Mortality Effects of Mosquito Anopheles Sp. Malaria Vectors Caused by Exposure to Burning Mosquito Drugs and Male Breadfruit Mat

Debby Dijola Moniharapon1*, Adrien Jems Akiles Unitly2, Maria Nindatu3, Mechiavel Moniharapon4, Veince B. Silahooy5, Beatrix Belina Sikafir6, Helena Djumaifin7

Biology Study Program, Department of Biology, Faculty of Mathematics and Natural Sciences, Pattimura University, Ambon

Corresponding Author: Debby Dijola Moniharapon debbydjola10@gmail.com

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ABSTRACT
This study aims to determine the effect of mortality on Anopheles sp. vector of malaria due to exposure to mosquito coils and male breadfruit mats. This study used a completely randomized design (CRD), in which 180 Anopheles sp. divided into 3 groups with 3 replications, Anopheles sp. placed in 9 plastic cups, where each plastic contains 20 Anopheles sp larvae, then reared until they become mosquitoes in a mosquito net box. 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. The results showed that the use of mosquito coils and electric mats for male breadfruit flowers had the same mortality effect, where the percentage of mortality was 100%.
INTRODUCTION

Malaria is a disease caused by protozoa of the genus *Plasmodium* which is characterized by fever, sweating, hemilitic anemia and splenomegaly. Female *Anopheles* mosquitoes are a causative factor for malaria which has been shown to contain sporozooids in their salivary glands. The spread of malaria is determined by several factors including *Agent*, *Host*, and the environment which interact with each other. The *agent* (parasite) lives in the human body (intermediate) and the body of the mosquito (definitive). In the body of the *Agent mosquito* develops into an infective form, ready to transmit to humans which functions as an *intermediate host* that is usually infected and becomes a place for development parasites (Mading & Kawzwaini, 2014). This can cause 90% of cases of death (Soedarto, 2009).

Control is one of the efforts to reduce or suppress the vector population. Control of disease vectors by using insecticides to kill adult mosquitoes, using abate as larvicides, and *repellents* to prevent mosquito bites (Sembel, 2010). Unfortunately, the use of synthetic organic insecticides is not accompanied by attention to the common side effects. The use of sublethal doses stimulates the adaptation of insects 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. Insecticides containing hazardous chemicals such as the use of organochlorines have been banned in Indonesia (WHO, 2005). The use of chemical insecticides for a long time and excessive doses will result in resistance to the target organisms.

THEORETICAL REVIEW

The most effective and popular vector control in society is the use of insecticides. The use of insecticides aims to kill vectors to break the chain of disease transmission. However, insecticides circulating in society are still chemical insecticides. Biological mosquito control is one of the safe ways to control the *Anopheles mosquito population* because this biological method does not use chemicals because it only uses compounds contained in a plant to kill *Anopheles mosquitoes*.

Male breadfruit flower is a vegetable insecticide because it contains saponins, tannins and flavonoids (Lumowa, 2013). The latest research on the use of male breadfruit flowers as a vegetable insecticide is male breadfruit biolarvicide which causes 76% mortality of *Anopheles* sp. mosquito larvae. (Moniharapon, Nindatu, Unitly & Sikafir, 2023) and the use of electric mats for male breadfruit flowers which causes 96.5% mortality of *Anopheles* sp. at the highest dose of 2gr (Moniharapon, Kaihena & Unitly, 2023), both of which only compared doses of male breadfruit flowers. Therefore it is necessary to carry out laboratory tests to compare the effect on the mortality of *Anopheles* sp. vector of malaria due to exposure to mosquito coils and electric mats for male breadfruit flowers.
METHODOLOGY
Research Design
This laboratory experimental study was conducted at the Zoology Laboratory, Faculty of Mathematics and Natural Sciences, Pattimura University from September to November 2022, using a completely randomized design (CRD) with 3 treatments and 3 replications. The population in this study were Anopheles sp. larvae. obtained from mosquito larvae breeding places in the house (in door). The samples in this study were 180 Anopheles sp. larvae. instar III each was placed in 9 plastic cups, each of which contained 20 Anopheles sp. larvae. instar III. The treatment group was K(-): negative control, namely the Anopheles sp. who were not exposed to mosquito coils and male breadfruit mats, ONB: Anopheles sp. mosquito group . those exposed to mosquito coils, and 2.5gr : Anopheles sp. exposed to male breadfruit mat 2.5 gr . The variables of this study were the independent variables, namely mosquito coils and breadfruit (Artocarpus altilis L.) pollen, and the dependent variable, namely the mortality of Anopheles sp.

Anopheles sp. Mosquito Larvae
Sampling of mosquito larvae inside the house (in door), observation of mosquito larvae inside the house was carried out at the breeding sites of mosquito larvae, then the larvae were taken using the single larva method (one scoop) using a dipper from a water reservoir (flower pot). ) then put into a bottle and labeled for further transport to the laboratory for identification. After that, an examination of Anopheles sp. mosquito larvae was carried out. following Lestari, Farhan, A., & Sulistiyono (2019), namely mosquito larvae were taken using a dropper pipette, placed on a glass object and covered with a cover glass, then identified mosquito larvae using a microscope with an objective lens magnification of 10x. after being identified, the larvae of Anopheles sp. kept until they become Anopheles sp. instar III in a mosquito net box.

Making Mat for Male Breadfruit Flowers
Breadfruit flowers are taken then washed as much as 1 kg then dried and then crushed using a blender until it becomes breadfruit flower powder then mat is made as follows:
1. Breadfruit flowers are wrapped in tissue paper containing 2.5 grams each to form an electric anti-mosquito plate (mat).
2. The packaging of the breadfruit flower mat is dripped with 1 ml of clean water.
3. The next 1.5 hours, another test was carried out on the mat. Dropping water is done every 1.5 hours up to 12 times dripping.
4. After drying, the breadfruit flower mat is ready to be used and attached to an electric heating device.

Use of Mosquito Burn Drugs
The mosquito coils used contain the active ingredient dimefluthrin 0.566% (4.2 mg/pcs), and the pyrethroids group are dallethrhin, allethrin,
pyrethrin, esbiothrin, terallethrin, transfluthrin. The active ingredient is effective in its nature as an insect repellent, even though the active ingredient only amounts to 1% of the mass of the mosquito coil.

**Anti-mosquito Observation**

Mosquito repellent observations were carried out on samples with a modified Lumowa work procedure, (2013), namely as follows:

1. Make sure the Glass Chamber is not contaminated
2. Heat the mosquito coil in the glass chamber for 3 minutes, and wait another 3 minutes before testing.
3. Remove and remove the mosquito coil from the glass chamber testing.
4. Release 20 mosquitoes into the glass chamber for testing.
5. Observe and record mosquitoes that die during exposure. Observations were made every 3 hours and the number of dead larvae stages was recorded in each treatment for 24 hours.
6. After 20 minutes of exposure, all mosquitoes were transferred to a plastic cup, stored or held for 24 hours.
7. Count or record the number of dead mosquitoes.

The percentage of larval mortality is calculated using the formula (Kundra, 1981) in Hasinu Runthe & Laisow (2014):

\[ M = \frac{a}{b} \times 100\% \]

Information: M: percentage of mosquito larvae mortality, a: number of dead mosquito larvae, and b: number of mosquito larvae used as supporting data

**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.

**RESULTS**

The results showed that there was a change in the mortality of *Anopheles* sp. mosquito larvae after exposure to breadfruit flower mat dose of 2.5gr compared to mosquito coils and negative controls are presented in table 1. The results of the 3rd hour observation showed that there was a significant difference between the 2.5gram dose and the negative control (\( P<0.05 \)), but not significantly different from the administration of mosquito coils (\( P>0.05 \)). The same thing happened at the 6th, 9th and 12th hour observations. This shows that the longer the exposure time, the 2.5gram dose of breadfruit mat and mosquito coils has the same effect on mosquito mortality, where the percentage of mortality reaches 100%.
Table 1. Average Mortality of *Anopheles* sp. Caused by Male Breadfruit Mats and Mosquito Coils Every 3 Hours for 24 Hours

<table>
<thead>
<tr>
<th>Treatment (Mean ± SD)</th>
<th>Observation Time (i-Hour)</th>
<th>Mortality Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>K(-)</td>
<td>0.00 ± 0.00 d</td>
<td>0.00 ± 0.00 d</td>
</tr>
<tr>
<td>ONB</td>
<td>11.00 ± 2.00 a.m</td>
<td>17.33 ± 0.57 a</td>
</tr>
<tr>
<td>2.5 gr</td>
<td>11.66 ± 1.52 a</td>
<td>17.00 ± 1.00 a.m</td>
</tr>
</tbody>
</table>

Note: Different superscript letters in one column show significantly different results (P <0.05) between treatment groups. K(-) : negative control, namely the *Anopheles* sp. those not exposed to male breadfruit mats and mosquito coils, ONB: *Anopheles* sp. mosquito group . those exposed to mosquito coils, and 2.5gr : *Anopheles* sp. exposed to male breadfruit mat 2.5 gr .

**DISCUSSION**

WHO (2005) states that insecticides are considered to have an effect if they cause the death of test mosquitoes by 10 - 95% for 24 hours, while the Pesticide Commission, (1995) states that the use of insecticides is said to be effective if it can kill 90-100% of test mosquitoes for 24 hours. Research shows that long-term use of mosquito coils can increase the risk of contracting ARI. This infection is characterized by several symptoms, such as coughing, runny nose, stuffy nose, sore throat, easy fatigue, dizziness, fever, and shortness of breath. Apart from causing ARI, harmful substances produced from burning mosquito coils, such as formaldehyde or formalin, can also trigger asthma attacks. The content of sulfur dioxide in insect repellent can also exacerbate asthma and bronchitis. This is allegedly due to harmful substances in mosquito coils such as carbon monoxide, sulfur dioxide, nitrogen dioxide, and even formaldehyde, which will be released into the air when the mosquito coil is turned on and finally inhaled. Other conditions that occur as a result of inhaling mosquito coils are headaches, sore eyes or eye irritation. Given the negative impact that can occur, it is necessary to use other alternatives. The use of vegetable insecticides is an alternative that can be done, because plant insecticides are safer because their residues are easily lost, and easily decomposed (biodegradable) so they do not pollute the environment (Kardinan, 2004).

Breadfruit (*Artocarpus altulis*) is a plant that contains insecticidal compounds such as saponins, tannins, and flavonoids which have plant insecticidal effects causing the mortality of *Anopheles* sp. mosquitoes. *Anopheles sp.* mosquito mortality. occurs due to poisoning when the electric mat is turned on causing evaporation of the active substances contained in breadfruit flowers which emit an aroma that mosquitoes do not like and can attack the mosquito's respiratory system (Lumowa, 2013).
Saponins are compounds found in male breadfruit flowers which can attack the mosquito’s nervous system. Ahmad & Fahmi, (2017) proved that breadfruit mat has saponins which can interfere with the mosquito's nervous system so that it can repel mosquitoes. The longer the exposure, the higher the mosquito mortality. It turns out that in addition to disturbing the nervous system, saponins also have a way of working as stomach poisons and inhibit the work of the cholinesterase enzyme in insects (Cania, 2013).

In addition to saponins, tannins contained in male breadfruit flowers are disliked and avoided by insects, and have insecticidal properties that can attack the respiratory system of mosquitoes. When using the electric method, tannins will have a direct impact on interfering with mosquito breathing and causing mosquito mortality (Qinahyu & Cahyati, 2016). One of the factors that is thought to influence mosquito mortality is the breadfruit pollen mat which is finer without fibers so that the water content dissolves more easily in the breadfruit powder mat and can affect the shape of the mat density. In addition to interfering with the respiratory system, tannins can reduce the ability to digest food by reducing the activity of digestive enzymes (proteases and amylase) (Haditomo, 2010). Apart from interfering with the digestive process, tannins can damage cell membranes (Novizan, 2002 and Unitly, Moniharapon, & Sapullete, 2021), which can result in death.

Flavonoids contained in male breadfruit flowers act as respiratory inhibitors so that when Anopheles sp. doing breathing, flavonoids will enter with the air (O2) through the respiratory apparatus (Lumowa, 2013). Flavonoids act as anticholinesterase. Anticholinesterase causes the cholinesterase enzyme to phosphorylate and become inactive. With the inactivity of the cholinesterase enzyme, it will cause an obstacle to the degradation process of acetylcholine 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 mosquitoes and tannins have a working mechanism in inhibiting or even killing mosquitoes.

Although the use of mosquito coils and male breadfruit mats has the same mortality effect on Anopheles sp. mosquitoes, the use of male breadfruit mats is a vegetable insecticide so it is good to use, because vegetable insecticides are safer because the residue is easily lost, and easily decomposed (biodegradable), so as not to pollute the environment (Kardinan, 2004). The use of male breadfruit flower powder as an electric mosquito repellent in killing mosquitoes is one of the most effective alternatives for reducing environmental pollution because plant insecticides decompose more quickly in nature so they do not cause resistance to targets. According to Qinahyu and in line with Cahyati & Sukowinarsih, (2010) and Cahyati, Sukendra, & Santik, (2016), pesticides in vegetable insecticides decompose more quickly in nature so they do not cause resistance. In addition, it can be made by yourself in a simple way and can economically reduce the cost of purchasing insecticides.
CONCLUSIONS AND RECOMMENDATION

The results showed that the use of mosquito coils and electric mats for male breadfruit had the same mortality effect, with both 100% mortality presentations.

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