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ACTIVITY OF CLOVE LEAVES (Syzygium aromaticum L.) LARVACIDES ON MORTALITY OF *Anopheles sp.*

Fenska Violenta Sapulette[1], Adrien Jems Akiles Unitly[1*], Debby D. Moniharapon[1]

[1]Department of Biology, Universitas Pattimura. Jl. Ir. M. Putuhena, Ambon 97233, Indonesia

**Corresponding Author* e-mail: *adebiologi@yahoo.co.id*

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ABSTRACT

This study used a completely randomized design with a sample of 525 Anopheles sp. larvae. which were divided into 7 groups and 3 replicates, which contained 25 Anopheles spper larvae. Except for the control, each group was given clove leaf infusion starting from a concentration of 0.50%, 1%, 2%, 4% and 6%, where the treatment was carried out for 24 hours. The results showed that there was a larvicidal effect of brewing clove leaves *(Syzygium aromaticum L.)* on the mortality of Anopheles sp mosquito larvae, where the effective concentration was 6% and had an LC50 value in killing Anopheles sp larvae of 0.89%. The overall phytochemical content of clove leaves which has a fast response to the mortality of Anopheles sp. are saponins, flavonoids and tannins which are capable of acting as larvicides through the mechanism of damaging cell membranes or interfering with larval metabolic processes. The content of saponins, flavonoids and tannins can increase the mortality of Aedes aegypti L. larvae through the mechanism of damaging cell membranes or interfering with larval metabolic processes as stomach poisoning.

Keywords: *anopheles*, larvae, *clove*.

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INTRODUCTION

Malaria is a disease caused by parasites caused by Plasmodium parasites and transmitted by Anopheles mosquitoes. Globally, its distribution is very wide, namely in the region between 600 north longitude and 400 south longitude, covering more than 100 countries with tropical and sub-tropical climates. The population at risk of contracting malaria is around 2.3 billion or 41% of the world's population. The 2005 WHO report stated that around the world the number of new cases of malaria ranged from 300-500 million people with deaths of 2.7 million people/year, mostly children under five years who are the most vulnerable group to disease and death from malaria with a number of endemic countries. malaria in 2004 as many as 107 countries (Lukman, 2011).

Indonesia is a country that has 1.8 million cases of malaria in 2006 and experienced an increase of 2.5 - 3 million cases in 2007 (Global Health Reporting, 2008). According to Hiswani (2004), 20 species of Anopheles have been found in Indonesia which are vectors of malaria, causing an increase in cases of malaria.

This is in line with Mardiana et al. (2005) and Ristiyanto et al. (2007) who stated that the high number of malaria cases was due to environmental conditions that strongly supported transmission, this was related to the life of the malaria vector, namely the Anopheles sp mosquito (Ristiyanto et al, 2007). One effort to control malaria is to control malaria vectors. The use of chemical insecticides as one of the eradication of malaria vectors is currently causing many new problems, namely environmental pollution, non-target insect deaths, target insect resistance, killing pets and even humans (Cavalcanti et al, 2004). Therefore, it is necessary to make an effort to find alternative insecticides that can kill the target insects but do not have side effects on the environment and human health.

Clove plants (Syzygium aromaticum L.) grow a lot in the Maluku Islands and are used by the community by the smoking method to kill mosquitoes which are vectors of malaria. According to Kardinan (2003), clove plants can be used as alternative insecticides to kill malaria vectors because they contain essential oils and eugenol (Kardinan, 2003). Eugenol in citronella essential oil has been studied to kill Anopheles aconitus larvae (Cahyati, 2005). Wijaya (2008) examined the extract of amethyst seeds (Datura mentel) which contains alkaloids and saponins which are larvicidal against Aedes aegypti L. larvae (Wijaya, 2009). In addition, the plant Ocimum gratisdsimum which contains eugenol is useful as a larvicide against Aedes aegypti L. (Cavalcanti et al, 2004). So that clove leaves contain essential oils and eugenol which can kill Anopheles sp. mosquito larvae. However, it is necessary to carry out laboratory tests to determine whether clove leaves have a larvicidal effect on the mortality of Anopheles sp. mosquito larvae.

METHOD

This study used a completely randomized design with 3 replications. The variables in this study consisted of two variables, namely the independent variable and the dependent variable. The independent variable was the steeping treatment and the ethanol extract of clove leaves while the dependent variable was the mortality of Anopheles sp. mosquito larvae. The tools used in this study were: analytical balance, blender, measuring cup, plastic tray, plastic container, glass beaker, glass stirring rod, pipette, evaporator and a set of stationery while the materials used in this study were: clove leaves, distilled water, honey , 70% ethanol, Anopheles sp. mosquito larvae.

The first stage is making clove leaf steeping. Clove leaves are dried at room temperature. After the leaves are dry, they are blended into a powder, then the doses of 0.09 gr/mL, 0.18 gr/mL, 0.36 gr/mL are weighed and brewed with boiling water. After that it is filtered and the dregs are removed.

The second stage was taking and grouping samples. The population in this study were Anopheles sp. larvae. which was obtained from several places within the Ambon Tiga Rumah Negeri community. The samples in this study were 525 Anopheles sp. larvae. which were divided into 7 groups and 3 replications, where each replicate was placed in a plastic cup containing 25 Anopheles sp. larvae as presented in table 1.

Table 1. Treatment of steeping clove leaves (Syzygium aromaticum L.) on Anopheles sp. mosquito larvae.

Concentration		**Repead**		
		U1	**U2**	**U3**
Control	0%	1	2	3
Leaf Stew Clove	0.5%	1	2	3
	1%	1	2	3
	2%	1	2	3
	3%	1	2	3
	4%	1	2	3
	6%	1	2	3

The third stage is treatment and observation. In this study, 5 variations of the concentration of clove leaf steeping (Syzygium aromaticum L.) were used, namely 0.5%, 1%, 2%, 4%, 6% and 0% (control). After being given a decoction of clove leaves (Syzygium aromaticum L.) on the Anopheles sp. mosquito larvae, observations were made every 3 hours for 24 hours and the number of dead larvae was recorded in each treatment. The results obtained were analyzed of ANOVA and continued with the Duncan test at the real rate

= 0.05 using SAS software and continued with the smallest significant difference test to determine the difference in the treatment given. Meanwhile, to determine the effective concentration (LC50) then used probit analysis.

DISCUSSION RESULT

The results showed that there were variations in the mortality of Anopheles sp. mosquito larvae which are presented in Table 2. All treatments of infusion of clove leaves *(Syzygium aromaticum L.)* on Anopheles sp. larvae were significantly different from the control (P<0.05). It can be seen that the higher the steeping concentration of clove leaves (Syzygium aromaticum L.), the higher the mortality of Anopheles sp. mosquito larvae. (P<0.05). Mortality of Anopheles sp. mosquito larvae. overall at a concentration of clove leaf steeping (Syzygium aromaticum L.) 0.5% occurred at 24 hours, 1% occurred at 18 hours, 2% also occurred at 18 hours, 4% occurred at 12 hours, 6% occurred at 12th hour, whereas in the control there was no mortality of Anopheles sp.

Table 2. Average mortality of Anopheles sp. larvae at various concentrations of steeping clove leaves (Syzygium aromaticum L.) every 3 hours for 24 hours of treatment.

Time Observation (i)	Concentration of Stewed Clove Leaves					
	0%	0.5%	1%	2%	4%	6%
3	0.0 ± 0.01^{d}	3.3 ± 0.01^{c}	6.0 ± 0.03^{b}	9.7 ± 0.01^{a}	12.0 ± 0.02^{a}	12.0 ± 0.02^{a}
6	0.0 ± 0.03^{e}	7.3 ± 0.07^{d}	8.7 ± 0.02^{d}	11.3 ± 0.02^{c}	18.0 ± 0.03^{b}	20.3 ± 0.05^{a}
9	0.0 ± 0.02^{e}	15.0 ± 0.01^{d}	17.7 ± 0.01^{c}	19.3 ± 0.04^{b}	22.0 ± 0.07^{a}	23.0 ± 0.03^{a}
12	0.0 ± 0.02^{e}	17.7 ± 0.01^{d}	20.3 ± 0.05^{c}	21.7 ± 0.09^{b}	25.0 ± 0.05^{a}	25.0 ± 0.08^{a}
15	0.0 ± 0.02^{e}	21.3 ± 0.03^{d}	23.0 ± 0.07^{c}	23.7 ± 0.07^{b}	25.0 ± 0.09^{a}	25.0 ± 0.04^{a}
18	0.0 ± 0.02^{c}	24.3 ± 0.05^{b}	25.0 ± 0.03^{a}	25.0 ± 0.04^{a}	25.0 ± 0.03^{a}	25.0 ± 0.07^{a}
21	0.0 ± 0.01^{c}	24.3 ± 0.02^{b}	25.0 ± 0.02^{a}	25.0 ± 0.02^{a}	25.0 ± 0.10^{a}	25.0 ± 0.02^{a}
24	0.0 ± 0.04^{b}	25.0 ± 0.01^{a}	25.0 ± 0.02^{a}	25.0 ± 0.02^{a}	25.0 ± 0.02^{a}	25.0 ± 0.02^{a}
PM(%)	0%	71.9%	75.4%	80.4%	88.5%	90.2%

Note: Numbers followed by different letters in the same row are significantly different (P < 0.05). PM: Percentage of mortality.

The larvicidal activity of clove leaf *(Syzygium aromaticum L.)* infusion on the mortality of Anopheles sp. mosquito larvae was determined based on the LC50 value which aims to determine the concentration that can kill 50% of Anopheles sp. mosquito larvae. Determination of LC50 using probit analysis showed that the LC50 value of steeped clove leaves (Syzygium aromaticum L.) was 0.89%. This means that at a concentration of 0.89% clove leaf infusion (Syzygium aromaticum L.) is effective in killing Anopheles mosquito larvae by 50%. The percentage of mortality of Anopheles sp. mosquito larvae at a concentration of 0% (control) was 0%, at a concentration of 0.5% the mortality of Anopheles sp. mosquito larvae was 71%, at a concentration of 1% the mortality of Anopheles sp. mosquito larvae. by 75.4%, at a concentration of 2% mortality of Anopheles sp. mosquito larvae. by 80.4%, at a concentration of 4% mortality of Anopheles sp. mosquito larvae. by 88.5% and at a concentration of 6% mortality of Anopheles sp. mosquito larvae. by 90.2%. This proves that the higher the mortality percentage of Anopheles sp. mosquito larvae. in line with the increasing concentration of clove leaf steeping (Syzygium aromaticum L.).

Mardiana et al. (2005) explained that the toxicity of an insecticide to a species is strongly influenced by the concentration of the chemical insecticide in the body of the target species (Mardiana et al, 2005). Increasing the concentration of the extract causes an increase in the content of the active ingredient in the substance which functions as a pesticide that can kill in large quantities (Kardinan 2003). This can be seen in research that steeping clove leaves at concentrations of 0.5%, 1%, 2%, 4 and 6% was able to kill larvae, but the highest lethal power was at concentrations of 4% and 6%. Clove leaves contain phytochemical compounds in the form of eugenol, saponins, flavonoids and tannins (Nurjanah, 2004). The distinctive aroma of clove oil is characterized by the presence of eugenol (Cahyati, 2005). Another ingredient that stands out is saponins, this is indicated by the fact that when shaken it creates foam. The presence of tannins is indicated by an astringent or astringent taste when in contact with the tongue. Flavonoids are thought to enter through the cuticle that coats the body of the larvae so that they can damage the respiratory system of the larvae. This

is in line with Hayatie et al. (2015) who stated that flavonoids enter the body of insects through their respiratory system and then cause damage to the respiratory system, causing mortality (Hayatie, 2015).

Polyphenols in clove leaves are thought to be able to inhibit insect digestion (Pratama, 2009). In addition to polyphenols, saponins in clove leaves are thought to be toxic in water and interfere with the digestion process of the larvae. Saponins have detergent-like properties so they are considered capable of increasing the penetration of toxins because they can dissolve lipophilic materials in water (Aminah, 2001). Saponins can also irritate the digestive tract mucosa. Saponins are thought to contain steroid hormones which reduce the surface tension of the mucous membranes of the digestive tract of the larvae so that the walls of the digestive tract become damaged. Saponins are bioactive compounds as toxins, included in the contact poison class because they can enter through the body wall of the larvae and stomach poisons through the mouth because the larvae usually take food from where they live. In addition, saponins also have a bitter taste, thereby reducing the appetite of the larvae and the larvae will die of starvation (Minarni et al. 2013).

Another substance contained in clove leaves is tannin and is thought to interfere with insects digesting food because tannins will bind to proteins in the digestive system that insects need for growth so that the process of absorbing protein in the digestive system is disrupted. Tannins form complexes with proline-rich proteins that cause inhibition of cellular protein synthesis (Aminah, 2001). Tannins suppress appetite, growth rate and survival ability. This is in line with Novizan's study (2002), which used yellow leaves for Ades Aegypti larvae (Novizan, 2002). Clove leaves have an essential oil in which there is eugenol, a compound that can interfere with the digestion of insects. Essential oils can cause death in larvae, and eugenol contained in essential oils affects the nervous system that is typical for insects and is not found in warm-blooded animals, causing the death of these insects (Cahyati, 2005). When viewed as a whole the phytochemical content of clove leaves has a fast response to the mortality of Anopheles sp. are saponins, flavonoids and tannins which are capable of acting as larvicides through the mechanism of damaging cell membranes or interfering with larval metabolic processes. According to Novizan (2002), the content of saponins, flavonoids and tannins can increase the mortality of Aedes Aegypti larvae through the mechanism of damaging cell membranes or interfering with larval metabolic processes as stomach poisoning or stomach poison (Novizan, 2002).

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