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Bioassay and efficacy of clove flower stalk extract of Afo variety as a natural larvicidal

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Abstract

This study aims to test the larvicidal bioassay of clove stalk extract against *Aedes aegypti* on a laboratory scale and field scale efficacy test in a bath. Samples of sun-dried clove flower stalks were made into water extracts and coarse powder measuring 8-10 mesh for efficacy tests in the bath. The experimental method with a completely randomized design model was used to test the effectiveness of the larvicide, which consisted of two stages: The aqueous extract was tested against *Ae. aegypti* third instar larvae for three concentrations, namely 10%, 20%, and 30%, plus control treatment with three repetitions; observations were made at 30 minutes and 60 minutes after application to see the number of dead larvae as stage one. The second stage was the efficacy test in the bath which was carried out by inserting clove coarse powder in packs of 20 grams, 30 grams, and 40 grams, as well as the control treatment with five replicates, observed every day to see the time of emergence of mosquito larvae in a matter of days. The results of the first stage test showed an effect of the concentration of clove flower stalk extract on the mortality rate of the larvae, which was indicated by a positive relationship, where the mortality rate of the larvae reached 100% at 60 minutes after application. In comparison, the second stage test showed that there was an effect of clove flower stalk coarse powder on the time of larvae emergence, where the average time for larvae emergence was up to 8.8 days in the treatment of 40 g of clove flower stalk coarse powder, 1.83 times longer than the control (4 .8 days). Clove flower stalk extract is effective as a larvicidal mosquito *Ae. aegypti*.

Keywords: Larvicides; Cloves; *Aedes aegypti;* Cloves of Afo variety

1. Introduction

The *Aedes aegypti* mosquito is a type of mosquito that is dangerous because this mosquito can cause Dengue Hemorrhagic Fever which can attack humans and cause death. The number of dengue cases in Indonesia is constantly increasing. Data for 2020 in Indonesia shows that there are 11 provinces with a DHF CFR (*Case Fatality Rate*) of 1% -> 1%; namely: Maluku Province; Central Java; North Maluku; North Kalimantan; North Sulawesi; Riau; Southeast Sulawesi; South Kalimantan; East Nusa Tenggara; Central Sulawesi; and South Sulawesi. In North Maluku Province; the increase in DHF cases in 2017-2020 was 37 cases in 2017; increased sharply to 487 cases in 2018; and continued to increase to 520 points in 2021; with the highest number of cases occurring in Ternate City. The increase in the number of issues needs to be a concern; and one preventive measure is to prevent mosquito larvae's development into mosquitoes.

Eradication and prevention efforts continue to be carried out to eradicate mosquitoes; including 3M Plus efforts; namely draining; closing; reusing used waste with economic value plus maintaining mosquito larvae-eating fish; using mosquito repellent; installing wire netting on windows and ventilation; cleaning the environment; checking water reservoirs; putting used clothes in closed containers; giving larvicides to water reservoirs that are difficult to drain; repairing

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channels and gutters that are not smooth and planting mosquito repellent plants. The most crucial prevention carried out at this time is the larval stage; considering that this stage is the most influential phase for the presence of mosquitoes [1].

Clove (*Syzygium aromaticum* (L.) Merr & Perry) is a type of spice plant that is widely available in the North Maluku region and has the potential to a larvicidal [2]. Using chemicals for a long time can affect non-target organisms and endanger environmental health [3]. In addition; complaints about using Abate powder (Temephos) include bad smell; rust in the water storage drum; the possible impact of resistance to mosquitoes; and the environmental impact if used continuously [4].

Based on the potential of clove as larvicides and to minimize the impact arising from Abate products; this research is essential to be carried out as a preventive measure; especially for people in the archipelago in North Maluku. If these products can be marketed on a broader scale; they can provide economic value for clove farming communities

2. Material and methods

This type of research is experimental research that tests the bioassay of *Ae. aegypti* aqueous extract of clove flower stalks and field-scale efficacy tests tested in baths. The tools and materials used are 1). Tool a. Water bath b. Glass Stirrer c. Glass Pipette d. Plastic Pipette d. Measuring Cup e. Test glass capacity of 350 mL e. pH meter e. thermometer f. Mortar g. Sieve 8 – 10 mesh 2). Material a. Sun Dried Clove Flower Stalk 100 grams b. Water from PDAM 100 L c. Aquades 300 mL d. Pack of Tea Bags, e. *Ae. aegypti* mosquito larvae third instar 360 individuals (rearing procedure followed [5]. with some modifications).

Procedure for the bioassay test, namely 1. A bowl with a capacity of 350 ml containing 250 ml of test medium (distilled water and clove flower stalk extract solution) and 30 late third instar larvae of *Ae. aegypti* that is homogeneous (5 days old and 4-5 mm long) [6]. *Ae. aegypti* was exposed to the test concentration range of 10%, 20%, 30% and control and counted the number of larvae that died every 30th and 60th minutes. The efficacy test procedure was 1. A sample of 100 grams of sun-dried clove flower stalks was coarsely ground with a size of ± 8 - 10 mesh 2. The bathtub is filled with 1000 Liters of water 3. Coarse clove flower stalk powder is put into the dip pack for 20, 30, and 40 grams each 4. Each treatment is repeated five times to obtain 20 experimental units.

Data analysis. Bioassay test data and efficacy tests use analysis of diversity and if there are differences, proceed with the BNT test.

3. Results

3.1. Larvicidal Bioassay Test Results

Test results showed that at 30 and 60 minutes after treatment, the number of dead larvae increased with increasing treatment concentration levels (Table 1). Increased larval mortality indicated the effect of clove flower stem extract on *Ae. aegypti* Larva.

Table 1 Comparison Test Results of Treatment Means in the Clove Flower Stem Extract Bioassay Test on the Mortality of *Ae. aegypti* Mosquito Larvae

Extract Concentration (%)	Larval mortality (%)	
	30 minutes	60 minutes
0	0.00[a]	3.33[a]
10	20.00[a]	53.33[b]
20	80.00[b]	93.33[c]
30	96.67[b]	100.00[c]
BNT value 5%	34.38	18.83

Noted: The average value of the treatment with the same letter notation is not significantly different

3.2. Efficacy test data of Clove Flower Stalk Coarse Powder in Bathtub

Endurance tests were carried out every week, namely observing the first time the larvae appeared in the bath, after being filled with water with a volume of 100 liters. The testing phase was carried out to find out how long it took for the mosquito larvae to appear after application using clove clove flower stem coarse powder. Coarse clove flower stem powder is packed in a tea bag, weighing 10 g/pack. Packages of clove stalk coarse powder were placed in a bath filled with water according to the treatment, namely, 2 packages, 3 packages, 4 packages each for each tub, plus a treatment control, namely a tub that did not use clove stalk coarse powder.

Table 2 Observation Results of Larvicidal Resistance Test, Time of the emergence of larvae (days)

Test	Treatment (g)			
	0	**20**	**30**	**40**
1	4	7	8	8
2	5	6	8	9
3	5	6	7	9
4	4	7	7	8
5	6	6	8	10
Total Treatment	24	32	38	44
Average	4.80	6.40	7.60	8.80

The results of observations for the larvicidal resistance test of clove stalk coarse powder are presented in Table 2 and Figure 1. The analysis results showed a positive correlation between the addition of clove stalk coarse powder and the length of time for larvae to emerge. This shows that the clove flower stalk coarse powder is effective as a larvicidal.

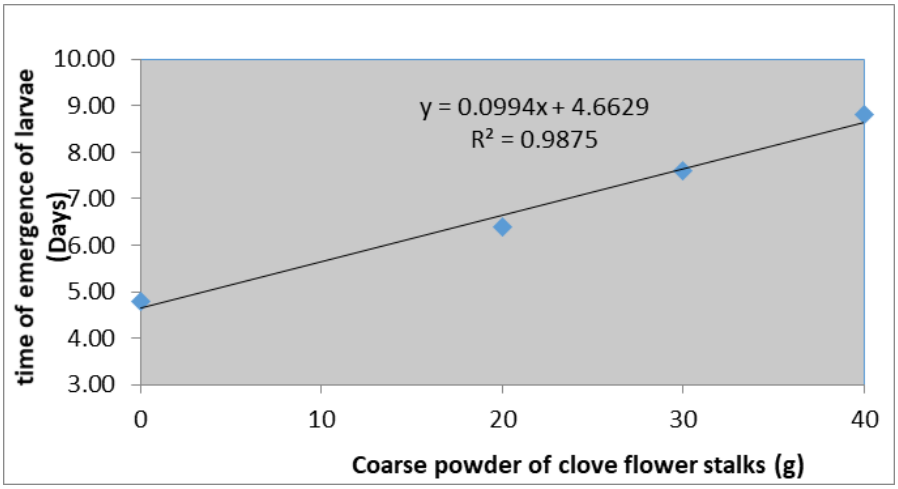

Figure 1 Relationship between the amount of clove stalk coarse powder and the time the larvae appeared

The results of the analysis of diversity showed that the treatment of clove flower stalks with coarse powder had a significant effect on the time of emergence of larvae (Table 3).

Ae. aegypti mosquito larvae will appear in the control bath in an average of 4.80 days, while for the bath with the treatment of clove, flower stem coarse powder in the 40 g treatment, it takes 8.80 days. This shows that the coarse clove flower stalk powder can inhibit the growth of *Ae. aegypti* mosquito larvae in the bath.

Table 3 Comparison test results of the mean treatment of clove flower stem coarse powder

Treatment (g)	Average Appearance of Larvae (days)
0	4.80[a]
20	6.0[b]
30	7.60[bc]
40	8.80[c]

Noted: The average value of the treatment with the same letter notation is not significantly different, the BNT value is 5% = 1.40

4. Discussions

The results of the bioassay test showed that there was an effect of clove flower stalk extract on the mortality of *Ae. aegypti*. This result is in line with the research of [7], who stated that the Zanzibar type of clover leaf is a larvicidal against *Ae. aegypti*. Afo cloves are a Zanzibar clove widely distributed in the Maluku and North Maluku islands. The presence of clover leaf compounds such as saponins, flavonoids, and tannins is thought to be able to act as a larvicidal through a mechanism of damaging cell membranes or interfering with larval metabolic processes [8].

The results of testing the efficacy of clove flower stalk powder in the bath showed an inhibition of the emergence time of mosquito larvae in the bath. This result is the same as that stated by [9], that the bathtub is generally the most preferred container for mosquitoes to lay their eggs. These results are similar to other studies in research from [10]. The type of landfill material that contains the most larvae is cement (86.7%), then iron (45.7%), and clay (40.0%). %), porcelain (9.5%), and plastic (7.4%). This indicates that the main breeding sites for *Ae. aegypti* are containers that are in or around houses or public places. This mosquito breeding place is a pool of water accommodated in a place or container. Whether there is *Ae. aegypti* in a container is influenced by several factors, including the type of container and container material [11].

It is suspected that the bathtub containing the coarse powder of clove flower stalks gave off a distinctive aroma of spices, making adult mosquitoes uninterested in laying their eggs on the tub wall. The average time required for larvae emergence was more than five days in treated water compared to the control, which was less than five days. This is proven following the research of [12], that the Aedes spp. The hatchability of Aedes spp eggs in regional PDAM media is better than in water originating from domestic waste.

5. Conclusion

An effect of the concentration of clove flower stalk extract on the mortality rate of the larvae was indicated by a positive relationship, where the mortality rate of the larvae reached 100% 60 minutes after application. In comparison, the second stage test showed that there was an effect of clove flower stalk coarse powder on the time of larvae emergence, where the average time for larvae emergence was up to 8.8 days in the treatment of 40 g of clove flower stalk coarse powder, 1.83 times longer than the control (4 .8 days). Clove flower stalk extract is effective as a larvicidal mosquito *Ae. Aegypti*.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Author's declaration

The authors hereby declare that the work presented in this article is original and that they will bear any liability for claims relating to the content of this article.

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