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Research Article

Environmentally Friendly Larvicides: A Post-Test Only Experimental Comparison of *Eleutherine palmifolia* and *Allium sativum* Extracts (0.6–1.8%) on *Aedes aegypti* Larval Mortality

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ABSTRACT

This study aims to identify the larvicidal potential of *Eleutherine palmifolia* (Dayak onion) and *Allium sativum* (garlic), which are rich in flavonoids and known to possess insecticidal properties. The study used a pure experimental design with a post-test control group. *Aedes aegypti* larvae were tested with *Eleutherine palmifolia* and *Allium sativum* extracts at concentrations of 0.6%, 1.2%, and 1.8%, respectively, along with abate as a positive control. Larval mortality was monitored for 24 hours with four replicates. The results showed that *Allium sativum* was highly effective at all concentrations (99–100% mortality), even at the 0.6% concentration. In contrast, *Eleutherine palmifolia* exhibited varying effectiveness, with mortality rates of 67%, 67%, and 84% at concentrations of 0.6%, 1.2%, and 1.8%, respectively. These findings confirm that *Allium sativum* has superior, consistent, and stable larvicidal effectiveness even at low doses, making it a promising alternative for mosquito control and dengue prevention.

***Keywords**: Aedes aegypti, Allium sativum, Biolarvicide, Eleutherine palmifolia, Mosquito Control*

Introduction

Dengue hemorrhagic fever (DHF), caused by the dengue virus and transmitted through the bite of the *Aedes aegypti* mosquito, continues to be a significant public health problem worldwide. In 2024, over 14 million dengue cases and more than 10,000 dengue deaths were reported globally (European Centre for Disease Prevention and Control, 2024). In Indonesia, the previous year (2023) saw 114,720 cases and 894 deaths. As of the 43rd week of 2024, a total of 624,194 dengue cases had been reported through SKDR, affecting 482 districts/cities. Additionally, the annual cycle of the disease has shortened in

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recent years, dropping from ten years to three years or even less (Kemenkes RI, 2024).

Efforts to control the *Aedes aegypti* mosquito population often focus on breaking its life cycle at the larval stage using synthetic larvicides such as abate (temephos). Unfortunately, long-term use of synthetic larvicides can lead to resistance among mosquito populations (Davila, 2024). Although some areas in Indonesia still have susceptible mosquito populations (Aprilia, 2022; Khaer, 2021; Ramly, 2022), resistance cases have been reported in several regions, including South Kalimantan (Andiarsa, 2020; Istiana, 2012), Surabaya (Rachmawati, 2020), Samarinda (Ekawati, 2019), and Semarang (Handayani, 2016). To address this growing threat to dengue control efforts, the development of environmentally friendly alternative larvicides is essential to enhance vector control strategies (Priya, 2023).

At the global level, trends show an increasing interest in natural-based bio-larvicides. This growing interest is motivated by the desire to reduce reliance on chemical larvicides and adopt more sustainable pest control practices (Priya, 2023; Tennyson, 2024). In Indonesia, various studies have shown the potential of plant-based materials as natural larvicides, such as *citronella* (Sudibyo, 2023), okra fruit (*Abelmoschus esculentus*) (Sari, 2018), African leaf (*Vernonia amygdalina*) (Hasibuan, 2022), sengkuang leaf (*Dracontomelon dao*) (Kurniawan, 2019), basil leaf (*Oncimum basilicum*) (Rendika, 2021), Dayak onion (*Eleutherine palmifolia*) (Rendika, 2021) and garlic (*Allium sativum*) (Ikhtiar, 2019; Kasim, 2019).

Although commonly used as cooking ingredients, *Eleutherine palmifolia* and *Allium sativum* have been shown in several studies to have potential as larvicides due to their various phytochemical compounds, including flavonoids, alkaloids, saponins, and tannins (Ningrum, 2022). Flavonoids act as toxins for mosquito larvae, leading to their death and reducing the risk of dengue disease in the environment (Aminu, 2022; Ikhtiar, 2019; Kasim, 2019).

Known as *Allium sativum*, garlic contains numerous active compounds that function as mosquito larvicides. When *Allium sativum* is crushed or cut, allicin emerges, one of the main compounds that exerts this effect (Fahmi, 2022; Nisar, 2021). Allicin has strong antimicrobial properties that can kill mosquito larvae. Other important organic sulfur compounds, such as ajoene, diallyl disulfide, and diallyl trisulfide, also contribute to *Allium sativum*'s larvicidal activity (Yearsi, 2021). By interfering with various larval biological functions, including energy metabolism, these compounds can inhibit larval growth. Saponins and flavonoids are two other important compounds found in *Allium sativum*. Saponins are known to damage larval cell membranes, while flavonoids inhibit enzymes essential for larval metabolism (Priya, 2023; Rajapaksha, 2024).

This study focuses on comparing the effectiveness of *Eleutherine palmifolia* and *Allium sativum* extracts against *Aedes aegypti* mosquito larvae, which has not been comprehensively conducted. Although previous studies have shown the potential of each plant as a larvicide, this study makes a new contribution by: Testing a direct comparison of the effectiveness of the extracts of both plants. The objective of this study is to identify the effectiveness of plant-based larvicides from *Eleutherine palmifolia* and *Allium sativum* against *Aedes aegypti* mosquito larvae and provide an environmentally friendly alternative larvicide to support more sustainable dengue control.

Methods

This research uses a true experiment approach, specifically a post-test only control group design. The sample consisted of *Aedes aegypti* larvae. The research was conducted from July to November 2024, encompassing stages from tool and material preparation to extract testing. The process of extracting *Eleutherine palmifolia* and *Allium sativum* as larvicides using the maceration method was carried out at the Pharmacology Laboratory, while the testing phase was conducted at the Parasitology Laboratory, both located at the Faculty of Medicine, Mulawarman University, Samarinda. The independent variables were the concentrations of *Eleutherine palmifolia* and *Allium sativum* (0.6%, 1.2%, 1.8%). The dependent variable was the mortality rate of mosquito larvae. The experiment was replicated four times. Mosquito larvae were observed and examined at 10-minute, 20-minute, 30-minute, 60-minute, and 24-hour intervals. The Shapiro-Wilk test was used to assess normality, and Levene's test was used to evaluate homogeneity of variance, both at a 5% significance level. Data analysis was conducted using IBM SPSS version 25.

Result and Discussion

Table 1 shows the mortality percentage of *Aedes* larvae after being treated with *Eleutherine palmifolia* extract at three different concentrations (0.6%, 1.2%, and 1.8%). Observations were made at time intervals of 10, 20, 30, and 60 minutes, as well as after 24 hours. At concentrations of 0.6% and 1.2%, no larval mortality was observed within the first 10 to 60 minutes. At the 1.8% concentration, larval mortality began to appear at 30 minutes (1 larva) and increased at 60 minutes (total of 4 larvae out of 4 replicates) (Figure 1). At concentrations of 0.6% and 1.2%, larval mortality was relatively similar, with approximately 16.75 larvae out of 25 test larvae dying, resulting in a mortality percentage of 67%. At the 1.8% concentration, mortality was higher, with an average of 21 larvae dead, equivalent to a mortality percentage of 84%.

The results of this study indicate that *Eleutherine palmifolia* extract has a larvicidal effect on *Aedes* larvae, which increases with concentration. The highest mortality occurred at the 1.8% concentration with a percentage of 84%, while the 0.6% and 1.2% concentrations had similar effects (67%). The absence of mortality at lower concentrations within the first 60 minutes suggests that the effect is more gradual rather than instantaneous. The 1.8% concentration was more effective in killing larvae, with a faster increase in mortality compared to the other concentrations. The 0.6% and 1.2% concentrations showed similar levels of effectiveness after 24 hours, indicating that there may be a threshold of effectiveness that was not met at these concentrations. If this extract is to be used as a natural larvicide, higher concentrations (≥1.8%) are recommended for optimal results.

Table 2 shows the mortality percentage of *Aedes* larvae after being treated with *Allium sativum* extract at three different concentrations (0.6%, 1.2%, and 1.8%). Observations were made within time intervals of 10, 20, 30, and 60 minutes, as well as after 24 hours. At the 0.6% concentration, no mortality was observed in the first 10 and 20 minutes, but larval death began to occur at 30 and 60 minutes. At concentrations of 1.2% and 1.8%, larval mortality started to appear at 30 minutes, with an increase at 60 minutes (up to 6 larvae dead in some replicates at the 1.8% concentration). All larvae died at the 1.2% and 1.8% concentrations after 24 hours (100% mortality). At the 0.6% concentration, the average mortality was 24.75 out of 25 larvae (99% mortality), indicating that nearly all larvae died.

The results of this study demonstrate that *Allium sativum* extract is highly effective in killing *Aedes* larvae, even at low concentrations (0.6%), with nearly all larvae dead after 24 hours. The 1.2% and 1.8% concentrations provided a faster effect compared to 0.6%, with mortality starting as early as 30 minutes (Figure 2).

When compared to *Eleutherine palmifolia*, *Allium sativum* exhibits higher effectiveness in a shorter time frame. All concentrations of *Allium sativum* approached or reached 100% larval mortality after 24 hours, whereas *Eleutherine palmifolia* at the 1.8% concentration only achieved 84%. *Allium sativum* extract shows great potential as a natural larvicide against *Aedes* mosquitoes, as even at low concentrations (0.6%), nearly all larvae died. With 100% effectiveness at 1.2% and 1.8% concentrations, this extract can serve as a natural alternative for mosquito control without requiring high concentrations. Further studies are needed to assess its ecological impact and whether this effect persists under natural environmental conditions.

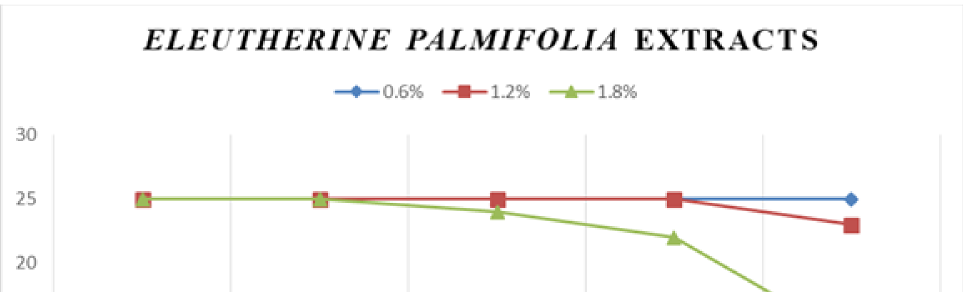

Figure 1. Observation and repetition time of Eleutherine palmifolia extracts

Table 1. Percentage of mortality of Aedes mosquito larvae on Eleutherine palmifolia extracts

Eleutherine palmifolia concentration	Mortality of larvae after 24 hours				Mean mortality	Mean percentage mortality (%)
	1	2	3	4		
0.6%	**17**	**17**	**15**	**18**	**16.75**	**67**
1.2%	**17**	**18**	**17**	**15**	**16.75**	**67**
1.8%	**21**	**20**	**22**	**21**	**21**	**84**

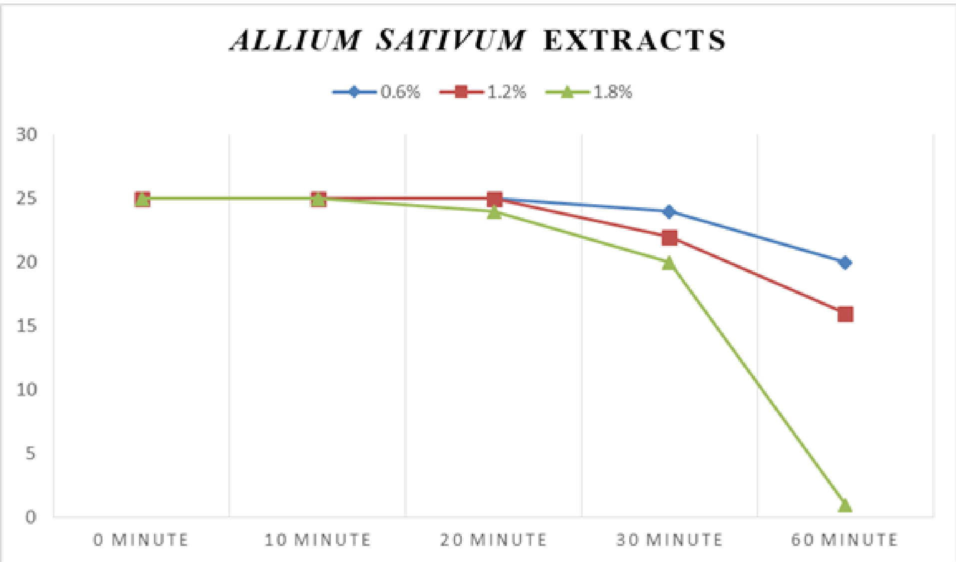

Figure 2. Observation and repetition time of Allium sativum extracts

Table 2. Percentage of mortality of Aedes mosquito larvae on Allium sativum extracts

Allium sativum concentration	Mortality of larvae after 24 hours				Mean mortality	Mean percentage mortality (%)
	1	2	3	4		
0.6%	**25**	**25**	**25**	**24**	**24.75**	**99**
1.2%	**25**	**25**	**25**	**25**	**25**	**100**
1.8%	**25**	**25**	**25**	**25**	**25**	**100**

The statistical analysis revealed a significant difference in the effectiveness of *Eleutherine palmifolia* at different concentrations (p = 0.001). This indicates that increasing the concentration of *Eleutherine palmifolia* significantly affects the mortality rate of *Aedes aegypti* larvae (Table 3). The median (IQR) larval mortality after 24 hours for *Eleutherine palmifolia* extract at concentrations of 0.6%, 1.2%, and 1.8% were 17.0 (15.0–18.0), 17.0 (15.0–18.0), and 21.0 (20.0–22.0), respectively.

In contrast, the *Allium sativum* extract showed consistently high effectiveness, with a median (IQR) mortality of 25.0 (24.0–25.0) across all concentrations tested (0.6%, 1.2%, and 1.8%), and no significant difference detected between them (p = 0.368). Furthermore, a Mann–Whitney U test demonstrated that *Allium sativum* extract produced significantly higher larval mortality compared to *Eleutherine palmifolia* (p < 0.001), supporting its superior larvicidal potential even at low concentrations.

The research results indicate that *Allium sativum* exhibits more consistent larvicidal effectiveness than *Eleutherine palmifolia*, achieving 100% larval mortality at a concentration of 0.6% without dose escalation. *Allium sativum* extract is a highly effective larvicidal agent, even at low concentrations, with an LC_{50} and EC_{50} value of 0.45%, while *Eleutherine palmifolia* demonstrated good larvicidal activity at certain concentrations, with an LC_{50} and EC_{50} of 0.28%

These findings align with several previous studies demonstrating the natural larvicidal efficacy of *Allium sativum* against mosquito larvae. A study comparing the effects of *Allium sativum* and anise essential oils revealed significant larvicidal activity against *Aedes* larvae, with *Allium sativum* oil being more effective at lower concentrations (Laojun, 2020).

Mulyono (2021) found that at a 0.6% concentration, *Allium sativum* solution caused 75% mortality in *Aedes* larvae, surpassing the 60% mortality achieved by *bajakah* leaf (*Uncaria acida*) extract. The results consistently show that *Allium sativum* solution is more effective in killing mosquito larvae compared to *Uncaria acida* extract at various tested concentrations. At the highest concentrations tested (1.3% and 1.5%), *Allium sativum* solution successfully killed all mosquito larvae, while *Uncaria acida* leaf extract achieved only 80% larval mortality.

Table 3. Results of Larval Mortality Statistical Tests

Statistical Test	*Eleutherine palmifolia*	*Allium sativum*	Extract Comparison
Normality Test (Shapiro-Wilk)	p = 0.406-0.683 (normal)	p = 0.001-1.000 (mixed)	p = <0.001 (not normal)
Homogeneity Test (Levene's Test)	p = 0.892 (homogeneous)	p = 0.405 (homogeneous)	p = 0.020 (not homogeneous)
One-Way Anova	p = 0.001 (**significant**)	-	-
Kruskal-Wallis Test	-	p = 0.368 (not significant)	-
Mann-Whitney U Test	-	-	p = 0.000013 (**significant**)

A study by Siregar (2023) revealed that *Allium sativum* extract processed into black garlic exhibited significant larvicidal effects on *Aedes* larvae at an effective concentration of 13% larval mortality reached 100%, while 5% concentration resulted in approximately 83% mortality, demonstrating effectiveness even at lower levels. Aminu (2022) evaluated the effects of extracts from *Allium sativum* (garlic), *Zingiber officinale* (ginger), and *Syzygium aromaticum* (clove) on *Aedes* larvae, finding that all three extracts showed potential as natural larvicides, with *Allium sativum* displaying the highest larvicidal activity among the tested extracts. The study reported an LC50 of 42.50% at a concentration of 2.685 mg/L.

Research by Sagala (2022) demonstrated that a 70% ethanol extract of *Allium sativum* was effective against third instar *Aedes* larvae, with a concentration of 6458 ppm killing 50% of the larvae within 24 hours. Furthermore, the ethanol extract of *Allium sativum* was effective not only against *Aedes aegypti* larvae but also against other mosquito species, such as *Aedes albopictus*, *Culex quinquefasciatus*, and *Anopheles*, with significant mortality observed at various concentrations (Dicken, 2022). Rajapaksha (2024) reported that *Culex quinquefasciatus* larvae were more sensitive to *Allium sativum* extract compared to *Aedes albopictus*.

The larvicidal effects of *Allium sativum* on mosquito larvae are attributed to its diverse active compounds. Substances such as flavonoids, saponins, and reducing sugars disrupt larval digestion, inhibit growth, and ultimately cause death (Dicken, 2022; Nasir, 2022). Additionally, sulfur compounds like diallyl sulfide and diallyl disulfide can damage the integrity of larval cell membranes (Dicken, 2022; Laojun, 2020).

The main active compound in *Allium sativum*, allicin, is known to have strong antimicrobial and insecticidal activity. Allicin is rapidly formed when *Allium sativum* is crushed or extracted, as a result of an enzymatic reaction between alliin and alliinase. This compound is chemically reactive, capable of disrupting insect cell membranes and damaging key protein structures, leading to larval death in a short time (Bhatwalkar, 2021; Muahiddah & Diamahesa, 2023).

In addition to allicin, *Allium sativum* also contains flavonoids, such as quercetin, which have antioxidant properties and can inhibit larval metabolism by interfering with digestive and respiratory enzymes (Khubber et al., 2020). The combination of allicin's cytotoxic effects and the enzyme-inhibiting activity of flavonoids explains why *Allium sativum* extract demonstrates a faster onset of action compared to other plants such as *Eleutherine palmifolia* (Batiha et al., 2020).

However, there are several challenges in applying *Allium sativum* extract in the field. First, allicin is unstable, easily degraded by heat, light, and oxygen, which can significantly reduce its effectiveness in open environments. Second, the strong odor of allicin may pose a barrier to large-scale application, as it can be unpleasant to humans or affect non-target animals. Third, the potential toxicity to non-target organisms and long-term environmental impact must be considered, as these aspects remain largely under-researched. Therefore, further studies on stable formulations, field efficacy, and ecological safety are essential before practical application can be recommended (Bhatwalkar et al., 2021; Khubber et al., 2020).

Our study found that 1.8% concentration of *Eleutherine palmifolia* was most effective in killing *Aedes* larvae, with an 84% mortality rate. While this confirms previous research on its larvicidal properties, it performed less well than sweet basil extract. *Eleutherine palmifolia* was less effective at lower concentrations (10-35% mortality) compared to sweet basil (30-50%). Even at higher concentrations, *Eleutherine palmifolia* didn't match sweet basil's 100% mortality rate at 1.5-1.8%. Although *Eleutherine palmifolia* shows potential as a natural larvicide, its performance is often less competitive compared to other plants such as *Allium sativum* and basil leaves (Rendika, 2021).

Conclusion

The results of this study support the potential of *Allium sativum* and *Eleutherine palmifolia* as natural and environmentally friendly alternatives for mosquito control. *Allium sativum* extract is a highly effective larvicidal agent, even at low concentrations, with an LC_{50} and EC_{50} value of 0.45%, while *Eleutherine palmifolia* demonstrated good larvicidal activity at certain concentrations, with an LC_{50} and EC_{50} of 0.28%, although further investigation is needed to confirm the consistency of its effects.

The presence of active compounds such as allicin and flavonoids in *Allium sativum* is believed to accelerate larval mortality by disrupting cell membranes and interfering with key metabolic enzymes. However, to support the practical application of these plant-based extracts as vector control agents, further studies are required to evaluate their toxicity to non-target organisms and the environmental degradation of their active compounds, ensuring ecological safety and long-term sustainability in field use.

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