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Biolarvicide Activity of Male Breadfruit Flower Causes Mortality of *Anopheles* sp. Mosquito Larvae Malaria Vector

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Abstract: This study aims to determine the biolarvicidal activity of male breadfruit pollen causing mortality of *Anopheles* sp. malaria vector. This study used a completely randomized design (CRD), in which 600 *Anopheles* sp. divided into 8 groups with 3 replications, *Anopheles* sp. placed in 24 plastic cups, where each plastic contains 25 *Anopheles* sp. larvae. The results obtained were analyzed by Analysis of Variance (ANOVA) and continued with the Duncan test at real rate $\alpha = 0.05$ using SAS software and continued with the smallest significant difference test to determine the difference in the treatment given. Then a LC50 probit analysis was performed to determine the concentration of breadfruit pollen biolarvicide which is good at 50% mortality of *Anopheles* sp. mosquito larvae. The results showed that male breadfruit powder concentrations of 0.5, 1, 1.5, 2, 4, and 6% had a biolarvicidal effect causing mortality of *Anopheles* sp. mosquito larvae with an effective concentration of 6% (97.2% mortality), of which 50% mortality (LC50) of *Anopheles* sp. occurred at a concentration of male breadfruit pollen at a concentration of 3.95%.

Keywords: *Anopheles* sp. Larvae; Biolarvicida; Male breadfruit

Introduction

Malaria is a disease transmitted by parasites from the genus Plasmodium through mosquito bites. It is known that there are 20 types of *Anopheles* sp. mosquitoes, which can transmit malaria (Sembel, 2010). Indonesia has 15 million cases of malaria, where the prevalence of malaria in eastern Indonesia is currently 70% of malaria cases, especially among Papua, West Papua, Maluku, North Maluku, Sulawesi and Nusa Tenggara (Kementerian Kesehatan RI, 2001). The eastern part of Indonesia, including the city of Ambon, is the heaviest malaria spreading area in Maluku Province and is a high malaria endemic area. The Ambon City Health Office stated that in 2012 there was an increase to 6,648 cases of clinical malaria and 1,660 positive malaria.

Currently, efforts to control malaria are still focused on finding and treating sufferers, while the vector aspect has not been optimally implemented. The use of sub-lethal doses of synthetic organic insecticides has side effects that can stimulate insect self-adaptation to insecticides. This trait will be passed on to the next generation, resulting in a new population that is resistant to a particular type of insecticide (Sembel, 2010). The development of natural insecticides is the best solution at this time because the continuous use of chemical insecticides can cause environmental pollution, the death of several types of living creatures, and the resistance of insects that are eradicated, so it is necessary to use natural insecticides that do not pollute the environment and are relatively safe for humans because they will quickly disappears in nature (Kardinan, 2011).

One of the plants that can be used as an insecticide is breadfruit (*Artocarpus altilis* L.) (Kardinan, 2004; Nurasia et al., 2020). Breadfruit flower is used empirically in the Tanimbar Islands and most of Maluku province as a mosquito repellent using the fumigation method (burning the breadfruit flower) to kill mosquitoes which are vectors for malaria (Herman & Henry, 2010). Breadfruit flower can also be used as a natural mosquito repellent and biolarvicide, because

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breadfruit flowers contain several chemical compounds, namely flavonoids, saponins, and tannins which act as respiratory inhibitors or respiratory poisons so that they can kill mosquitoes (Lumowa, 2013; Qinahyu & Cahyati, 2016; Ahmad & Fahmi, 2017; Ernawati & Cahyati, 2018). Therefore it is necessary to carry out laboratory tests to determine the activity of breadfruit pollen as a biolarvicidal cause of death of *Anopheles* sp. mosquito larvae malaria vector.

Method

Research Design

This research was conducted from February 2022 to September 2022 at the Zoology Laboratory, Faculty of Mathematics and Natural Sciences, Pattimura University, using a completely randomized design (CRD), divided into 8 concentration groups with 3 replications.

Research Samples

The research samples were *Anopheles* sp. larvae which in this study used 600 larvae of *Anopheles* sp. placed in 24 plastic cups. 24 plastic cups are 8 concentration groups repeated 3 times. Each plastic cup contains 25 *Anopheles* sp. larvae.

Production of Biolarvicides

Production of Biolarvicides have done by taken the male breadfruit flowers, and then washed as much as 1 kg, then dried and then crushed using a blender to become breadfruit flower powder.

Biolarvicidal Observations

Biolarvicidal observations were carried out on samples with work procedures following Sapulette et al. (2019) as follows:

1. This study used concentrations of 0% (A), Abate 1% (B), Breadfruit powder concentrations of 0.5% (C), 1% (D), 1.5% (E), 2% (F), 4% (G), and 6% (H), where concentration loading is carried out by weighing 0.5 g of male breadfruit powder in a glass beaker and adding 100 ml of distilled water, little by little while stirring until the solution becomes unified. Furthermore, the same procedure was carried out to make concentrations of 1, 1.5, 2, 4, and 6%. As for the control, 100 ml of distilled water was used.
2. Breadfruit powder is put into plastic cups filled with water. After that, enter the *Anopheles* sp. larvae. A total of 25 tails at each concentration while in the control group given distilled water.
3. Each plastic cup was labeled with breadfruit flower pollen and *Anopheles* sp. larvae.
4. Observations were made every 3 hours and the number of dead larvae stages was recorded in each treatment for 12 hours.

Data Analysis

The results obtained were analyzed by Analysis of Variance (ANOVA) and continued with the Duncan test at real rate $\alpha = 0.05$ using SAS software and continued with the smallest significant difference test to determine the difference in the treatment given. Then do the LC50 probit analysis using excel.

Result and Discussion

Results

The results showed that there was a change in the mortality of *Anopheles* sp. mosquito larvae. After giving breadfruit powder concentrations of 0.5, 1, 1.5, 2, 4, and 6% compared to giving Abate 1% which is presented in Table 1.

Table 1. Average Mortality of *Anopheles* sp. Larvae at Various Concentrations of Breadfruit Powder Every 3 Hours

Treatment	Observation Time (i-Hour)				Mortality Percentage (%)
	3	6	9	12	
0 %	0 ± 0 ^{d}	0 ± 0 ^{e}	0 ± 0 ^{e}	0.6 ± 0.57 ^{e}	2.4
Abt. 1%	20 ± 0 ^{a}	25 ± 0 ^{a}	25 ± 0 ^{a}	25 ± 0 ^{a}	100
0.5%	0 ± 0 ^{d}	0 ± 0 ^{e}	0 ± 0 ^{e}	1.67 ± 0.57 ^{e}	6.68
1%	0 ± 0 ^{d}	0 ± 0 ^{e}	0 ± 0 ^{e}	5.3 ± 0.57 ^{d}	21.2
1.5%	0 ± 0 ^{d}	0 ± 0 ^{e}	0 ± 0 ^{e}	10 ± 0 ^{c}	40
2%	0 ± 0 ^{d}	2.67 ± 0.57 ^{d}	5 ± 1 ^{d}	11 ± 1 ^{c}	44
4%	2.67 ± 0.57 ^{c}	6 ± 1 ^{c}	9 ± 1 ^{c}	21 ± 1 ^{b}	84
6%	4.67 ± 0.57 ^{b}	12 ± 2 ^{b}	19 ± 1 ^{b}	24.3 ± 0.57 ^{a}	97.2

Information: Different superscript letters in one column showed significantly different results ($P < 0.05$) between treatment groups. 0% = not given breadfruit flower powder and abate, Abt. 1% = 1% abatement, 0.5% = 0.5% breadfruit powder, 1% = 1% breadfruit powder, 2% = 2% breadfruit powder, 4% = 4%, 6% breadfruit powder = Provision of 6% breadfruit flower powder.

For the 3rd hour observation time, there was no significant difference between the concentrations of 0.5, 1, 1.5, 2, and 0% (negative control) ($P < 0.05$), but significantly different from Abate % (positive control), concentration 4 and 6% were significantly different from 0% control and 1% Abate. For the 6th hour of

observation, there was no significant difference between concentrations of 0.5, 1, 1.5, and 0% concentration (negative control) ($P < 0.05$), but significantly different from Abate 1% (positive control) ($P < 0.05$), concentrations of 2, 4, and 6% were significantly different from 0% concentration (negative control) ($P < 0.05$).

Anopheles sp. mosquito larvae for the 9th hour observation time at concentrations of 0.5, 1, 1.5, 2, 4, and 6% were significantly different from Abate 1%, where concentrations of 0.5, 1, and 1.5% were not significantly different ($P > 0.05$) with 0% (negative control). The average mortality of *Anopheles* sp. mosquito larvae for the 12th hour of observation at concentrations of 1, 1.5, 2, 4, and 6% were significantly different from 0 and 1% Abate, but 6% concentration was not significantly different ($P > 0.05$) with 1% Abate. This shows that the higher the concentration of breadfruit pollen, the higher the mortality of *Anopheles* sp. mosquito larvae (Figure 1). In addition, it shows that the concentration of 6% at 12 hours of treatment has the same mortality as Abate. The same thing can be seen in the average percentage of mortality of *Anopheles* sp. mosquito larvae. After giving breadfruit powder concentrations of 6 and 9% had a percentage close to 1% Abate (positive control). Increased mortality of *Anopheles* sp. mosquito larvae in line with the increase in concentration where the mortality rate of 97.2% is close to Abate 1%, namely 100% at the 12th hour (Figure 2).

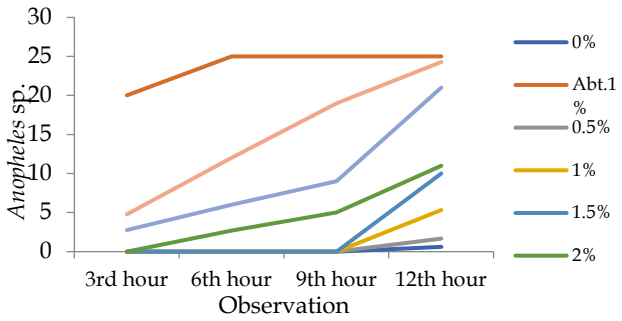

Figure 1. Graph of mortality of *Anopheles* sp. mosquito larvae in each treatment group

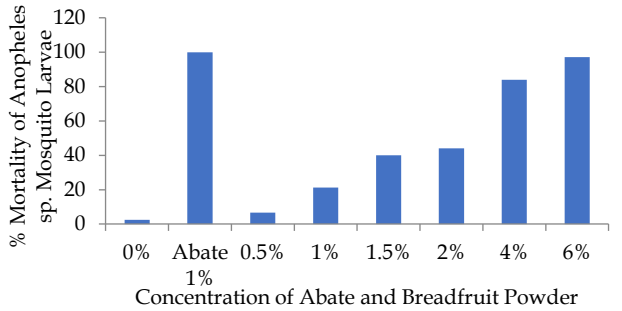

Figure 2. Graph of the percentage mortality of *Anopheles* sp. mosquito larvae in each treatment group

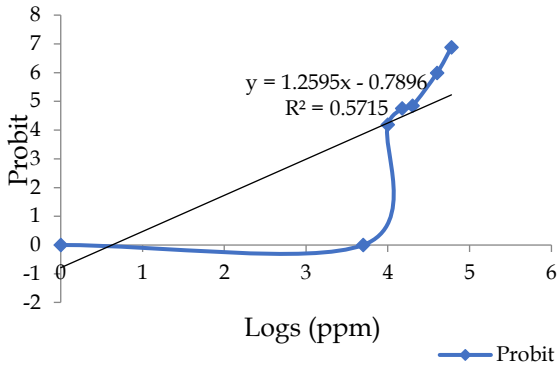

Figure 3. Graph of probit analysis LC50 Concentration of Male Breadfruit Powder

Equality:	y =	ax + b
	5 =	1.2595x - 0.7896
	x =	4.596881
LC50 = antilog (x)	=	39525.87 ppm
LC50	=	3.952587 %

Based on Probit Analysis and LC50 Calculation (Figure 3), the LC50 value is 3.95%.

Discussion

The results showed that the lowest larval mortality was seen in the 0 % negative control that was not given breadfruit powder. While the treatment of male breadfruit pollen concentrations of 0.5, 1, 1.5, 2, 4, and 6%, showed biolarvicidal ability, where the highest lethal power was at a concentration of 6%. The mortality of *Anopheles* sp. mosquito larvae after being given male breadfruit powder, it is suspected that it is due to the active substances of breadfruit flowers which can function as biolarvicides such as flavonoids, saponins, polyphenols, hydrocyanic acid, acetylcoline, tannins, riboflavin, and phenols (Sitorus et al., 2014). Flavonoid compounds function as anticholinesterase. Anticholinesterase which causes the cholinesterase enzyme to be phosphorylated and becomes inactive. Inactivity of the cholinesterase enzyme can cause an obstacle to the process of acetylcholine degradation resulting in the accumulation of acetylcholine in the synaptic cleft. Furthermore, there is an increase in the transmission of stimuli, which causes the respiratory muscles to contract continuously resulting in respiratory muscle spasms and causes the death of mosquito larvae. Flavonoid dioscorine can also cause spiracle damage, as a result the insect cannot breathe and eventually dies.

Anopheles sp. larvae allegedly due to exposure to breadfruit pollen flavonoids which work to inhibit mitochondria in cells, whereas mitochondria function as a place where respiration processes occur, namely

electron transport and the Kerbs cycle. If the mitochondria are disrupted, ATP production will be hampered, so that the binding to oxygen is low in the end the use of oxygen by the mitochondria is not optimal resulting in breathing disorders causing death. Flavonoids have a number of hydroxyl groups, or a sugar, causing them to tend to dissolve easily in water so that they can enter the body of the larvae through the digestive system. Saponins reduce the activity of digestive enzymes and absorption resulting in the death of *Anopheles* sp. larvae (Hayatie et al., 2015).

Breadfruit flowers also contain saponins which are harmful to *Anopheles* sp. larvae, because it is toxic. Aminah et al. (2001) stated that saponins have detergent-like properties so they can increase the penetration of toxins because they can dissolve lipophilic materials in water. This can cause the death of *Anopheles* sp. larvae. According to Ahdiyah et al. (2015), saponins can inhibit the action of enzymes causing a decrease in the use of protein which can cause the death of *Anopheles* sp. larvae. 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. Saponins irritate the mucosa of the digestive tract and reduce the surface tension of the mucous membrane of the digestive tract of the larvae so that the digestive wall becomes corrosive. Saponins can destroy red blood cells through hemolysis reactions, causing death (Aminah et al., 2001). Research by Utami (2010) found that saponins can cause the death of larvae because they have a bitter taste which reduces appetite resulting in hunger resulting in death.

Apart from saponins, tannins in breadfruit flowers are thought to have biolarvicidal properties. This is in line with the results of research by Novizan (2002), Muftiah et al. (2019), Unitly et al. (2021), and Moniharapon et al. (2023b) that tannins have biolarvicidal abilities through the mechanism of damaging cell membranes or interfering with larval metabolic processes causing a lack of appetite, stunted growth and reduced survival so that larvae experience death. According to Ridwan et al. (2010), tannins are polyphenolic compounds that can form complex compounds with proteins. Tannins cannot be digested by the stomach and have a binding capacity with proteins, carbohydrates, vitamins, and minerals. According to Yunita et al. (2009), Cania & Setyanimgrum (2013), Waskito & Cahyati (2018) , tannins interfere with insects digesting food because tannins will bind to proteins in the digestive system that insects need for growth, so it is estimated that the digestive process of the larvae can be disrupted which causes death. This is in line with research by Aminah et al. (2001) that tannins form complexes with proline-rich proteins which cause inhibition of cell protein synthesis. Tannins can suppress appetite, growth rates, and the ability to survive so that the larvae experience death.

In addition, the phenol contained in male breadfruit pollen is also thought to be a biolarvicidal agent for *Anopheles* sp (Moniharapon et al., 2023c). Usman et al. (2020), Moniharapon et al. (2023a) in his research found phenol compounds which were thought to be toxins that caused the death of mosquito larvae. He added that the process of mortality or death of the larvae is caused by contact poisons (biolarvicida) phenolic compounds that enter the body of the larvae through the skin, natural gaps/holes in the body (sipon). This is in line with Wahyuni & Loren (2015) that phenolic compounds have the properties of a dehydrating poison (Desiscant), where the poison is a contact poison which can result in continuous death. Larvae exposed to this poison will die due to lack of fluids (Jones et al., 2012; Lestari et al., 2019).

Biolarvicidal activity of male breadfruit pollen on the mortality of *Anopheles* sp. mosquito larvae determined based on the LC50 value, 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 male breadfruit powder was 3.95%. This means that at a concentration of 3.95%, effective male breadfruit pollen has 50% mortality against *Anopheles* sp. mosquito larvae.

Conclusion

Based on the research results obtained, it was concluded that male breadfruit pollen concentrations of 0.5, 1, 1.5, 2, 4, and 6% had a biolarvicidal effect causing mortality of *Anopheles* sp. mosquito larvae with an effective concentration of 6% (97.2% mortality), of which 50% mortality (LC50) of *Anopheles* sp. occurred at a concentration of male breadfruit pollen at a concentration of 3.95%.

Author Contributions
Debby D. Moniharapon and Adrien Jems Akiles Unitly conceptualized the research idea, designed of methodology, management and coordination responsibility, analyzed data, conducted a research and investigation process; Veince B. Silahooy conducted literature review and provided critical feedback on the manuscript.

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Conflicts of Interest
All authors declare no conflicts of interest.

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