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Original Article

Effectiveness of Exterminator Anopheles Spp Larvae from Clove Leaf Waste (Syzygium Aromaticum)

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ABSTRACT

Objective: The current reliance on chemical vector control methods raises concerns about their long-term environmental impact. To address these concerns, we need to explore alternative vector control strategies that are environmentally friendly, effective, efficient, and safe for human health. One promising approach involves the use of plant essential oils. This study aims to determine the effectiveness of residual waste clove leaf oil (*Syzygium aromaticum*) as an exterminator of *Anopheles sp*. larvae

Methods: This study used experimental methods carried out at the Entomology Laboratory of Donggala Health Research and Development Institute. It used third-instar *Anopheles sp.* larvae obtained from the field. The material used is clove leaf oil, which is a by-product of the distillation of clove leaves. The solution used to kill *Anopheles sp.* larvae uses five different concentrations, specifically 0.006%, 0.007%, 0.008%, 0.009%, and 0.01%.

Results: This study shows the ability of waste clove leaf oil (*Syzygium aromaticum*) to eradicate *Anopheles sp.* larvae with lethal power LC50 at a concentration of 0.005%. The study found that the number of *Anopheles sp.* larvae that died varied significantly depending on the concentration of the solution. The difference was statistically significant ($P < 0.05$).

Conclusion: Waste clove leaf oil (*Syzygium aromaticum*) can be used as an ingredient to eradicate *Anopheles sp.* larvae.

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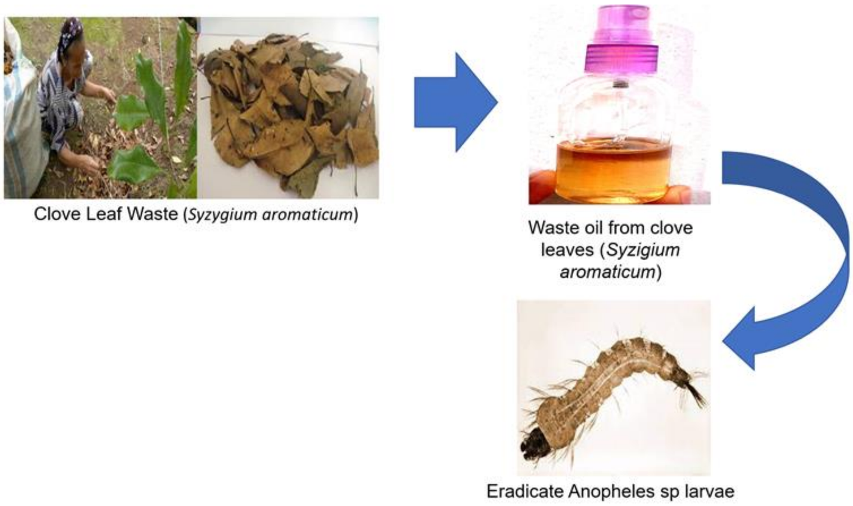
GRAPHICAL ABSTRACT



Introduction

Vector is a threat to global public health [1, 2]. One of the highly contagious and fast-spreading animal vectors is the mosquito [3]. The global burden of vector-borne diseases, especially malaria, is 429,000 deaths per year and 212 million cases per year [4]. The incidence of malaria cases (cases per 1,000 population at risk) decreased from 80 (238 million cases) in 2000 to 57 (229 million cases) in 2019. Meanwhile, the death rate (deaths per 100,000 population at risk) decreased from 25 (736,000 deaths). in 2000 to 10 (409,000 deaths) in 2019 [5].

Indonesia every year, several hundred thousand infections and around 2,000 deaths occur in Indonesia due to malaria. Progress has been made in eradicating malaria in the western part of the country, but has stalled in Papua province, which accounts for 74% of the cases reported each year. Control and eradication of malaria in collaboration with the Ministry of Health, the National Malaria Control Program (NMCP), the United Nations Children's Fund (UNICEF), and the World Health Organization (WHO) [6].

The control of malaria vectors frequently carried out until now still focuses on control using chemicals, for example controlling the egg and larval stages through killing techniques, while the control of mosquito vectors adults is carried out using nebulization techniques [7, 8]. Continuous use of chemicals can harm living things, including humans, animals, plants, and the environment. Apart from that, chemical control apparently has created resistance to mosquitoes and requires a lot of energy and costs [7, 8]. Concerning the dangers of using chemicals to control mosquito vectors, methods to control mosquito vectors should use controls made from natural extracts or essential oils of plants that are more environmentally friendly and safe for survival of humans, animals, plants and the surrounding environment [9]. The compounds contained in plants can be an alternative in the control of mosquito vectors in both the larval and adult phases [10, 11].

Indonesia, as a country rich in plant species, makes it easier for experts to conduct research on the use of natural ingredients to control mosquito vectors. The active compounds contained in various types of plants are toxic to insects in general and to mosquitoes in particular because they can inhibit their metabolic, biochemical and physiological processes [12]. Clove (*Syzygium aromaticum*) holds promise as a natural mosquito vector control agent due to its rich composition of active compounds, including eugenol (87.24%), eugenyl acetate (5.8%), b-caryophyllene (3.85%), α-cadinol (2.43%), myrcene (1.84%), and methyl eugenol (1.8%).

These compounds exhibit toxicity, effectively eliminating mosquito vectors [13-15]. This study aims to determine the effectiveness of residual waste clove leaf oil (*Syzygium aromaticum*) as an exterminator of *Anopheles sp.* larvae.

Materials and Methods

This study was an experimental study conducted in the laboratory using third-stage *Anopheles sp.* mosquito larvae collected from the field. The laboratory observation and testing methods for bio larvicides were in accordance with the WHO Guidelines for Larvicide Standards [16].

Preparation of used clove leaf oil

Waste clove leaf oil is obtained from clove farmers who have oil refining factories. This distillation process is usually carried out by placing dried clove leaves in a large oven and then heating them for 8 hours and then taking the distillate that comes out of combustion.

Collection of anopheles sp. larvae

The larvae collection process is carried out by taking larvae that are available in the field, taking into account that for *Anopheles* type mosquitoes it is very difficult to carry out the breeding/reproduction process because they are normally only capable of surviving until the first offspring/filial because when entering the second phase of offspring, many usually die.

Apart from that, the place where the larvae are collected must be close to the laboratory where the study is carried out because they die very easily if they are moved a long distance. The larvae used are *Anopheles sp.* larvae in stage III or early stage IV.

Biolarvicide tests

This bioassay test was carried out at the Entomology Laboratory of Donggala Health Research and Development Institute. The biolarvicide testing process uses the WHO Guidelines for Standard Laboratory Observation and Testing of Larvicides. The concentration of waste clove leaf oil (*Syzygium aromaticum*) used is the concentration that has been carried out in the preliminary tests, specifically 0.006%, 0.007%, and 0.008%, concentration of 0.009% and 0.01%. This test was carried out for 24 hours with 4 repetitions to determine the level of larval death using 25 test larvae in each observation bowl. In this study, a control was also used where the test vessel was given only water without adding waste clove leaf oil (*Syzygium aromaticum*).

Data analysis

The larval mortality data was analyzed following standard statistical guidelines. The mortality criteria for larval susceptibility were established as follows [16]: a) Mortality > 98% (vulnerable species); b) Mortality of 80-98% (a tolerant species); and c) Mortality < 80% (a resistant species). The Probit test was employed to analyse the test outcomes of deceased *Anopheles sp.* larvae for determining the LC50 value, and the Kruskal-Wallis test was conducted utilizing the SPSS software.

Results and Discussion

Table 1 and Figure 1 shows that the mortality rate of *Anopheles sp.* mosquito larvae increases with increasing concentrations of waste clove leaf oil (Syzygium aromaticum). At a concentration of 0.006%, the mortality rate was 84%, while at a concentration of 0.01%, the mortality rate was 100%. This suggests that waste clove leaf oil is a potential larvicide for *Anopheles sp.* mosquitoes. The probit test results on the mortality rate of *Anopheles sp.* larvae using waste clove leaf oil (*Syzygium aromaticum*) obtained an LC_{50} value of 0.005%, which means that waste clove leaf oil (*Syzygium aromaticum*) can mortality 50% of *Anopheles sp*. from a concentration of 0,005%.

Meanwhile, in the Kruskal Wallis test, P = 0.001 ($P < 0.05$) (Table 2), it can be concluded that there is a significant difference in the number of deaths of *Anopheles sp.* larvae between concentrations.

Table 1: The mortality rate of *Anopheles sp* larvae after 24 hours of exposure

Concentration (%)	Number of Test (Larvae)	Replications				Larval Mortality Rate (Larvae)	Larval Mortality Percentage (%)	Criteria
		1	2	3	4			
0	25	0	0	0	0	0	0	Control
0,006	25	22	22	20	20	21	84	Effective (Tolerant)
0,007	25	22	22	20	20	21	84	Effective (Tolerant)
0,008	25	22	23	22	25	23	92	Effective (Tolerant)
0,009	25	23	22	24	25	23,5	94	Effective (Tolerant)
0,010	25	25	25	25	25	25	100	Effective (Vulnerable)

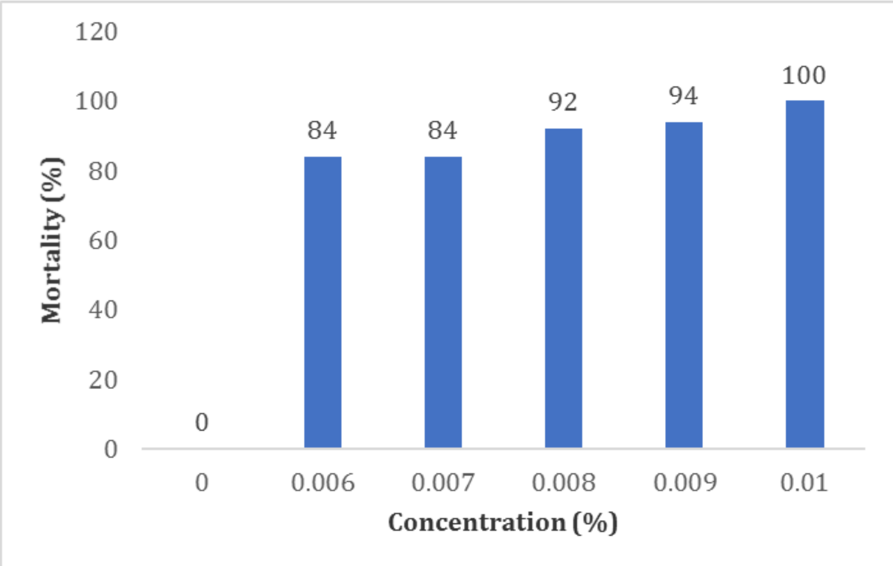


Figure 1: Mortality percentage of *Anopheles sp* larvae for 24 hours

Table 2: Kruskall wallis test results *Anopheles sp* Larvae

Concentration (%)	*Anopheles sp*					*P*-value
	Mean	SD	Median	Min	Max	
0	0.00	0.00	0.00	0.00	0.00	0.001
0.006	21.00	1.15	21.00	20.00	22.00	
0.007	21.00	1.15	21.00	20.00	22.00	
0.008	23.00	1.41	22.50	22.00	25.00	
0.009	23.50	1.29	23.50	22.00	25.00	
0.010	25.00	0.00	25.00	25,00	25.00	

The test results showed that the oil concentration was directly proportional to the death rate of the larvae, meaning that the higher the concentration of waste clove leaf oil (*Syzygium aromaticum*), the higher the death rate, death of the larvae. The results of this research agree with other research on the ability of clove plants (*Syzygium aromaticum*) to kill Anopheles sp larvae. Eugenol from clove oil is capable of killing *Anopheles stephensi* larvae with LC_{50} (57,49 ppm) and LC_{90} (93.14 ppm) [17]. Clove essential oil (*Syzygium aromaticum*) has LC_{50} (17.527 µg/ml) against

Anopheles gambie **(18)**. Furthermore, the essential waste clove leaf oil (*Syzygium aromaticum*) has an effect on the mortality of *Anopheles aconitus* larvae with LC_{50} (54.145 ppm) [18]. Another research also found that *Syzygium aromaticum* essential oil could kill 86,96% of *Anopheles stephensi* larvae [17].

Essential oils naturally form a layer on top of water due to their lower density. This barrier prevents mosquito larvae from reaching the surface to breathe, leading to their suffocation and death [19]. This hypothesis is also a plausible explanation for the mortality of the test larvae, as the material is derived from petroleum, a known larvicidal substance. The primary factors contributing to larval death typically fall into two categories: internal and external. Internal factors encompass the larvae's innate ability or tolerance to resist the effects of the provided essential oil, while external factors revolve around the composition of active compounds present in waste clove leaf oil (Syzygium aromaticum). The toxicity or toxicity of an active ingredient is determined by variations in the type and amount of chemical composition it contains. The greater the variation and the higher the active compound content, the more it will influence the bioactivity of the larvae.

Eugenol is one of the active compounds of cloves (*Syzigium aromaticum*) which has a distinctive odor (special aroma) that has a high insecticidal content and antimicrobial properties, which is why it is widely used in the control of insect pests and pathogens [19,20]. Eugenol enters the body of the insect, and then attacks the respiratory system and nervous system and is a neurotoxin, contact poison and stomach poison that causes death in mosquitoes (insects) [20].

Conclusion

Waste clove leaf oil (Syzygium aromaticum) exhibits larvicidal activity against *Anopheles sp.* larvae, with an LC50 value of 0.005%. This implies that waste clove leaf oil (Syzygium aromaticum) can effectively eliminate 50% of *Anopheles sp.* larvae at a concentration of 0.005%. Moreover, a statistically significant difference in larval mortality was observed across the tested concentrations ($P < 0.05$).

These findings suggest that waste clove leaf oil (Syzygium aromaticum) holds promise as an effective and eco-friendly larvicidal agent for controlling *Anopheles sp.* mosquito populations.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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