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Effectiveness of Clove Oil (*Syzigium aromaticum*) as Biolarvacide of *Aedes aegypti*

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The vector control that is often done so far is by chemical control, which will continually have a negative impact. Therefore, it is necessary to control methods that are more friendly to the environment, effective and efficient, and safe for health, one of which is the use of essential oils from plants. Clove (Syzigium aromaticum) is one of the plants that can be used as an alternative larvicide to kill mosquito vectors because it contains many chemical compounds, including eugenol compounds, eugenol acetate, methyl eugenol, b-caryophyllene, methyl eugenol, saponins, flavonoids and larvicidal tannins. This study aims to determine the effectiveness of clove leaf waste oil (Syzygium) as a biolarvicide for the Aedes aegypti mosquito. The research method used is experimental with a Quasi-Experimental Design approach and Post-test Control Group Design. The object of this research is clove leaf waste oil (Syzigium aromaticum) that has been obtained from the distillation of dried clove leaf waste. The object of this research is the third-stage larvae and adult Aedes aegypti mosquitoes obtained by rearing or self-reproducing mosquitoes in the laboratory. The bioassay test method used a glass test vessel with a volume of 200 ml of solution consisting of a mixture of water (aquades) with clove leaf waste oil. This study used a treatment concentration of 0.006%; 0.007%; 0.008%; 0.009% and 0.01%. The control used aquades (0%). Then put 25 Aedes aegypti larvae in the test container and then observe the number of mortality larvae during 24 hours of observation. Results: The results showed that clove (Syzygium aromaticum) leaf waste oil was effective as a biolarvicide with LC50 at a concentration of 0.005% and Kruskal Wallis test value of 0.000 (P<0.05) which means that there is significant difference in the number of deaths of Aedes aegypti mosquito larvae at each concentration. Clove leaf waste oil (Syzygium aromaticum) is effective as a biolarvicide for Aedes aegypti mosquitoes.

Keywords: Aedes aegypti; Biolarvicides; Effectiveness; Syzygium aromaticum.

Vector disease is one of the diseases that pose a threat to public health worldwide[1,2]. Mosquitoes are an important and primary vector in the spread of disease and are highly contagious to humans[3].

Each year around 50 million cases of dengue occur and around 500,000 patients are hospitalized, predominantly children[4-6]. In 2019, regions of the Americas and 16 countries reported an increase in dengue cases at the national level, such

as Antigua and Barbuda, Argentina, Brazil, Chile, Colombia, El Salvador, Guatemala, Guadeloupe, Guyana, Honduras, Jamaica, Martinique, Mexico, Paraguay, San Martin, and Venezuela. 99,998 cases of dengue were reported (incidence rate of 10.2 per 100,000 population), including 28 deaths. Of the total number of reported cases, 25,333 were examined in the laboratory and 632 were categorized as severe cases of dengue (0.63%)[7,8]. Overall, all regions of Indonesia have high dengue cases (incidence rate of 78.0) and an increase in dengue cases spreading to cities and regencies in 34 provinces of Indonesia[9].

Vector mosquito control can be carried out mechanically, physically, biologically and environmentally, both in developing mosquitoes and in adult mosquitoes [(10)(11)(12)]. Today, the control that is frequently carried out is chemical control using chemicals, including organophosphorus, organochlorine, carbamate and pyrethroid group compounds. However, the continued use of these chemicals will have negative impacts, such as the death of non-target organisms, environmental contamination, and danger to public health. In addition, this method is also costly and may cause resistance in mosquitoes[10-12].

Various efforts have been made to prevent the emergence of resistance in mosquitoes to various chemicals. Therefore, it is necessary to control the methods, especially the insecticides that are more friendly to the environment, effective and efficient, and safe for health[13]. Biological control can be an alternative to mosquito vector control by reducing the mosquito vector population and using natural materials[14,15].

Natural larvicides/insecticides have been shown to make a significant contribution as a new alternative in an effort to reduce the number of diseases caused by mosquito vectors. The content of eugenol, alkaloids, flavonoids, saponins and other active principles of plants can be toxic for Aedes aegypti larvae. Plant essential oils can interfere with the metabolic, biochemical, physiological and behavioral processes of insects[16].

Clove plants (*Syzigium aromaticum*), which are widely cultivated in Indonesia, have potential to be used as alternative larvicides/ insecticides to kill vector mosquitoes because they contain many chemical compounds reaching up to 40 chemical compounds, including eugenol compounds (87.24%), eugenyl acetate (5.8%), b-caryophyllene (3.85%), á-cadinol(2.43%), myrcene (1.84%), methyl eugenol (1.8%)[17-22].

Expert research shows that cloves (*Syzigium aromaticum*) have compounds that have the potential to be used as larvicides for *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes with mortality rates greater than 85%[18]. This study aims to determine the effectiveness of clove leaf waste oil (*Syzygium aromaticum*) as a bio larvicide for the *Aedes aegypti* mosquito.

MATERIALS AND METHODS

Types of Research

The research method used is experimental with a Quasi-Experimental Design approach and Post-test Control Group Design. This research was carried out for 3 months starting from the preparation of tools and materials including the process of rearing Aedes aegypti larvae and the process of making clove leaf waste oil (*Syzigium aromaticum*).

Acquisition of clove leaf waste oil

The clove leaf waste oil procurement process is carried out at the clove leaf waste oil refining factory located in Donggala regency, Central Sulawesi province. The distillation process requires 20 kg of dried clove leaf residue with a distillation time of 8 hours.

Collection of mosquito larvae

The process of collecting Aedes aegypti mosquito larvae is carried out by self-breeding (rearing) at the Donggala Health Research and Development Center Entomology Laboratory located in Labuan Village, Labuan Subdistrict, Donggala District, Central Sulawesi province. The stage used is stage III. The larvae used for the test should be normal and not exhibit a different body color from normal larvae. Larvae are classified as dead if they do not move after being touched with a sexy needle at the siphon (cervix), or continue to move but cannot reach the surface of the water or do not show a typical diving reaction when agitated Water.

Biolarvicide tests

Observation and laboratory test methods for biolarvicides refer to the WHO Standard Guidelines for Larvicide Testing[23] on mosquito larvicide testing. This test used 20 mg of abate

powder (themefos) as a positive control and 200 ml of aquadest as a negative control. The number of test larvae used was 25 stage III and they were placed in a container with a volume of 200 ml of solution. The treatment used a dose of 0.006%; 0.007%; 0.008%; 0.009% and 0.01% and observations were made for 24 hours with 4 replications.

Statistic analysis

The results of the larval susceptibility test were grouped with the following larval mortality criteria [20]:

a. Mortality > 98% indicates a vulnerable species
b. 80 – 98% mortality indicates a tolerant species
c. Mortality < 80% indicates resistant species

Data from all replicas must be collected for analysis. The results of the observation of dead larvae were analyzed by Probit analysis to determine the LC_{50} value and Kruskal Wallis analysis using the SPSS software.

Ethical approval

This study was approved by the Health Research Ethics Committee of the Faculty of Public Health, Hasanuddin University, Makassar, Indonesia. Number: 3106/UN4.14.1/TP.02.02/2021.

RESULTS

The results of testing the mortality rate (mortality) of *Aedes aegypti* larvae during 24 hours of observation can be seen in the following table and graph.

The table and graph above shows that the average mortality rate of Aedes aegypti mosquito larvae using clove leaf oil (*Syzigium aromaticum*) at a concentration of 0.006% was 77% (19.3 larvae), at a concentration of 0.007% it was 76% (19 larvae). Whereas at a concentration of 0.008% to 0.01%, all larvae tested experienced 100% kill.

The results of the probit analysis of the clove leaf (*Syzigium aromaticum*) used oil test on the mortality of Aedes aegypti mosquito larvae obtained an estimated LC_{50} value of 0.005%. Clove (*Syzigium aromaticum*) leaf waste oil can kill 50% of *Aedes aegypti* larvae from concentrations of 0.004% to 0.006%.

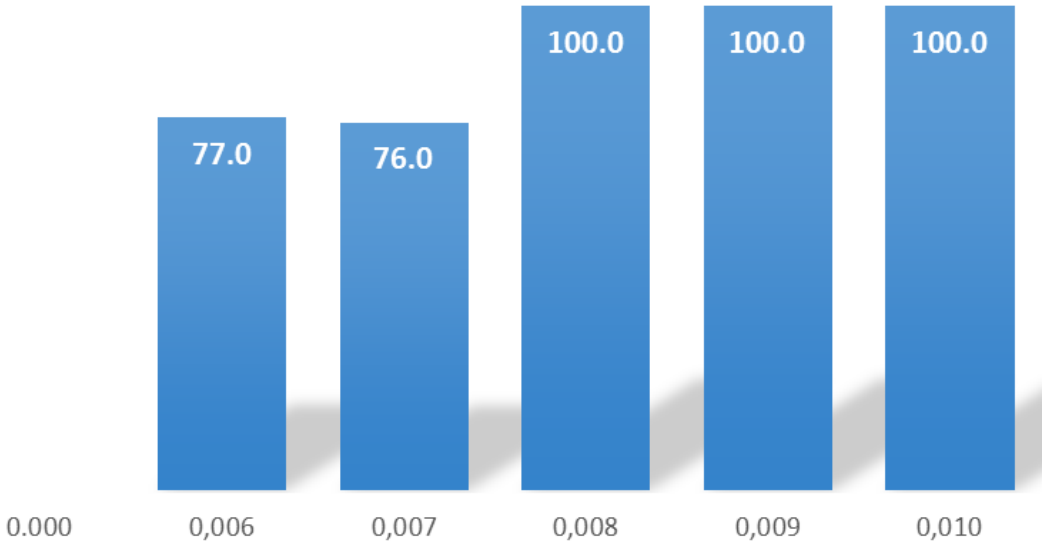
The value obtained in the Kruskal Wallis test was P = 0.000, (P <0.05), so it can be concluded that there was a significant difference between the number of deaths of *Aedes aegypti* larvae and an increase in the total concentration of clove leaf waste oil (*Syzigium aromaticum*).

Table 1. Mortality Percentage of *Aedes aegypti* Larvae for 24 hours

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	21	18	18	20	19,3	77	Effektive (Tolerant)
0,007	25	20	23	17	16	19	76	Effektive(Tolerant)
0,008	25	25	25	25	25	25	100	Effektive (vulnerable)
0,009	25	25	25	25	25	25	100	Effektive (vulnerable)
0,010	25	25	25	25	25	25	100	Effektive (vulnerable)

Table 2. Krusskall wallis test results *Aedes aegypti* larvae

Concentration (%)	*Aedes aegypti*					*P*
	Mean	SD	Median	Min	Maks	
0	0,00	0,00	0,00	0,00	0,00	0,000
0,006	19,25	1,50	19,00	18,00	21,00	
0,007	19,00	3,16	18,50	16,00	23,00	
0,008	25,00	0,00	25,00	25,00	25,00	
0,009	25,00	0,00	25,00	25,00	25,00	
0,010	25,00	0,00	25,00	25,00	25,00	



Graph 1. Mortality Percentage of *Aedes aegypti* Larvae for 24 hours

DISCUSSION

According to the results of laboratory research, clove leaf waste oil (*Syzigium aromaticum*) has the ability to biolarvicide against the larvae of the Aedes aegypti mosquito. The test results showed an increase in mortality (mortality) of Aedes aegypti mosquito larvae along with increasing concentrations.

This study showed that the mortality rate of 50 percent of Aedes aegypti larvae (LC_{50}) at a concentration of 0.005% so the results of this study were more effective compared to previous studies where other studies[15] stated that essential oil from *Syzigium aromaticum* had an LC_{50} at a concentration of 92.56 mg/ l (0.009256%) for *Aedes aegypti* larvae and LC_{50} at a concentration of 124.42 mg/l (0.012442%) for *Culex quinquefasciatus* larvae for 24 hours. Furthermore, the research[24] also found that the essential oil of *Syzigium aromaticum* can kill the larvae of *Anopheles stephensi* by 86.96%.

In principle, essential oils always float on the surface of the water (specific gravity lower than water) so they are effective as mosquito larvae larvicides, because mosquito larvae live in water and have the behavior of approaching or hang from the surface of the water to breathe[25].

The mortality of *Aedes aegypti* mosquito larvae is influenced by 2 factors, namely internal factors and external factors. Internal factors are the ability of the body of mosquito larvae or the resistance of the body to resist the influence of the given essential oil. While the external influence is the substance contained in the clove blade itself. The difference in the level of toxicity of a compound is determined by the chemical variation of the oil composition that will determine the bioactivity of the plant against mosquito larvae.

Clove (*Syzigium aromaticum*) has eugenol as its main component, which gives rise to a distinctive aroma. It has been reported to have high insecticidal and antimicrobial properties that have been included in many formulations to control insect pests and pathogens[25–27]. Eugenol acts by affecting the nervous system of insects and can act as a contact poison, a stomach poison, a respiratory poison, and a neurotoxin that can cause death in mosquitoes [26].

CONCLUSION

The results of this study indicate that clove (*Syzigium aromaticum*) leaf waste oil is effectively used as a biolarvicide for Aedes aegypti mosquito larvae. It is recommended that further research can examine the content of clove leaf waste oil to know which active substance can kill *Aedes aegypti* larvae.

Conflict of Interest

There are no conflict of interest.

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