

REPELLENCE OF *Aedes aegypti* BASED ON *CITRUS AURANTIFOLIA* AND *ANNONA MURICATA*

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Abstract

Aedes aegypti is the main vector of dengue virus infection. Natural control of the vector aims to reduce resistance and disturbance to the environment. This research developed natural insecticides from the peel of *Citrus aurantifolia* S. and leaves of *Annona muricata* L. The Purpose of the study determine the concentration of *Citrus aurantifolia* S. and *Annona muricata* L. extracts as repellents *Aedes aegypti*. This study used a complete randomised design on the factorial pattern, factor A (level A1: peel of *Citrus aurantifolia* S. extract and A2: leaf of *Annona muricata* L. extract) and factor B (concentration level of b1:15%, b2:30%, b3:45%, and b4:60%). The data was analysed using two-way ANOVA. The results of data analysis showed factor A=F (0.030)<Ftable (4.49), there was no significant effect, factor B=F (5.423)>Ftable (3.24) there was a significant difference, factor AB=F (0.751)<Ftable (3.24), there was no significant difference. The concentration of botanical insecticides has a significant effect on the *Aedes aegypti* mosquito repellent. Leaf of *Annona muricata* extract with a concentration of 60% has the most effective ability as a repellent for *Aedes aegypti*.

Keywords: *Aedes aegypti*, dengue fever, bioinsecticide, repellent.

INTRODUCTION

Dengue Haemorrhagic Fever (DHF) is an arbovirus disease, which is a virus transmitted from Arthropods to humans through the bite of a female *Aedes aegypti* mosquito which is often found in tropical and subtropical regions (Banneheke *et al.*, 2016; Balière *et al.*, 2023). Dengue fever has become an international concern with worldwide cases reaching 50 million per year (Tuiskunen Bäck & Lundkvist, 2013). The World Health Organisation estimates that between 50 and 100 million dengue infections occur each year and nearly half of the world's population lives in countries where dengue vectors are endemic (Chan, 2012; Tukan et al., 2017).

Dengue hemorrhagic fever was first reported in Indonesia in 1968, in the city of Surabaya and later DKI Jakarta (Siregar and Makmur, 2018; Karyanti *et al.*, 2014).

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Furthermore, reports from several regions have increase cases until now with the number of cases fluctuating and difficult to eliminate completely (Sazali *et al.*, 2024). The increase in dengue hemorrhagic fever cases has been widespread in several areas, in addition to being found in urban areas with high residential conditions, it is also found in suburban and rural areas (Sazali & Lubis, 2024; Alenou *et al.*, 2023; Dom *et al.*, 2016; Wen *et al.*, 2015).

One of the key strategies of the dengue haemorrhagic fever vector control program is to reduce the spread (transmission) of dengue virus vectors (Sazali *et al.*, 2014; Sazali and Astuti, 2018; Adesina *et al.*, 2019; Alenou *et al.*, 2023; Sazali and Astuti, 2018). Control of the spread of dengue virus in residential areas has been carried out by using insecticides to suppress the number of viral vectors on a large scale (Meliyane *et al.*, 2017; Tainchum *et al.*, 2018). Insecticides and synthetic chemicals are among the ingredients used to control larvae and adult mosquitoes *Aedes aegypti* known as fogging programs (Meliyane *et al.*, 2017; Sazali *et al.*, 2020). However, many reported active ingredients in the form of insecticides released into the environment cause many problems. Therefore, chemical compounds that are safe for human health and the environment are needed.

Some examples of plants that contain vegetable insecticides are lime peel and soursop leaves (Susanti *et al.*, 2018; Dzulhijja, W and Prastowo, 2020). Lime (*Citrus aurantifolia*) contains elements of beneficial chemical compounds, one of which is the compound d-limonene. D-limonene compounds in lime peel can be used as insect repellents, one of which is that it can provide an insecticidal effect against several types of ticks. In addition, *Citrus aurantifolia* contains substances such as saponins, citronella, linallyl acetate, flavonoid compounds and other substances.

The leaves of *Annona muricata* L. are known as vegetable insecticides (Isabela *et al.*, 2019; Bestari *et al.*, 2020). Active compounds in the form of acetogenin, annonacin, flavonoids and tannins as well as secondary metabolic compounds of alkaloid and triterpenoid saponin groups have cytotoxic and neurotoxic effects on larval and insect cells, causing death in larvae. At high concentrations acetogenin compounds can be antifeedants for insects, causing reduced appetite, while low concentrations of acetogenin compounds, are stomach toxins that can cause insects to die (Salessy *et al.*, 2022).

Based on the results of observations made in Jempong Baru, Sekarbela District, Mataram City directly by observing the flying behavior of *Aedes aegypti*. Control of *Aedes aegypti* still uses chemicals such as cipermethrin, the active ingredient Malathion and Lamdacyhalotrin. Cypermethrin is an insecticide that belongs to the Pyrethroid group. The use of these chemicals will certainly damage the environment and human health. Therefore, vegetable chemicals are environmentally friendly and easy to obtain (Sazali *et al.*, 2020; Sazali & Rizki, 2017).

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Research using lime peel as a fly repellent insecticide has been conducted by Hariyanti et al, (2015). The results of the study that the peel of lime fruit (*Citrus aurantifolia*) is effective as a vegetable insecticide against flies that perch on the flesh. In the treatment using lime peel, 13 flies landed on the flesh. Lime peel was also used in other studies as an insecticide against *Aedes aegypti* (Ekawati, 2017; Nurhaifah & Sukesi, 2015; Wahyuni & Adiwanito, 2019).

This study used *Aedes aegypti* and lime peel extract and soursop leaves. This plant-based insecticide is considered economical and does not cause environmental damage and health problems and is easily decomposed compared to chemical pesticides. Therefore, vegetable insecticides as repellent *Aedes aegypti* from Lime Peel (*Citrus aurantifolia*) and Soursop Leaves (*Annona muricata*) need to be developed.

METHOD

This type of research is experimental research with a descriptive quantitative approach. The population in this study was all *Aedes aegypti* mosquitoes bred in a medium (container). The samples used in this study were 10 adult *Aedes aegypti* for each concentration. This study will be used with a non-random sampling technique, so that the number of samples are 240 *Aedes aegypti*.

The research design used in this study is quasi experimental design, with the research design being Complete Random Design (RAL) factorial pattern consisting of 2 factors. Factor A is treatment with level A1: lime peel and A2: soursop leaves, while factor B (concentration) with levels B1 (15%), B2 (30%), B3 (45%), and B4 (60%).

Data were analyzed using SPSS 16 with a significance level of 5%. The extraction of repellent materials was carried out at the Chemistry Laboratory, University of Mataram and the identification of the behavior of *Aedes aegypti* mosquitoes was carried out by the Integrated Laboratory of the State Islamic University of Mataram. The type and flow of research should be presented in this section with image captions. Image captions are put into part of the image title (figure caption) instead of being part of the image. The methods used in completing the research are also listed in this section.

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RESULTS AND DISCUSSION

The test results for 1 hour showed that lime peel extract with the lowest concentration of 15% had an average rejection of 72.78%, the highest concentration of 60% had a rejection of 94.99%, while the lowest concentration of soursop leaf extract of 15% had a rejection of 75.55%, the highest concentration of 60% had a rejection of 97.77%.

Table 1. The repellent power of lime peel extract and soursop seeds against the flight behaviour of *Aedes aegypti*.

Repetition	Prepellent compound								T
	a	a	a	a	a	a	a	a	
1	6	9	9	9	9	9	9	1	7
2	8	8	8	9	6	6	8	9	6
3	7	8	8	9	7	7	9	9	6
Total	2	2	2	2	2	2	2	2	2
Avera	7	8	8	9	7	7	9	9	

Note:

a₁ : *Citrus*

a₂ : *Annona*

b₁ : 15%

b₂ : 30%

b₃ : 45%

b₄ : 60%

The test results for 1 hour showed lime peel extract the lowest concentration of 15% had an average rejection of 72.78%, the highest concentration of 60% had a rejection of 94.99%, while the soursop leaf extract of the lowest concentration of 15% had a rejection of 75.55%, the highest concentration of 60% had a rejection of 97.77%.

The analysis showed that in factor A (vegetable insecticide) there was no significant difference in the effect of lime peel extract and soursop leaf extract seen from a significant $0.547 > 0.05$, while treatment B (concentration) there was a significant difference in the

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concentration of lime peel extract and soursop leaves, namely 15%, 30%, 45%, and 60% seen from a significant $0.00 < 0.05$. While the interaction of factors A and B has no effect seen from the significant $0.377 > 0.05$. Then further tests were carried out using LSD to determine the real difference from the concentration.

Further test results showed differences in the effectiveness of concentrations of lime peel extract and soursop leaves. The concentration of 15% has a significant difference with a concentration of 45% and 60% (value of $\text{sig} < 0.05$), then a concentration of 30% has a significant difference with a concentration of 60% (value of $\text{sig} < 0.05$), then a concentration of 45% has a significant difference with a concentration of 15% (value of $\text{sig} < 0.05$), while concentration of 60% has a significant difference with concentrations of 15% and 30% (value of $\text{sig} < 0.05$).

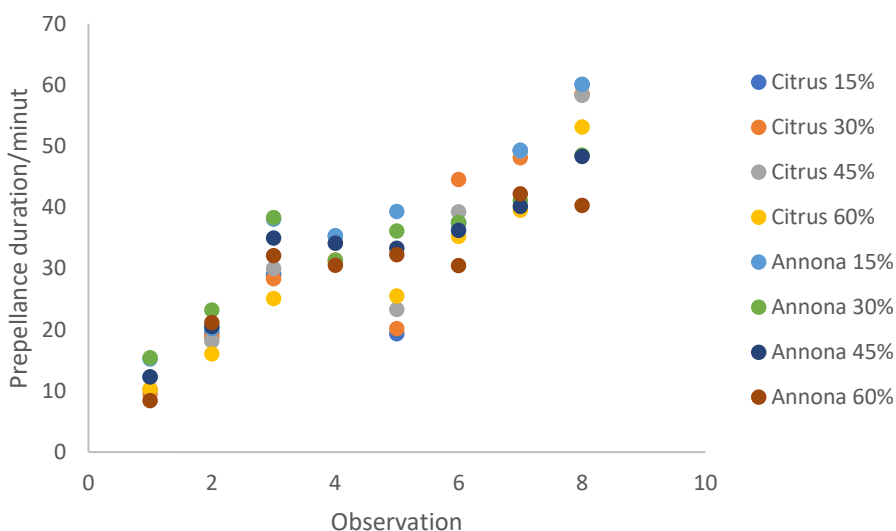


Figure 1. Duration of *Aedes aegypti* mosquito preparation with lime peel extract and soursop seeds based on 15 minutes of observation. Prepulsion is determined by observing the behaviour of flying away from the repellent source.

Based on this study, there was no significant influence on the type of vegetable insecticide from lime peel and siirsak leaves. Therefore, lime peel and soursop leaves can act as vegetable insecticides. Tangerines, kaffir lime and lime peel extracts can function as prepelements, because they contain essential oils including citronellol, geraniol, and linalool acting as mosquito repellents (Susanti et al., 2018).

Annona muricata L. leaf and seed extracts have active compounds in the form of acetogenin, annonacin, flavonoids and tannins. In addition, there are secondary metabolic

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compounds of alkaloid and triterpenoid saponin groups with cytotoxic and neurotoxic effects on larval and insect cells. The effect caused is death in the larvae. At high concentrations, acetogenin compounds can be antifeedants for insects, causing reduced appetite, and low concentrations of acetogenin, stomach toxins that can cause insects to die (Dzulhijja et al., 2020; Isabela et al., 2019).

Annona muricata L leaves and seeds can be used as vegetable insecticides (Bestari et al., 2020), The active ingredients in soursop are acetogenin larvicide, insecticide, accharicide, antiparasitic, and bactericidal. This study used soursop seeds as an insecticide. *Aedes aegypti* mosquitoes on average experienced the lowest mortality at a concentration of 15%, while concentrations that can kill 50% to 90% of mosquitoes were concentrations of 35% and 54%.

Based on Figure 1. The above shows that in this study the repulsion power of lime peel extract has been seen starting from the first observation, the duration for 10-60 minutes lime peel 15% already has prepellence power, soursop leaves 15% slowly show the presence of prepellence properties at minutes 20-40, the lowest concentration is 15% with an average repulsion power of 72.78%, soursop leaf extract 75.55%, this is because the insecticide extract used is low so it can still be tolerated. Concentration 30% lime peel 88.33%, soursop leaves 78.89%. Concentration 45% lime peel 86.11%, soursop leaves 92.77%. The highest concentration is 60% lime peel 95.00%, while soursop leaves 97.78%. Lime peel extract is not constant because there is a decrease in the 45th minute, while the higher the concentration of soursop leaves the higher the repulsion and the most effective concentration is 60% of the repulsion of *Aedes aegypti* of 97.78%, this is because the higher the concentration of the vegetable insecticide, the higher the toxins contained in the extract.

This study used lime peel and soursop leaves as prepellent mosquitoes *Aedes aegypti*. The use of prepellent generally does not directly kill insects, but is more functional to resist the presence of insects, especially with a pungent smell seen from the perch of the insect. Insects, especially *Aedes aegypti*, have chemical receptor organs found in the palps and antennae. Prepellent control, both chemical and botanical, targets receptor organs where they are very sensitive to chemical compounds.

Table 2. Results of observations of the flight response of *Aedes aegypti* on prepellent with concentration variations within 60 minutes.

Prepelle nt	Prepellent Duration/minut							
	P	P	P	P	P	P	P	P
	1	2	3	4	5	6	7	8

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	1	1	2	3	1	3	4	6
Citrus	2	9	9	5	9	7	9	0
15%	,	,	,	,	,	,	,	,
	2	5	0	3	3	4	3	0
	7	1	8	3	3	2	3	7
	9	1	2	3	2	4	4	5
Citrus		8	8	1	0	4	8	8
30%	,	,	,	,	,	,	,	,
	5	8	3	3	1	5	1	5
	2	3	3	6	6	7	3	2
	1	1	3	3	2	3	4	5
Citrus	0	8	0	4	3	9	9	8
45%	,	,	,	,	,	,	,	,
	2	1	0	1	3	2	1	3
	5	6	1	5	3	5	6	3
	1	1	2	3	2	3	3	5
Citrus	0	6	5	1	5	5	9	3
60%	,	,	,	,	,	,	,	,
	2	0	1	1	5	2	5	1
	8	7	1	2	4	5	4	6
	1	2	3	3	3	3	4	6
Annona	5	0	8	5	9	7	9	0
15%	,	,	,	,	,	,	,	,
	2	1	0	3	3	4	3	0
	3	8	8	3	3	2	3	7
	1	2	3	3	3	3	4	4
Annona	5	3	8	1	6	7	1	8
30%	,	,	,	,	,	,	,	,
	4	2	3	3	1	5	1	5
	1	4	3	6	6	7	3	2
	1	2	3	3	3	3	4	4
Annona	2	0	5	4	3	6	0	8
45%	,	,	,	,	,	,	,	,

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	2	5	0	1	3	2	1	3
	8	2	1	5	3	5	6	3
		2	3	3	3	3	4	4
Annona	8	1	2	0	2	0	2	0
,	,	,	,	,	,	,	,	,
60%	4	1	1	5	2	5	1	3
	3	8	2	2	5	1	8	4

Observation of the behaviour of *Aedes aegypti* that has been contaminated by lime peel extract and soursop leaves shows decreased activity and less aggression. Such symptoms are characterised by a lack of activity and weakened movements. The skin is generally impermeable and is the boundary that separates an organism from its environment. Chemicals absorbed through the skin are usually in large quantities, so that they will cause systemic effects. Small organisms generally have a relationship between the surface of the body with relatively large body weight, so that if there is contact between the surface of the body and a substance, the active compounds can be easily absorbed.

The way vegetable insecticides work is that if these compounds enter the body of insects will disrupt the digestive tract and inhibit olfactory receptors in the insect mouth area (Vinauger *et al*, 2014). Lime peel extract and soursop leaves that enter the body of insects can also enter the digestive organs then absorbed intestinal walls and flow with blood which will interfere with metabolism. Disturbed metabolism can cause insects to lack energy for their lives. Plant-based insecticides can disrupt the nervous system. Insects are usually paralyzed and can return to normal if they can neutralize the poison, and vice versa will die if they cannot neutralize the poison, then the nerve network will be disrupted and cause insect fatality.

This research focused on repellent on *Aedes aegypti*, with different methods. This study intends to see the difference in the effectiveness of vegetable insecticides of lime peel and soursop leaves as repellents or repellent *Aedes aegypti* using 4 concentrations, namely 15%, 30%, 45% and 60%, 1 hour observation was carried out with a time span of 15, 30, 45, and 60 minutes.

The data from this study showed that there was an effective concentration of lime peel extract and soursop leaves as a repellent of *Aedes aegypti*. Lime peel extract at a concentration of 15% around 72.78% while soursop leaves 75.55%, then at a concentration of 30% lime peel extract around 88.33% while soursop leaves 77.89%, then at a concentration of 45% lime peel extract decreased 86.11% while soursop leaves increased to 92.77%, and at a concentration of 60% lime peel extract increased to 95.00% while soursop

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leaves increased to 97.78%. The distribution of lime peel extract is not constant, while soursop leaves are constant, as can be seen in the schematic figure 1.

The behaviour of *Aedes aegypti* to plant-based insecticides affects flight power. *Aedes aegypti* factor has an olfactometer that can respond to certain chemical compounds. Olfactory mosquitoes are disturbed by the presence of folatyl compounds produced from extracts of citrus fruit peels and soursop fruit. Therefore, the behaviour of *Aedes aegypti* away from repellent sources of different durations based on concentration variations.

CONCLUSION

There was no significant effect on the type of vegetable insecticide prepellent of *Aedes aegypti* mosquitoes. Insecticides of *Citrus aurantifolia* S. and leaves of *Annona muricata* L. both act as prepellent. There is a significant effect on the concentration of lime peel extract and soursop leaves, shown in soursop leaf extract with a concentration of 60% has the most effective ability as a prepellent. Therefore, the behavior of *Aedes aegypti* away from prepellent sources of different durations based on concentration variations.

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