex mosquitoes, and larval susceptibility testing (*bioassay test*).

Extraction material

Extraction of soursop leaves and ylang ylang flowers using the maceration method. Each ingredient (approximately 1 kg) is washed with clean running water. The fresh leaves are then arranged in trays and dried in the air for seven days, without exposure to direct sunlight. After drying, cut it into small pieces, then crush it using a grinder and sift it so that the results are smoother. The extraction process is carried out by soaking 30 grams of dried leaf powder each using 70% alcohol in a closed glass jar for 2×24 hours. The filtrate was separated after 48 hours of soaking and filtered using filter paper. The entire filtrate is then concentrated through an evaporation process using a 500 ml beaker filled with water and then heated using a spirit burner to obtain a thick solution. Dilution uses distilled water to obtain the specified concentration. Then the step is to weigh the extract formula so that it matches the specified dosage. The *Culex* mosquito larvae used in the research came from cultures grown in plastic buckets. In each experiment, 25 larvae were used, so that the total number of *Culex* larvae used during the study was 700 larvae.

BioassaysTest

Bioassay tests carried out based on WHO (2005). A total of 28 test glasses containing 100 ml of clean water were prepared for the experiment. In each test glass, plant extract is added according to the specified formula and dosage and stirred until completely dissolved. A total of 25 *Culex* mosquito larvae were put into each test glass and observed for 12 hours. The death of *Culex* mosquito larvae was recorded every hour during the 12-hour experiment, in each test glass. Larval death is determined based on visuals and the condition of the larvae, namely that they sink to the bottom of the container, do not move, and do not respond to stimulation in the form of touch using a stick on the siphon (Putri *et al*., 2022). The experiment was carried out with three repetitions.

Data analysis

Experimental data in the form of observations of the effect of biolarvicides on the mortality of mosquito larvae which have been calculated according to the formula are then entered into the observation table. Mortality calculation follows the formula:

$$\textbf{Average}: \frac{Total\ larval\ mortality}{Number\ of\ Repetitions}$$

Analysis datausing the Kruskal Wallis test and Duncan test. The Kruskal Wallis test is a statistical test that aims to determine whether there is a significant difference between two or more groups of independent variables and the dependent variable, while the Duncan test is a follow-up test for any middle value that is not the same when testing homogeneity so that it gives results that reject the hypothesis. zero and accept the results of the alternative hypothesis. All analyzes were carried out at a 95% confidence level, using statistical tools, namely IBM SPSS Statistics 23.

3. RESULTS AND DISCUSSION

1 kg each of fresh soursop leaves and ylang-ylang flowers were air-dried for ± 1 week to obtain a yield of 500 g of dried leaves and flowers. Grind 200 g using a grinder, extract and 70% alcohol are macerated in a ratio of 1:10, so that each uses 30 g of crushed soursop leaf and ylang ylang flower extract and 300 ml of 70% alcohol. The maceration process lasts for 2×24 hours. Then filtration was carried out using filter paper to produce 300 ml of filtrate. The evaporation process involves evaporating the filtrate at a temperature of 70℃ using a 500 ml beaker, resulting in 16 g of thick extract from soursop leaves and ylang-ylang flowers.

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In F1 1% with a ratio of 75% : 25% so the required dose is 7.5 g soursop leaves : 2.5 g ylang ylang flowers, F2 1.5% with a ratio of 50% : 50% so the required dose is 5 g leaves soursop: 5 g ylang-ylang flowers, and F3 2.5% with a ratio of 25%: 75% so the required dose is 2.5 g soursop leaves: 7.5 g ylang-ylang flowers. Next, make the extract according to the specified dose by weighing the combination of the two extracts as much as 10 g, then dissolving it using distilled water as much as 100 ml. At D1 1% the required dose is 1 ml, at D2 1.5% the required dose is 1.5 ml, and at D3 2.5% the required dose is 2.5 ml. Then the predetermined dose is dropped into each plastic cup containing 10 ml of water and 25 mosquito larvae. The results of mosquito larvae mortality are recorded at five times, namely 30 minutes, 1 hour, 3 hours, 6 hours, 9 hours and 12 O'clock.

Table 1. Observation Results of Mosquito Larval Mortality

Concentration %	Number of Dead Larvae (Minutes)						Total Dead Larvae	Average
	30 minutes	1 hour	3 hours	6 Hours	9 hours	12 hours		
Controls	0	0	0	0	0	0	0	0
	0	0	0	3	13	2	19	
F1D1	0	0	1	3	13	3	20	20
	0	0	0	2	15	4	21	
	0	0	1	2	15	2	20	
F1D2	0	0	1	3	14	1	19	20.3
	0	0	1	2	13	2	22	
	0	0	0	3	13	1	20	
F1D3	0	0	1	3	14	2	20	21
	0	0	2	1	17	3	23	
	0	0	0	0	19	5	24	
F2D1	0	0	0	2	16	3	21	21.67
	0	0	0	2	17	1	20	
	0	1	3	1	15	5	25	
F2D2	0	0	2	1	14	3	20	21.3
	0	0	0	3	12	4	19	
	1	0	1	3	16	1	22	
F2D3	1	0	0	2	13	4	20	21.67
	1	0	0	2	17	3	23	
	0	0	3	2	14	5	24	
F3D1	0	0	0	8	12	0	20	21
	0	0	0	5	10	4	19	
F3D2	0	0	0	2	17	5	24	22.3
	0	0	2	5	10	4	22	

In table 1, the results of mosquito larvae mortality were obtained using 10 trials and 3 repetitions so that the number of samples used was 28 containers. The initial number of larvae was 25, in the control experiment not a single larva died because they were not given a dose of the extract. In FID1 with a formula ratio of 7.5 g soursop leaves: 2.5 g ylang-ylang flowers with a dose of 1 ml, the average result was 20, FID2 with a formula ratio of 7.5 g soursop leaves: 2.5 g ylang-ylang flowers with a dose of 1, 5 ml produces an average of 20.3, FID3 with a formula ratio of 7.5 g soursop leaves: 2.5 g ylang ylang flowers with a dose of 2.5 ml produces an average of 21, F2D1 with a formula ratio of 5 g soursop leaves: 5 g of ylang-ylang flowers with a dose of 1 ml produces an average of 21.67, F2D2 with a formula ratio of 5 g of soursop leaves: 5 g of ylang-ylang flowers with a dose of 1.5 ml produces an average of 21.3, F2D3 with a formula ratio of 5 g soursop leaves: 5 g ylang ylang flowers with a dose of 2.5 ml produces an average of 21.67, F3D1 with a formula ratio of 2.5 g soursop leaves: 7.5 g ylang ylang flowers with a dose of 1 ml produces an average of 21 , F3D2 with a formula ratio of 2.5 g soursop leaves : 7.5 g ylang ylang flowers with a dose of 1.5 ml produced an average of 22.3, and F3D3 with a formula ratio of 2.5 g soursop leaves : 7.5 g flowers ylang-ylang with a dose of 2.5 ml produced an average of 23.67.

From 10 trials and 3 repetitions. In the first 30 minutes the number of mosquito larvae that died was still quite small, as many as 25 mosquito larvae died with the highest time after 9 hours of observation and the time of death decreased at 12 hours of observation. In the middle of the observation period, more mosquito larvae died compared to the last time limit that had been determined. Mosquito larvae were still actively moving at the beginning of the observation period because they could still take oxygen to the surface of the water, while in the middle of the observation period (9 hours), mosquito larvae began to move slowly, and many died because it was increasingly difficult to get oxygen. At 12 hours of observation, there was a decrease. The death of mosquito larvae is because on average mosquito larvae have died in the middle of the observation period, this is due to exposure to toxic natural larvicide compounds. This is in line with research by (Ishak *et al.*, 2020), that natural larvicides derived from plants contain secondary

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metabolite compounds that are produced in plant tissue and play a role in growth, such as saponins, terpenoids, alkaloids and flavonoids. This compound can be toxic to insects, one of which is by reducing their ability to digest food.

Ranks

	ekstrak daun sirsak dan bunga kenanga	N	Mean Rank
jumlah larva mati	F0	3	2.00
	F1	9	13.06
	F2	9	17.44
	F3	9	20.50
	Total	30	

Figure 1. Kruskal-Wallis Test Results on a Combination of Soursop Leaf Extract and Ylang Ylang Flower Extract

The results of the Kruskal-Wallis Test (Figure 1) showed that F3 had the highest average number, while the lowest average number was found in F1. In F3 the required amount is 2.5 g soursop leaves : 7.5 g ylang ylang flowers, while in F1 the required amount is 7.5 g soursop leaves : 2.5 g ylang ylang flowers, so it can be concluded from the combination of these extracts which contains quite effective compounds, namely ylang ylang flower extract compared to soursop leaf extract. Because ylang ylang flowers contain alkaloids, phenols, terpenoids, flavonoids, steroids and tannins. This is in line with research by (Ikhsanudin *et al*., 2022), the effectiveness of biolarvicide from ylang-ylang flower extract is based on the activity it contains. These substances include alkaloids, phenols, terpenoids, flavonoids, steroids and tannins. The high number of compounds in ylang ylang flowers can cause death of mosquito larvae, one of which is tannin. According to (Dhenge *et al*., 2021), tannin compounds will reduce the ability of protease enzymes to convert amino acids. Tannins can be used to inhibit protease enzymes. The enzyme digestion process which is influenced by tannins causes enzyme function to be disrupted. As a result, all metabolic processes are disrupted, and the larvae experience nutritional deficiencies. Thus, larval growth will be hampered, and if this process continues it will have a negative impact on larval development.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
F0-F1	-11.056	5.789	-1.910	.056	.337
F0-F2	-15.444	5.789	-2.668	.008	.046
F0-F3	-18.500	5.789	-3.195	.001	.008
F1-F2	-4.389	4.094	-1.072	.284	1.000
F1-F3	-7.444	4.094	-1.818	.069	.414
F2-F3	-3.056	4.094	-.746	.455	1.000

Figure 2. Results of Nonparametric Tests: Independent Samples on the Combination of Soursop Leaf Extract and Ylang Ylang Flower Extract

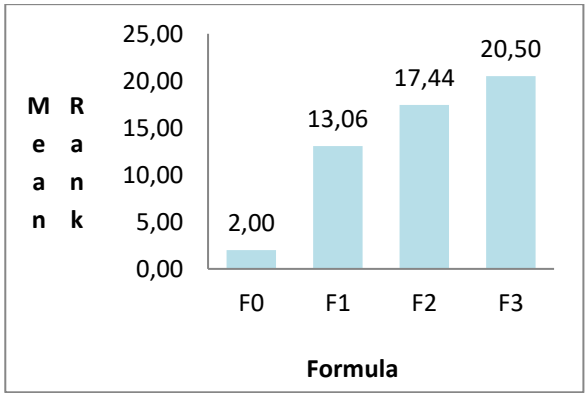

Figure 3. Diagram of Kruskal-Wallis Test Results for Combination of Soursop Leaf Extract and Ylang Ylang Flower Extract

The formula for the combination of soursop leaf extract and ylang-ylang flower extract (Figure 2) showed that F0-F2 was significant because t < 0.05, so the hypothesis was rejected, meaning that the formula for soursop leaf extract and ylang-ylang flower extract was effective against mosquito larvae mortality. Meanwhile, F0-F2 had significant results with numbers that were not too far from F0-F3, F0-F3 was significant because t < 0.05, so the hypothesis was rejected, meaning that the formula for soursop leaf extract and ylang-ylang flower extract was effective against larval mortality mosquito. This means that the formula of F3 contains compounds that are quite effective compared to F1, F3 is dominated by ylang-ylang flower extract with a ratio of 2.5 g soursop leaves: 7.5 g ylang-ylang flowers. This is in line with the research of (Simbolon *et al*., 2020), that the greater the concentration of the combination of extracts, the more active compounds they contain. So, more poison is consumed by mosquito larvae. The effectiveness of biolarvicide is based on the

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structured content of ylang ylang flowers such as alkaloids, phenols, flavonoids, terpenoids, steroids and tannins. Alkaloids cause stomachache or stomach poison in *Culex* mosquito larvae. The phenol contained in ylang ylang extract can cause larval lysis, allowing the phenol to enter the cells and preventing the oxidation of metabolites. Later, the phenol contained in the cells can disrupt the cell's working system. Terpenoids, also known as terpenes, kill larvae by affecting their secondary metabolism. This can affect the ovoposition of mosquito larvae, repellants, larvicides and Culex eggs. Flavonoids are plant compounds that can function as inhibitors of glucocorticoids or glucocorticoid poisons. Together with flavonoid alkaloids, tannins and saponins, the hormone produces a combination that is beneficial in its action as a larvicide against larvae. Tannin as a biolarvicide works by preventing larvae from eating their food (Ikhsanudin *et al*., 2022).

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
D0-D1	-12.833	5.789	-2.217	.027	.160
D0-D2	-14.389	5.789	-2.485	.013	.078
D0-D3	-17.778	5.789	-3.071	.002	.013
D1-D2	-1.556	4.094	-.380	.704	1.000
D1-D3	-4.944	4.094	-1.208	.227	1.000
D2-D3	-3.389	4.094	-.828	.408	1.000

Figure 4. Results of Nonparametric Tests: Independent Samples on Soursop Leaf and Ylang Ylang Flower Extract Doses

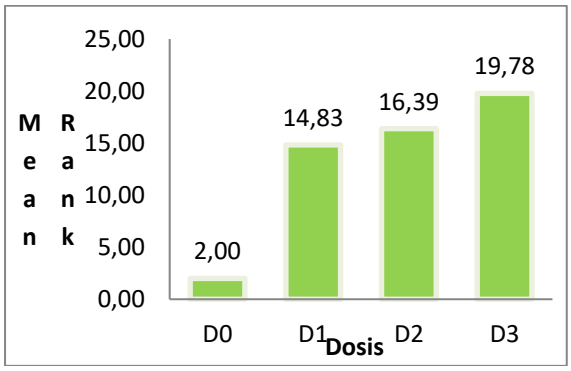

Figure 5. Diagram of Kruskal-Wallis Test Results for Soursop Leaf and Ylang Ylang Flower Extract Doses

The dose of the combination of soursop leaf extract and ylang-ylang flower extract (Figure 4) showed that D0-D3 was significant because t < 0.05, so the hypothesis was rejected, meaning that the dose of soursop leaf extract and ylang-ylang flower extract was effective on mosquito larvae mortality. D3 has the highest effectiveness with a dose of 2.5 ml. The more larvae that die, the higher the treatment dose. This is caused by the presence of chemicals in the natural larvicide extract which contain species with high toxicity. However, there are warnings that must be heeded when using chemicals in water because these factors have the potential to have a negative impact on other organisms in the water (Kusumawati *et al*., 2018).

Soursop leaf extract contains almost the same compounds as ylang-ylang flowers, for example acytogenin, alkaloids and flavonoids, but when combined the extract from ylang-ylang flowers is more effective than soursop. This is influenced by the compound content, formula measurements, and dosage. According to (Ahyanti *et al*., 2023), alkaloids are substances that can cause thinning and rupture of membranes, as well as weaken the larval system by inhibiting the activity of the acetylcholinesterase enzyme. Due to the presence of alkaloid compounds, the color of the larvae becomes more transparent, and the number is smaller due to the slow change in touch. Saponins can interact with proteins and lipid membranes, causing changes in membrane structure up to the final membrane, resulting in hemostasis and osmotic power: intracellular lipid lysis. Saponin is a type of flavonoid that can inhibit enzyme activity, especially in the early stages of metabolism when ATP is needed so that larvae can survive and develop, becoming weak and dying. Flavonoids function as antifeedants by inhibiting larval receptors in the surrounding environment, so that larvae can avoid sensory stimulation and cannot differentiate between food around them. Due to reduced energy, larvae cannot grow and develop. The high energy requirements of larvae are carried out during the biotransformation process. The biotransformation process is the body's response to the presence of toxins. Apart from inhibiting receptors, flavonoids are used as a defense mechanism against larvae. The presence of flavonoids in the

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larval environment is caused by hydrolysis of hydroxyl groups (-OH) between flavonoids and proteins important for larval membranes through hydrogen ionization. This condition causes the active transport of Na+-K+ to become unstable. Cell membranes swell and rupture due to intracellular hyperosmolarity in intracellular fluid imbalance. If the larvae consume flavonoids, Na+-K+ transport will be disrupted, and the larval tubules will be damaged. Rupture of the membrane causes death of the larva.

4. CONCLUSION

The most effective treatment is F3 with the required dose is 2.5 g of soursop leaves: 7.5 g ylang ylang flowersand the lowest in F1 with the required dose is 7.5 g of soursop leaves: 2.5 g of ylang-ylang flowers. Meanwhile, the most effective dose is 2.5 ml of D3, and the lowest dose is 1 ml of F1. The highest number of mosquito larvae deaths was at 9 hours of observation and the lowest was at 12 hours of observation. So, the natural larvicide soursop leaf extract and ylang-ylang flower extract are effective against mosquito larvae mortality.

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