Effectiveness of Methanol Extract Combination of *Cymbopogon citratus* Stem and *Syzygium aromaticum L.* Leaf as a Larvacide Against *Aedes aegypti*

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Abstract.

Dengue Hemorrhagic Fever (DHF) is a common disease in tropical countries, including Indonesia. The control of Ae. aegypti vectors relies on chemical insecticides, which can cause resistance and environmental pollution. Therefore, the use of biolarvicides is considered a safer alternative. Lemongrass (Cymbopogon citratus) stems and clove (Syzygium aromaticum L.) leaves are known to contain active compounds with larvicidal potential. This study aimed to determine the effectiveness of a 1:1 methanolic extract combination of lemongrass (Cymbopogon citratus) stems and clove (Syzygium aromaticum L.) leaves as a larvicide against Ae. aegypti based on different concentration levels. The study used an experimental method with a longitudinal prospective design. Seven treatment concentrations (0,5%; 0,75%; 1,5%; 3%; 5%; 7%; and 10%) were tested on Ae. aegypti third-instar larvae with four replications. Larval mortality was observed every hour for 12 hours, and probit analysis was used to determine LC_{50}, LC_{90}, LT_{50}, and LT_{90} values. Larval mortality increased linearly with higher extract concentrations. The LC_{50} value was 0,0385%, and the LC_{90} value was 7,885%. At the lowest concentration (0,5%) the LT_{50} and LT_{90} values were 3,292 hours and 6,970 hours, respectively, while at the highest concentration (10%), both values <1 hours. There was a linear relationship between the concentration of the tested extract and larval mortality. The 1:1 methanolic extract combination of lemongrass stems and clove leaves proved effective as a biolarvicide against Ae. aegypti as indicated by the LC_{50}, LC_{90}, LT_{50}, and LT_{90} values.

Keywords : *DHF, Aedes aegypti, biolarvicide, Cymbopogon citratus, Syzygium aromaticum L.*

I. INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is a global health problem that commonly occurs in tropical regoins[1]. The *World Health Organization* (WHO) reported that 2023 recorded the highest number of DHF cases in history, with over 6.5 million cases and more than 7,300 deaths worldwide. Asia remains the region with the highest dengue incidence[2]. In Indonesia, which is endemic to mosquito-borne diseases, more than 457 mosquito species have been identified, and the number of DHF cases continues to increase[2]. The increasing population density and rapid urbanization have contributed to the spread and potential outbreaks of DHF[1].The dengue virus is transmitted to humans through the bites of *Ae. aegypti*, *Ae. albopictus*, and several other mosquito species[3]. Currently, no specific antiviral treatment or fully effective vaccine is available, making vector control the main preventive measure. Vektor control can be carried out chemically and non-chemically[4]. Chemical control typically involves larvicides and repellents, while non-chemical control includes community based programs such as “3M Plus” (draining, covering, and recycling) and the “G1R1J” movement[5]. Chemical insecticides remain the most widely used method in mosquito control programs due to their effectiveness and easy application[6]. Temephos an organophosphate larvicide, is commonly used but poses risks of toxicity, environmental contamination, and insecticide resistance when used excessively[7, 8]. Several studies have reported Ae. aegypti resistance to various synthetic insecticides in Indonesia[9].To reduce these negative impacts, researchers have developed environmentally friendly alternatives such as plant-based biolarvicides.

Natural larvicides are considered safer because they are biodegradable and leave no harmful residues[10]. They work by disrupting the life cycle of Ae. aegypti larvae before they develop into adults[11]. One of the plants with potential larvicidal activity is lemongrass (*Cymbopogon citratus*), which contains alkaloids, tannins, saponins, and flavonoids[12]. Another is clove (*Syzygium aromaticum L.*), which belongs to the *Myrtaceae* family and contains eugenol, alkaloids, flavonoids, and saponins[13]. Clove leaf extract at

concentrations of 0.008–0.01% has been shown to cause up to 100% larval mortality[13].This study examines the effectiveness of a 1:1 methanolic extract combination of lemongrass stems and clove leaves as a natural larvicide against Ae. aegypti. These plants were selected due to their availability, affordability, and previously demonstrated larvicidal potential. Methanol was chosen as the extraction solvent because methanol produced a higher flavonoid yield (9.25 mg QE/g) compared to ethanol[14]. Previous others research indicated that lemongrass and clove extracts showed larvicidal activity comparable to temephos, both individually and in combination[6]. Another study found that a 6% clove leaf extract concentration exhibited the highest larvicidal effect[15]. Therefore, this study aims to strengthen empirical evidence and propose a sustainable, plant-based alternative for dengue vector control.

II. METHODS

This study used an experimental laboratory method with a longitudinal prospective design. The research aimed to test the larvicidal activity of a 1:1 methanolic extract combination of lemongrass (*Cymbopogon citratus*) stems and clove (*Syzygium aromaticum L.*) leaves against *Ae. aegypti* larvae. The test subjects were *Ae. aegypti* third-instar larvae obtained from a controlled breeding colony maintained in the entomology laboratory. The test materials consisted of methanolic extracts of lemongrass stems and clove leaves prepared using the maceration method. Methanol was selected as the solvent due to its polarity and high extraction efficiency for flavonoid compounds. The extracts were then combined in a 1:1 ratio to form the test solution.

Seven concentrations of the extract combination were prepared 0,5%; 0,75%; 1,5%; 3%; 5%; 7%; and 10%. Each concentration was tested on 10 *Ae. aegypti* third-instar larvae with four replications. A control group using distilled water was included. Larval mortality was observed at hourly intervals for 12 hours. Dead larvae were identified by their immobility and lack of response to mechanical stimulation.The number of dead larvae was recorded at each observation period to determine the mortality rate.Data were analyzed using Probit analysis to determine the LC_{50} and LC_{90} (Lethal Concentration) values, as well as the LT_{50} and LT_{90} (Lethal Time) values. LC_{50} and LC_{90} represent the concentration required to kill 50% and 90% of larvae, respectively, while LT_{50} and LT_{90} represent the time required to reach the same mortality levels. Statistical analysis was conducted using SPSS software.

III. RESULT AND DISCUSSION

The observation results of *Ae. aegypti* larval mortality after treatment with the 1:1 methanolic extract combination of lemongrass (*Cymbopogon citratus*) stems and clove (*Syzygium aromaticum L.*) leaves are shown in **Table 1**. In the first hour, the highest mortality occurred at the 10% concentration with an average of 9.75 larvae (97.5%), while the lowest mortality was found at 0.5% with an average of 3 larvae (30%). At hours 9 to 12, all concentrations reached 100% larval mortality.The relationship between extract concentration and larval mortality is shown in **Figure 1**, indicating that mortality increased proportionally with concentration. The probit analysis showed a significant effect of extract concentration on larval mortality (coefficient 0,241; sig. p=0.000). The LC_{50} and LC_{90} values were 0.0385% and 7.885%, respectively on **Table 2**, indicating high larvicidal effectiveness at low concentrations.The time analysis also showed a significant relationship between exposure time and larval mortality (coefficient 0.348; sig. p=0.000). The LT_{50} and LT_{90} values at 0.5% concentration were 3.292 hours and 6.970 hours, while at higher concentrations (10%), mortality occurred faster, even <1 hour observation was completed (**Table 3**). Overall, higher extract concentrations resulted in shorter larval mortality times.

Table 1. Percentage of Ae. aegypti Larval Mortality at 0.5%; 0.75%; 1.5%; 3%; 5%; 7%; and 10% Concentrations After Exposure to the 1:1 Methanolic Extract Combination of Lemongrass (*Cymbopogon citartus*) Stems and Clove (*Syzygium aromaticum L.*)

Time (Hour)	Percentage of Dead Larvae at the Concentration of the Methanolic Extract Combination of Lemongrass (*Cymbopogon citratus*) Stems and Clove (*Syzygium aromaticum L.*) Leaves (%)						
	0.5%	0.75%	1.5%	3%	5%	7%	10%
1	30	50	72.5	80	82.5	92.5	97.5
2	35	55	72.5	80	87.5	97.5	100

3	55	57.5	77.5	85	92.5	100	100
4	60	77.5	92.5	95	97.5	100	100
5	62.5	85	100	97.5	100	100	100
6	72.5	92.5	100	100	100	100	100
7	82.5	100	100	100	100	100	100
8	97.5	100	100	100	100	100	100
9	100	100	100	100	100	100	100
10	100	100	100	100	100	100	100
11	100	100	100	100	100	100	100
12	100	100	100	100	100	100	100

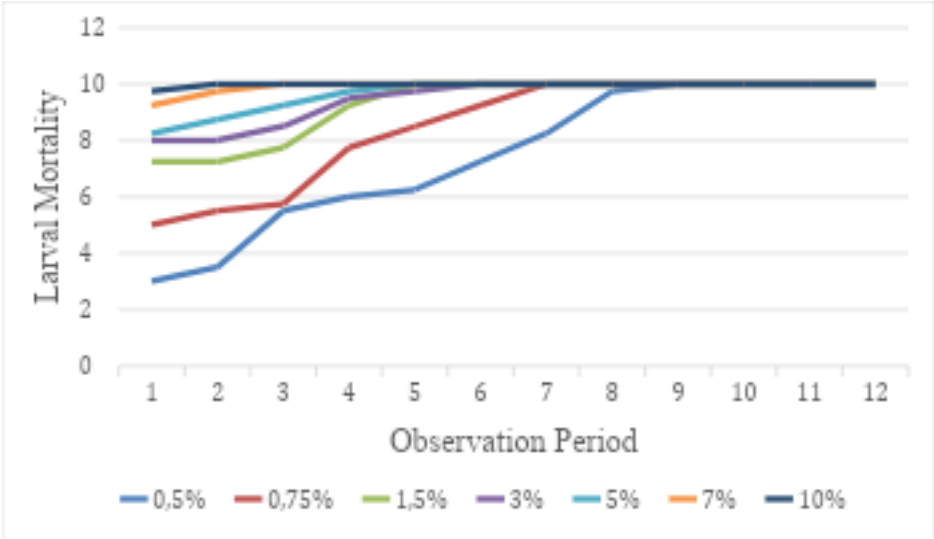

Fig 1. Comparison Chart of *Ae. aegypti* Larval Mortality at 0.5%; 0.75%; 1.5%; 3%; 5%; 7%; and 10% Concentrations after Exposure to the 1:1 Methanolic Extract Combination of Lemongrass (*Cymbopogon citratus*) Stems and Clove (*Syzygium aromaticum L.*) Leaves Over 12 Hours

Table 2. LC_{50} and LC_{90} Probit Analysis of the 1:1 Methanolic Extract Combination of Lemongrass (*Cymbopogon citratus*) and Clove (*Syzygium aromaticum L.*)

M (%)	LC_x	LC (%)	Confidence Interval	
			Lower	Upper
50	-3.257	0.0385	-9.753 (0.0000581)	-1.300 (0.273)
90	2.065	7.885	1.014 (2.757)	3.405 (30.114)

Table 3. LT_{50} and LT_{90} Probit Analysis at Different Concentrations of the 1:1 Methanolic Extract Combination of Lemongrass (*Cymbopogon citratus*) and Clove (*Syzygium aromaticum L.*)

Concentration	M(%)	LT_x	Confidence Interval	
			Lower	Upper
0.5%	50	3.292	2.300	4.153
	90	6.970	6.015	8.264
0.75%	50	1.649	0.419	2.645
	90	5.326	4.329	6.561
1.5%	50	-0.189	-1.846	1.089
	90	3.489	2.272	4.798
3%	50	-0.758	-2.598	0.647
	90	2.920	1.577	4.299
5%	50	-1.612	-3.766	0.031
	90	2.066	0.456	3.636
7%	50	-3.664	-7.002	-1.039
	90	0.014	-2.757	2.544
10%	50	-4.610	-8.989	-1.050
	90	-0.932	-4.777	2.565

Discussion

As shown in **Table 1**, larval mortality of Ae. aegypti increased proportionally with extract concentration, indicating a linear relationship between concentration and mortality rate. The 1:1 methanolic extract combination of lemongrass (*Cymbopogon citratus*) stems and clove (*Syzygium aromaticum L.*) leaves demonstrated strong larvicidal activity. The probit analysis results presented in **Table 2** show LC_{50} and LC_{90} values of 0,0385% and 7,885%, respectively, confirming high larvicidal potential. These findings differ from those reported, who found an LC_{50} of 0,412% for *Cymbopogon citratus* extract on Culex sp. larvae[16]. Compared to the single extract, the combined extract demonstrated higher effectiveness[16]. The study also reported an LC_{50} value of 0,005% for clove extract, indicating strong toxicity potential[13]. Differences in LC values between studies may be attributed to variations in plant origin, harvesting period, storage methods, and larval biological conditions[17].The probit analysis of LT values (**Table 3**) revealed that higher concentrations shortened the time required for larval mortality. At the lowest concentration (0.5%), LT_{50} and LT_{90} were 3,292 and 6,970 hours, respectively. As concentration increased, both LT_{50} and LT_{90} values decreased sharply, reaching <1 hour at 10%. **Figure 1** visually supports this pattern, showing that nearly all larvae died within the first hour at higher concentrations. These results suggest a strong dose-dependent effect and faster larvicidal action at higher concentrations. This aligns with findings, who also reported decreasing LT values with increasing concentrations [18, 19].The high larvicidal activity observed may be due to the synergistic effect of active compounds present in both plants. Lemongrass contains citronellal, geraniol, flavonoids, saponins, and tannins, which cause dehydration, disrupt digestive cell integrity, and affect the nervous system of larvae[16].

Clove leaves contain eugenol, flavonoids, alkaloids, and terpenoids that act as neurotoxins and stomach poisons, leading to paralysis and death[13, 20]. These active metabolites may work synergistically, increasing toxicity through mechanisms such as enzyme inhibition, respiratory disturbance, and neuromuscular interference[21].Compared to temephos, a standard synthetic larvicide recommended by WHO, the combined extract showed relatively competitive activity. Temephos has LC_{50} and LC_{90} values of 0,004 and 0,010, with LT_{50} and LT_{90} of 12,283 and 20,140 hours, respectively[22]. Although temephos remains more potent, its continuous use may cause resistance and environmental contamination. Therefore, the methanolic extract combination offers a safer and environmentally friendly alternative biolarvicide.Larval mortality may also be affected by intrinsic and extrinsic factors. Intrinsic factors include larval resistance and age, while extrinsic factors are related to environmental variables such as temperature, pH, humidity, and light[23, 24]. In addition, solvent type influences the extraction of active compounds. Methanol, with a higher dielectric constant (33,62) than ethanol (24,3), dissolves more polar compounds like saponins and flavonoids, resulting in higher larvicidal potency[25, 26].Overall, the 1:1 methanolic extract combination of lemongrass and clove shows promising potential as a plant based biolarvicide. Based on the LC value classification, with LC_{50} and LC_{90} values below 10%, the extract is categorized as “toxic,” demonstrating strong larvicidal capability[20]. This plant based combination supports environmentally sustainable vector control programs and aligns with the One Health approach[27]. Future studies should focus on formulation optimization and field application to enhance its practical use.

IV. CONCLUSION

There is a linear relationship between the concentration of the 1:1 methanolic extract combination of lemongrass (*Cymbopogon citratus*) stems and clove (*Syzygium aromaticum L.*) leaves and larval mortality, indicating that higher extract concentrations result in greater larval death rates.The 1:1 methanolic extract combination of lemongrass stems and clove leaves proved effective as a larvicide against Ae. aegypti, as demonstrated by the obtained LC_{50}, LC_{90}, LT_{50}, LT_{90} values.The Lethal Consentration (LC_{50} and LC_{90}) values were 0.0385% and 7.885% respectively. The Lethal Time (LT_{50} and LT_{90}) values at the lowest concentration (0.5%) were 3.292 hours and 6.970 hours, while at the highest concentration (10%) both LT_{50} and LT_{90} values <1 hour.

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