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The Effect of Lemongrass Leaves and Stalks Extracts using Methanol as The Eco-friendly Larvicides on Fourth Instar *Aedes aegypti* Larvae

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Abstract

BACKGROUND: *Aedes aegypti* is one of the most vicious mosquitoes, known for its role in several deadly diseases, including dengue fever and Zika. Several strategies have been developed over decades to prevent vector-borne diseases; one of them is insecticide to control the mosquito population. However, this strategy would not last long due to the elevation of resistance, environmental problem, and some issues regarding human health. Natural products have become major options to combat the glitches. Lemongrass (*Cymbopogon citratus*) has known for its toxic effect on mosquito larva.

AIM: The objective of this study was to assess the effect of *C. citratus* extract against fourth instar of *A. aegypti* larvae.

METHODS: Three extract concentrations, 2.5%, 5%, and 7.5%, were used. The larvicide activity was evaluated at 2, 4, and 6 h. Our data indicated that all concentrations contributed to increase larvae mortality.

RESULTS: Lethal Concentration (LC) 50 and LC90 were achieved at 2.5% and 4.1% of extract, respectively.

CONCLUSION: *C. citratus* extract has a larvicide activity against fourth instar *of A. aegypti.*

Introduction

Mosquitoes have been known for hundreds of years as the crucial arthropod vectors for pathogen transmission, which cause fatal diseases to millions of people worldwide. Malaria, dengue fever, Zika, and filariasis are some of the threads of public health transmitted through mosquito bites. *Aedes aegypti* has been known as the dengue vector, yellow fever, and chikungunya and is extensively found in tropical and subtropical regions [1]. Dengue fever is a re-emerging disease in most tropical areas, posing nearly a third of the human population at risk of infection [2]. In Indonesia, dengue fever incident is still high in ratio, especially in the rainy season. Since 1948, Indonesia has experienced a 700-fold increase in incidence over 45 years [3]. Thus, the research of vector control in Indonesia is definitively vital.

One of the features of mosquitoes' life cycles is the immature aquatic stage. This phase is essential in mosquito control strategy using larvicidal. Various synthetic larvicidal kinds are already used in the trial, ranging from direct applications to insect growth regulators [4]. However, there are several threads using synthetic insecticides, such as environmental pollution and insect resistance [5], [6]. Natural insecticides are more sustainable and have been used widely as the latest option for insect control. For decades, the interest to develop natural-environmentally friendly insecticides has significantly increased, followed by the development of bio gradable, cheap, and safe for humans [7]. Many studies have resulted in insect repellents derived from plant extracts, such as *Eucalyptus citriodora (E. citriodora), Cymbopogon nardus, Cymbopogon citratus (C. citratus), Curcuma longa, Pogostemon cablin (P. cablin)*, *and some more,* have been proven its efficiency against *Aedes* spp., *Culex* spp. and *Anopheles* spp [8], [9], [10], [11].

Lemongrass *(C. citratus)* has known for its toxic effect on mosquito larvae. This effect relates to the citronella chemical compound inside the stalk and leaves, which reaches 35% and becomes the densest chemical compound inside lemongrass. Citronella

affects the mosquito through direct contact. The mosquito in contact with citronella would lose its body fluid and die [12]. *C. citratus* has no effect on first instar larvae but did inhibit growth and increase mortality in *A. aegypti's* later developmental stages [13]. In this study, lemongrass leaves were extracted using methanol. The extracts were tested against fourth instar *A. aegypti* larvae to observe its larvicide effect.

Methods

Study setting

All treatments were replicated 4 times.

Larvae

The laboratory F-21 strain was obtained from the Faculty of Veterinary Universitas Syiah Kuala, Banda Aceh. The fourth instar larvae were reared in plastic containers containing a sterilized diet (40 mesh chick chow powder/yeast, 80:20).

Extraction of C. citratus extract

Leaves and stalks of *C. citratus* (3 kg), obtained from a traditional Banda Aceh market, were crushed and extracted twice using 90% methanol (10 L) at room temperature then filtered after 24 h. The extracts then were concentrated to dryness using a rotary evaporator at 50°C. The result was 30 ml of pure extract; then, the extract was diluted using aquadest and stored at 4°C.

Bioassay

Concentrations of the extracts, 2.5%, 5%, and 7.5%, were prepared by serial dilution of a stock extract. In each plastic container, 40 early fourth-instar larvae of *A. aegypti* were placed into a with each test solution (50 ml). Negative controls received the aquadest only and positive control groups received abate powder with three different concentrations (2.5%, 5%, and 7.5%). Treated and control larvae were detained at the same conditions. The larvicidal activity was evaluated 3 times in 2, 4, and 6 h after treatment. Larvae were defined dead if the appendages did not move when prodded with a wooden dowel. No mortality was detected in the negative control group.

Statistical analysis

The percentage of mortality of each group was compared using analyze variance test.

Results

Methanolic extract of *C. citratus* had larvicidal activity. The mortality rate was observed every time frame used: 2, 4, and 6 h after treatment (Table 1). It induced 100% mortality at 7.5% extract concentration, 95% at 5%, dan 55% at 2.5% extract concentration (Figure 1).

Table 1: Mortality Rate of Fourth Instar *A. aegypti* Larvae per hours after *C. citratus* Extract Treatment

C. citratus Concentrate (%)	Experiment Number	Time			Mortality (n)	Percentage
		2 h	4 h	6 h		
2.5	1	1	3	3	22	55
	2	0	2	3		
	3	1	2	3		
	4	0	1-	3		
5	1	1	5	4	38	95
	2	2	4	4		
	3	2	4	3		
	4	2	3	3		
7.5	1	9	1	0	40	100
	2	9	1	0		
	3	7	2	1		
	4	8	2	0		

A. aegypti: Aedes aegypti, C. citratus: Cymbopogon citratus.

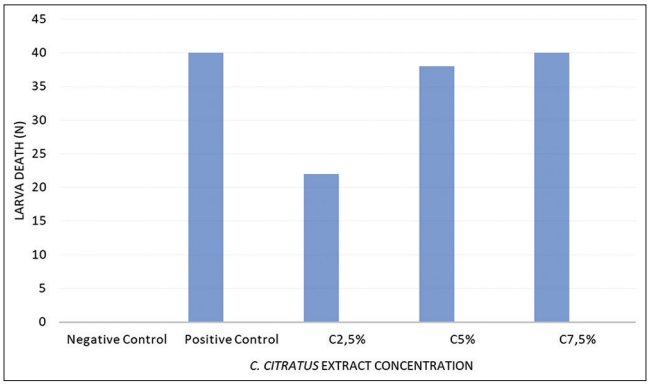

Figure 1: The effect of Cymbopogon citratus extract on mortality of fourth instar Aedes aegypti larvae

The lethal concentration (LC) 50 and LC90 achieved at a concentration of 2.5% and 4.1% of lemongrass extract, respectively. Lemongrass extract of *C. citratus* at a concentration of 2.5% and 4.1% were effective as a larvicide.

Discussion

Our study showed that *C. citratus* had significant larvicidal activity against the fourth-instar larvae of *A. aegypti*. Since *C. citratus* is eco-friendly and may serve as a suitable alternative to synthetic larvicide, as it is safe, low-priced, and widely available in Indonesia. Furthermore, this information might initiate the broader screening of the compounds responsible for different bioactivities and assess their molecular mechanism of action.

In a previous study, *C. citratus* oil was effective against the immature stages of *A. aegypti* and the larvicidal properties were reported in LC50, means the mortality of larvae reach 50% after treatment compared with control group [14]. *C. citratus* has five major compounds: citral (14.4%), citronellal (15.1%), eucalyptol (4.7%), limonene (13.1%), and myrcene (27.5%) [14], [15].

One of the crucial compounds is citronella, which has larvicidal properties and can be used as a repellant in essential oil form [15]. A study using the citronella extract found that the LC50 on *Anopheles* mosquito larvae was 2.590 ppm (0.2590%) and LC90 at 111.123 ppm (11.11%) [16]. Another study demonstrated that *C. citratus* exhibited LC50 at 69 ppm (0.0069%) on third and fourth *A. aegypti* larvae [17]. It might be related directly to the larvicidal effect of *C. citratus*. Other compounds might have similar or better effects on larvicide, as well as the combination of the major compounds. The variability of the concentrations among studies might depend on the method and part of the plant used for the experiment.

Cavalcanti *et al*. (1991) conveyed that *C. citratus* caused significant growth inhibition and mortality in the later developmental stages of *A. aegypti* [17]. Further experiments are warranted to determine the major compound fraction of *C. citratus* as the larvicide.

Conclusion

This study demonstrated the larvicidal potential of the lemongrass extracts (C. citratus) against the dengue vector A. aegypti. The results showed that C. citratus extract has a larvicide activity against fourth instar of A. aegypti. This study is significant to develop eco-friendly larvicide from C. citratus to curb mosquito larva which can provide an alternate to synthetic chemical insecticides.

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