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Research Article

Comparative Study on the Effectiveness of Papaya Leaf *(Carica papaya Linn)* and Citrus Leaf *(Citrus sp.)* Infusions in Reducing Aedes aegypti Larvae Mortality

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ABSTRACT

Background: Dengue Hemorrhagic Fever (DHF) is an infectious disease caused by the dengue virus and transmitted through the bite of the Aedes aegypti mosquito. Vector control efforts have thus far relied heavily on chemical larvicides, which can cause resistance and pollute the environment. This study aims to determine and compare the effectiveness of papaya leaf (Carica papaya Linn) and citrus leaf (Citrus sp.) infusions as natural larvicides against Aedes aegypti larvae.

Method: This study was a laboratory experiment with a post-test-only control group design, using 150 Aedes aegypti instar III larvae divided into six test containers, each containing 25 larvae, with three replicates for papaya leaf infusion and three replicates for 40% concentrated citrus leaf infusion. The infusion was made from dried leaves, which were ground into powder, then boiled at 90°C for 15 minutes before being filtered. Each treatment was tested by adding 5 mL of infusion to a container containing 100 mL of water. The 24-hour mortality data were analyzed using a two-sample independent T-test or Mann–Whitney U test if the data were not normal, with a significance level of $p < 0.05$.

Results: The results showed that there was a significant difference in larval mortality between papaya leaf infusion and citrus leaf infusion ($p = 0.017$). Citrus leaf infusion had a higher lethality rate than papaya leaf infusion. Citrus leaf infusion caused 100% larval mortality within 12 hours, while papaya leaf infusion showed an average mortality rate of 11.66 larvae within 24 hours.

Conclusion: Infusions of citrus leaves and papaya leaves have been shown to have potential as natural larvicides against Aedes aegypti larvae. However, citrus leaf infusions are more effective.

Keywords: Aedes aegypti; Lemon leaves (*Citrus sp.*); Natural larvicide; Papaya leaves (*Carica papaya Linn*)

INTRODUCTION

Dengue fever (DF) is one of the infectious diseases that poses a serious health problem in tropical and subtropical regions, including Indonesia. DF is an infectious disease caused by the dengue virus and transmitted by the Aedes aegypti mosquito. Data show that approximately 390 million infections occur each year, of which 96 million exhibit clinical symptoms. In addition, the prevalence of DBD is estimated to reach 3.9 billion people at risk of infection.[1,2] The highest number of dengue fever cases worldwide was recorded in 2019, with widespread distribution across almost all regions, including Afghanistan, where it was reported for the first time. In the Americas, there were approximately 3.1 million cases, of which more than 25,000 were severe. In Asia, the incidence rate was also relatively high, with Bangladesh reporting 101,000 cases, Malaysia 131,000, the Philippines 420,000, and Vietnam 320,000. Meanwhile, in 2021, dengue fever outbreaks were still found in various countries, including Brazil, Colombia, India, Kenya, Paraguay, Peru, the Philippines, Vietnam, as well as in several island regions such as the Cook Islands, Fiji, and Reunion.[3,4,5]

Dengue Dengue fever cases in Indonesia rise annually, with increasing patient numbers. According to the WHO Health Emergencies report for 2024, Indonesia reported 164,673 dengue cases across 480 districts or cities in 38 provinces. The highest number of cases was recorded in Bandung, Tangerang, Depok, Bekasi, and Malang. The national death toll from dengue reached 926, with the highest case fatality rates found in Malinau, Purworejo, Mandailing Natal, Barru, and Surakarta City. Additionally, by week 20 of 2024, the Early Warning and Response System (SKDR) detected a total of 481,589 suspected dengue cases.

High incidence of dengue fever is influenced by several factors, including poor environmental conditions, low public awareness of dengue, and unhealthy public behavior. In addition, the presence and proliferation of Aedes aegypti mosquitoes, the primary vector, increase the risk of this disease transmission.[6] Therefore, appropriate and effective control measures are needed to reduce the mosquito population that causes dengue fever. Prevention efforts can be implemented in various ways, including controlling dengue vectors through biological, physical, and chemical methods. One commonly used method is control using chemical insecticides. However, long-term use of chemicals can harm human health and lead to mosquito resistance.[7]

Several previous studies have identified alternatives to chemical insecticides, including patilawa leaf extract (Lantana camara Linn.), cinnamon extract (Cinnamomum sp.), ethanol extract from beluntas leaves (Pluchea indica L.), and lime fruit peel extract (Citrus aurantifolia). These studies have shown that such extracts contain substances like ethanol, saonin flavonoids, cinnamaldehyde, eugenol, and other compounds capable of killing Aedes aegypti larvae. Natural insecticides are considered safer because they do not leave residues in soil or agricultural products, as they break down naturally. This helps maintain ecosystem balance and biodiversity in agroecosystems, slows the development of pest resistance, and supports the resilience and sustainability of farming practices.[8,9,10,11,12]

One plant that can be used as a natural insecticide is papaya leaves *(Carica papaya Linn)*. Based on phytochemical tests of papaya leaf extracts, papaya leaves contain secondary metabolites, including tannins, methanol, glycosides, saponins, flavonoids, and alkaloids, which can be used as natural insecticides.[13] A study found that papaya leaf methanol extract

had a significant effect on Culex quinquefasciatus larvae.[14] The use of papaya leaf infusion as a natural larvicide is environmentally friendly and can reduce the risk of resistance. [15]

In addition, citrus leaves contain active substances such as limonoids/limonene, triterpenoids, tannins, saponins, and flavonoids that can serve as alternatives to chemical larvicides. Several studies have found that lime leaf extract is effective in killing Aedes aegypti larvae.[16,17] Flavonoids in lime leaf extract denature proteins in the larvae's cell walls, thereby inhibiting nutrient absorption and ultimately causing death. Saponins can damage the protective protein layer of the body, making it easier for toxic substances to enter the larvae's tissues, which can be fatal. Meanwhile, alkaloids contribute to the damage or degradation of larval cell membranes.[8,9,18,19]

Based on the above description, research on the use of plants as Aedes aegypti larvicides is essential to develop an environmentally friendly, readily available, and effective botanical insecticide for killing dengue vector mosquitoes. The use of plants available in residential areas is a potential option, including papaya and citrus leaves, which are known to contain phytochemicals such as flavonoids, alkaloids, tannins, and saponins that can inhibit the growth and kill Aedes aegypti larvae. However, the two plants have different active substances, for example, papain and carpaine in papaya leaves, and limonoids and citral cein citrus leaves, so a comparative study is needed to determine the effectiveness of each and identify the most optimal, economical, and applicable botanical larvicidal material for the community. The infusion form was chosen because this extraction method is simple, does not require chemical solvents, is environmentally safe, and can extract water-soluble active compounds, thereby enabling direct application of the research results to sustainable community-based dengue vector control.

METHOD

The design of this study was an experimental, post-test-only control-group study comparing the effectiveness of papaya leaf infusion *(Carica papaya Linn)* and citrus leaf infusion *(Citrus sp.)* on the mortality of Aedes aegypti larvae. The study involved 150 Aedes aegypti larvae, with each fish placed in six treatment containers—three for papaya leaf infusion and three for citrus leaf infusion—at the same concentration (40%), with three replications. Inclusion criteria included healthy, active third-instar larvae. The tools used included a blender, analytical scales, a water bath, a measuring cup, plastic containers or test tubes, filter cloth, a knife, a cutting board, and a stopwatch. The research materials consisted of fresh papaya leaves, fresh citrus leaves, well water or distilled water, and Aedes aegypti larvae.

The infusion process involves two stages: preparing the injection and conducting a trial use.

1. Stages of Infusion Preparation

   The first step taken is to select fresh papaya leaves and citrus leaves, then wash them under running water to remove dirt and contaminants. The cleaned leaves are dried in an oven. Once dry, the leaves are ground using a blender without adding water until an herbal powder is obtained. A total of 52 grams of each leaf powder (papaya and citrus) is weighed and mixed with 200 ml of water as the solvent. The mixture is heated in a water bath at 90°C for 15 minutes, with stirring every 5 minutes. After heating, the mixture is filtered

through a cloth while still hot to obtain the infusion filtrate. This step is carried out for each leaf. The resulting infusion has a concentration of 40% and is then stored in separate sterile containers until used for testing.

1. Steps for Testing the Use of Infusions

   A total of six test containers were prepared and labeled according to treatment groups, with three replicates for papaya leaf infusion and three for citrus leaf infusion. Each container was filled with 100 ml of healthy water. In each container, 25 Aedes aegypti larvae were placed. Then, 5 ml of infusion was added to each container according to the treatment group (papaya leaf infusion or citrus leaf infusion).

   Larval mortality was observed at 15, 30, 45, 90, 2, 4, 6, 8, 12, and 24 hours. The number of dead larvae was recorded cumulatively at each interval. All observation data were then entered into an observation table for further analysis regarding the effectiveness of each infusion on Aedes aegypti larval mortality.

The comparison of the effectiveness of papaya leaf infusion and lime leaf infusion over 24 hours was analyzed using a two-sample independent T-Test. At the same time, the Mann–Whitney U test was applied if the normality assumption was not met. A p-value < 0.05 was set as the significance threshold to determine a meaningful difference between the two treatment groups.

RESULTS

Testing the Effectiveness of Papaya Leaf Infusion

The effectiveness of papaya leaf infusion as a control agent for Aedes aegypti mosquito larvae was tested in three experiments. Larval mortality data are shown in Table 1.

Table 1. Observation of Larvae Mortality with 40% Papaya Leaf Infusion Treatment

Test Container	No.of Larvae	Number of Larvae That Die Within Hours									
		15 Minutes	30 Minutes	45 Minutes	90 Minutes	2 Hours	4 Hours	6 Hours	8 Hours	12 Hours	24 Hours
I	25	0	0	1	1	2	2	3	4	5	15
II	25	0	0	0	1	1	1	1	1	2	9
III	25	0	0	0	0	1	2	2	2	3	11
Total		0	0	1	2	4	5	6	7	10	35

Based on the observation, the effectiveness of papaya leaf infusion as an agent for controlling Aedes aegypti mosquito larvae was highest in the first experiment (P1), with 15 dead larvae, and lowest in the second experiment (P2), with only 9 dead larvae after 24 hours.

According to Figure 1, mortality started at 45 minutes in experiment 1 and increased over 24 hours, with the highest mortality occurring in that experiment. From the three experiments above, the average mortality of larvae at specific time intervals can be calculated to provide an overview of the effectiveness of papaya leaf infusion.

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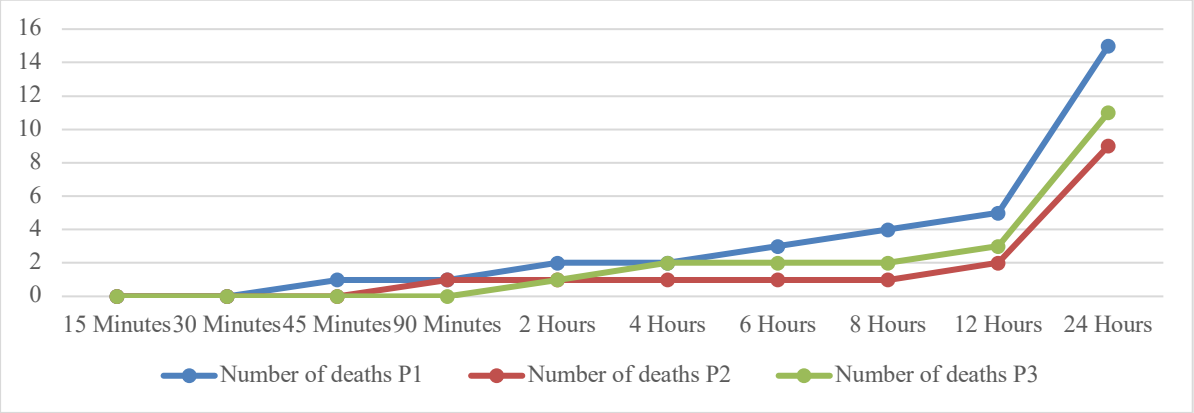

Figure 1. Graph of 24-Hour Larval Mortality in Papaya Leaf Infusion

Table 2. Average Larvae Mortality in Papaya Leaf Infusion

No.	Observation at the hour of	Average Larva Mortality	Larvae Mortality Percentage (%)
1	15 minutes	0	0
2	30 minutes	0	0
3	45 minutes	0.33	1.32
4	90 minutes	0.67	2.68
5	2 hours	1.33	5.32
6	4 hours	1.67	6.68
7	6 hours	2	8
8	8 hours	2.33	9.32
9	12 hours	3.33	13.32
10	24 hours	11.66	46.64

Table 2 shows that during the first 15- and 30-minute intervals, no larvae died, with an average larval mortality rate of 0, indicating that papaya leaf infusion did not exert a lethal effect within a very short time. Conversely, at the 24-hour interval, the average larval mortality reached its peak at 11.66, indicating that papaya leaf infusion is effective in killing mosquito larvae after sufficient time to act.

Testing the Effectiveness of Citrus Leaf Infusion

The effectiveness of citrus leaf infusion against Aedes aegypti mosquito larvae was also tested three times. Larva mortality data can be seen in the Table 3.

Table 3. Observation of Larvae Mortality with Citrus Leaf Infusion Treatment at a Concentration of 40%

Test Containe	Number of Larvae	Number of Larvae That Die Within Hours									
		15 Minutes	30 Minutes	45 Minutes	90 Minutes	2 Hours	4 Hours	6 Hours	8 Hours	12 Hours	24 Hours
I	25	0	0	0	0	0	5	8	8	25	25
II	25	0	0	0	0	0	5	7	10	25	25
III	25	0	0	0	0	0	5	5	13	25	25
Total		0	0	0	0	0	15	20	31	75	75

Based on Figure 2, the highest larval mortality occurred in the first experiment (P1) and the third experiment (P3), both of which reached 25 dead larvae after 24 hours, while the lowest

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mortality occurred in the second experiment (P2), which only reached 10 dead larvae in the same period.

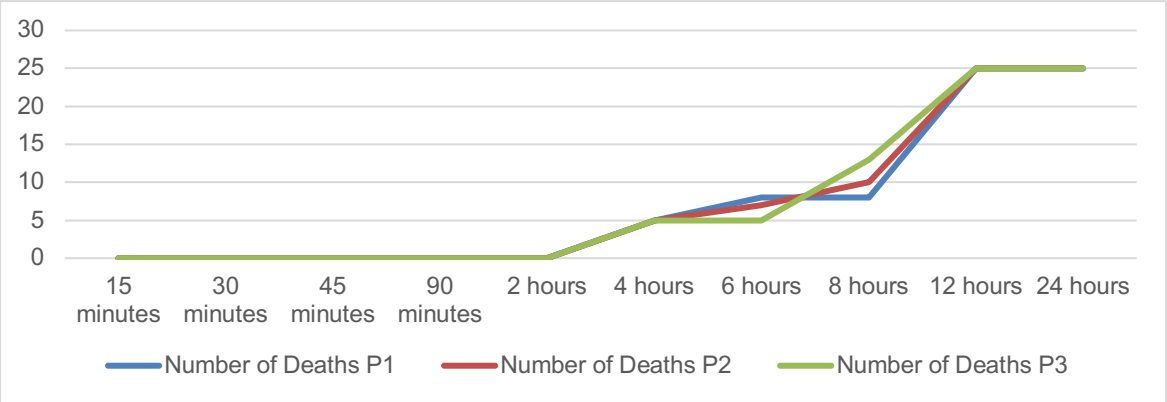

Figure 2. Graph of Larvae Mortality within 24 Hours after Citrus Leaf Infusion

Based on Table 4, there were no deaths during the first 0–2 hours of observation. Deaths started to occur at 4 hours, with numbers similar across all three experiments (P1, P2, P3). Later, the number of fatalities gradually increased at the 6th and 8th hours, with P3 showing a slightly higher count than P1 and P2. The highest mortality occurred at the 12th hour, when all larvae in the three experiments (25 per experiment) died, and this condition continued until the 24th hour.

Table 4. Average Larval Mortality

No.	Observation at the hour of	Average Larva Mortality	Larvae Mortality Percentage (%)
1	15 minutes	0	0
2	30 minutes	0	0
3	45 minutes	0	0
4	90 minutes	0	0
5	2 hours	0	0
6	4 hours	5,00	20
7	6 hours	6,67	26.68
8	8 hours	10,33	41.32
9	12 hours	25,00	100
10	24 hours	25,00	100

Based on Table 4, the average larval mortality above showed no mortality at intervals of 15 minutes to 2 hours, with an average of 0 dead larvae, indicating that the treatment did not have a short-term larvicidal effect. Deaths began to appear at the 4th hour with an average of 5 larvae, then increased to 6.67 larvae at the 6th hour, and rose sharply to 10.33 larvae at the 8th hour. At the 12th hour, the average larval mortality reached 25 larvae, or 100% of the total, and this condition persisted for 24 hours. This indicates that the treatment takes several hours to start working but can achieve full effectiveness, with total mortality, within 12 hours after application.

The results of the two-sample independent t-test indicated a significant difference in larval mortality between papaya leaf infusion and citrus leaf infusion after 24 hours of exposure ($t = -7.56$; $p = 0.017$). The group receiving the citrus leaf infusion had more dead larvae than the

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group receiving the papaya leaf infusion. These results suggest that citrus leaf infusion is more effective as a larvicide under the tested conditions.

Effectiveness of Papaya Leaf and Citrus Leaf Infusions Based on 24-Hour Mortality

Table 5. Results of the T-Test on Aedes aegypti Larvae Mortality

Treatment Group	*n*	Mean ± SD	Min-Max	*t*	*df*	*p*	Mean Difference
Papaya Infusion	3	11.67 ± 3.06	9-15	-7.56	2	0.017	-13.33
Citrus Infusion	3	25.00 ± 0.00	25-25	-	-	-	-

The results of the two-sample independent t-test indicated a significant difference in larval mortality between papaya leaf infusion and citrus leaf infusion after 24 hours of exposure ($t = –7.56$; $p = 0.017$). The group receiving the citrus leaf infusion had more dead larvae than the group receiving the papaya leaf infusion. These results suggest that citrus leaf infusion is more effective as a larvicide under the tested conditions.

DISCUSSION

Based on the research results, papaya leaf infusion (Carica papaya Linn) at 40% concentration shows potential as a natural larvicide against Aedes aegypti larvae. However, its mechanism of action and effectiveness are lower than those of citrus leaf infusion (Citrus sp.) at the same concentration. Papaya leaf infusion only began to show effects after 45 minutes of exposure, with an average mortality rate of only 0.33 larvae. Unlike citrus leaves, papaya leaf infusion also showed larvicidal activity, but results varied across experiments. The average larval mortality rate at 24 hours was 11.66 larvae (46.64%), indicating that this infusion requires more time and higher concentrations to produce consistent lethal effects. These findings are consistent with several previous studies showing that papaya leaf infusion (Carica papaya Linn) can cause mortality in Aedes aegypti larvae. A previous study found that papaya leaf infusion at 20% and 30% concentrations significantly increased the mortality of Aedes aegypti larvae.[20] Meanwhile, A study found that the methanol fraction of papaya leaves had an LC_{50} of approximately 4,929 ppm against third- and fourth-instar larvae of Aedes aegypti after 24 hours of exposure.[21,22] A study by Pakan et al. (2023) also found that high concentrations of papaya leaf extract achieved 100% mortality within 12 hours, whereas lower concentrations showed decreased effectiveness.[23]

Papaya leaf infusion slowly kills Aedes aegypti larvae. This can be seen in the characteristics of dead larvae: they do not respond when touched, their bodies turn white or pale yellow, and they appear elongated. This effect is related to the larvicidal properties of papaya leaf extract (Carica papaya), which come from its secondary metabolite content. The results of research conducted by Dhenge et al. (2021) indicate that papaya leaf extract contains alkaloids, flavonoids, saponins, and tannins, which are known to disrupt the physiological systems of larvae, leading to death.[24]

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Various active compounds contained in the infusion, such as papain, carpaine alkaloids, flavonoids, saponins, tannins, and steroids, act as natural larvicides against mosquito larvae. The proteolytic enzyme papain breaks down essential proteins in the larvae's bodies, disrupting their metabolism and growth. At the same time, the alkaloid carpaine inhibits acetylcholinesterase, disrupting the nervous system and leading to convulsions and death in larvae.[25, 26] Flavonoids work by damaging the nervous and respiratory systems of larvae, while saponins damage the cuticle layer and disrupt the body's fluid balance (osmoregulation). Tannins and steroids enhance the toxic effects by inhibiting digestive enzymes and slowing metabolism, causing the larvae to lose their ability to survive.[27,28,29] The combination of these compounds causes complex physiological disturbances in larvae, making papaya leaves an effective, environmentally friendly, readily biodegradable, and safe natural larvicide.

However, the analysis showed a significant difference in larval mortality between papaya leaf infusion and citrus leaf infusion after 24 hours of exposure ($p = 0.017$). The group given the citrus leaf infusion had more dead larvae than the papaya leaf infusion group. This indicates that there is variability in Aedes larvae's responses to infusions, which may be due to factors such as infusion concentration, environmental conditions, and individual larval characteristics.[30] Another factor that may affect the effectiveness of papaya leaf infusion on Aedes aegypti larval mortality in this study is that most of the papaya leaves used were not from the apical meristem or the active growth area at the tips of the plant's shoots and roots. Leaves on the apical meristem contain more secondary metabolites than older leaves. Secondary metabolites and enzymes play essential roles in leaf growth, development, and cell division. As leaves age, their activity decreases, lowering the concentration of secondary metabolites in the plant. Older leaves are generally located from the sixth leaf down from the shoot, while younger leaves consist of the top three leaves on the shoot.[31]

This confirms that papaya leaf infusion has the potential to be a natural larvicide, although its effectiveness remains lower than that of synthetic larvicides such as Abate. An essential advantage of papaya leaves is that they are environmentally friendly, biodegradable, and relatively safe for humans and animals compared to chemical insecticides, which can lead to resistance and environmental pollution.[32,33]

Regarding The Effectiveness of Citrus Leaf Infusion (Citrus sp.) as a Larvicide, based on the experimental results, the citrus leaf infusion in the three experiments began to show effects after 4 hours of exposure. In addition to larval deaths, several larvae became less active. Maximum effectiveness was achieved at 12 hours, with 100% mortality among the test larvae, faster than with the papaya leaf infusion. The results of this study are in line with recent research showing that ethanol extract in lime leaves (Citrus aurantiifolia) with the addition of 5% polyethylene glycol (PEG) at concentrations of 2.5%, 5%, and 10% effectively caused 100% mortality in Aedes aegypti larvae in just 6 hours of exposure. The effectiveness of this lime leaf infusion is equivalent to that of abate as a positive control, although the mechanisms of action differ. The flavonoid, saponin, and alkaloid content in lime leaves plays a vital role in damaging cell membranes, disrupting nutrient absorption, and increasing toxicity, which causes larval mortality.[34,35] Research by Taufik et al. (2023) shows that lime leaf extract (Citrus aurantifolia) can cause up to 100% mortality in Aedes aegypti larvae at a concentration of 3%. It is effective as a larvicide based on WHO standards. This study confirms that the active compounds in lime leaves, such as limonoids and flavonoids, play a crucial role in inducing

toxic effects on larvae, acting through both stomach and contact poisoning mechanisms.[36] In addition, lime leaf extract (*Citrus aurantifolia*) has been reported to cause up to 100% mortality in *Aedes aegypti* larvae at a concentration of 3% and is considered effective as a larvicide based on WHO standards. This effectiveness is supported by the presence of active compounds such as limonoids and flavonoids, which play a crucial role in inducing toxic effects on larvae through both stomach poisoning and contact poisoning mechanisms.[9] This reinforces the findings in this study that citrus leaf infusion works faster and more consistently in causing mortality in Aedes aegypti larvae compared to papaya leaf infusion, with the results of the two-sample independent T-test showing that there is a significant difference in larval mortality between papaya leaf infusion and citrus leaf infusion after 24 hours of exposure ($t = -7.56$; $p = 0.017$).

Lemon leaves contain limonoids that can enter the body of larvae. In this study, lemon leaf infusion can cause death in larvae, but it takes 4 hours to have an effect. Limonoids must enter the larvae's bodies in sufficient doses to disrupt their metabolism. These doses accumulate in the larvae's bodies from the extract they eat or from osmosis through the larvae's skin. Citrus leaf extract (Citrus sp.) mixed with water produces a homogeneous solution. Because it is homogeneous, the solution can enter the larval digestive system upon ingestion and/or swallowing. Even if the extract is not ingested by the larvae, it can still enter through the larval skin because it is evenly mixed with the water that serves as the medium for larval development. This substance disrupts the larvae's metabolism and nervous system, causing convulsions, paralysis, and ultimately death.[37, 38]

In addition to effectively killing mosquito larvae, citrus leaf infusion has an essential advantage over chemical larvicides, namely its biodegradability. Active compounds, such as limonoids, are derived from natural organic materials and degrade rapidly when mixed with water. This makes citrus leaf infusion safe for the environment, as it leaves no harmful residues and does not pollute the surrounding water and soil.[39, 40] Unlike synthetic pesticides, which can promote resistance and harm ecosystems, citrus leaf infusion supports environmentally friendly vector control. However, because it is natural, citrus leaf infusion has a limited shelf life, as its organic ingredients quickly decay and lose their effectiveness when stored for too long. Nevertheless, its safety for humans and ecosystems makes citrus leaf infusion a potential alternative as a sustainable natural larvicide.

Comparatively, citrus leaf infusion has been proven to be faster and more consistent in causing mortality in Aedes aegypti larvae than papaya leaf infusion. However, both shows promise as safer, more environmentally friendly alternatives to natural larvicides. Optimizing concentration, extraction methods, and selecting the appropriate plant parts are essential factors in enhancing the effectiveness of both types of infusions in efforts to control the Aedes aegypti mosquito vector.

CONCLUSION

Based on the study results, it can be concluded that there is a significant difference in larval mortality between papaya leaf infusion and citrus leaf infusion after 24 hours of exposure. Papaya leaf infusion (Carica papaya Linn) and citrus leaf infusion (Citrus sp.) are both effective as natural larvicides against Aedes aegypti larvae. Citrus leaf infusion was proven to be faster

and more consistent in causing larval mortality, with a mortality rate reaching 100% within 12 hours, while papaya leaf infusion showed a slower effect with an average larval mortality of 11.66 larvae (46.64%) after 24 hours. Both types of infusions are natural, readily biodegradable, and safe for humans and the environment, making them potential alternatives as environmentally friendly, sustainable botanical larvicides to support the control of mosquito vectors causing dengue fever (DBD).

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Declarations

Authors' contribution

All authors actively participated in the research and writing of this manuscript and are accountable for its content. All authors have read and approved the final manuscript. The specific contributions of each author are as follows: Ardian S. Leky: Original Draft Preparation, Writing, Data Collection, Methodology, Conceptualization, Data Curation, Statistical Analysis, Data Interpretation, Visualization, Validation, Supervision, Review, and Editing. Arif Andriyanto: Conceptualization, Methodology, Data Collection, Writing - Original Draft Preparation, Statistical Analysis, Data Interpretation, Review and Editing.

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