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The Effectiveness Of The Leaf Extract Of Lime And Lemon Grass As Natural Mosquito Insecticide

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ABSTRACT
One of the prevention and eradication of mosquitoes that is safe to be used is natural ingredients from plants. Extract lime leaves and lemon grass contain compounds limonoida, sitronela, and geraniol which are toxic to mosquitoes. The purpose of this study was to determine differences in the effectiveness of extract of lime leaves and lemon grass as natural mosquito insecticide. This study used experimental research and the research method was posttest-only control design. It was an experimental design that consisted of two groups, each of which was selected randomly, namely the control group and the experimental group. The research sample number 5 mice per group with 6 repetitions. Bivariate data analysis using a paired t-test and multivariate data analysis using ANOVA test with α value = 0.05. The results showed that lime leaf extract (sig = 0.000) leaf extract, lemon grass (= 0.002), as well as extract mix lime leaves and lemongrass (sig = 0,000) were effective in killing adult mosquitoes. However, there was no significant difference in the number of adult mortality between minute 10 and minute 20 in the experimental group. The mixture of lime leaves and lemon grass extract were the most influential ingredients. It was effective against adult mortality levels. This extract contained compounds citronellal and geraniol twice to kill more mosquitoes.
Keywords : Extract, Lemongrass leaf, Lime leaf, Mosquito.

INTRODUCTION
Mosquitoes are insects which are more dangerous to human health. [1] Diseases caused by a mosquito bite tropical disease with incidence rates tend to rise. Data from around the world shows that Asia becomes the first rank in the number of patients with a disease caused by a mosquito each year. In Indonesia, the disease caused by the mosquito is still one of the major public health problem. [2]

Until now, the efforts to prevent and eradicate the mosquitoes have been carried out starting from mosquito nest eradication (PSN), fogging, larviciding/mosquito larvae eradication (larvasidasi), and the use of mosquito (including insecticides). Insect or mosquito repellent is commonly used by the majority of Indonesian society to eradicate mosquitoes in the room. Mosquito coils have a variety of types, including liquid, fuel or coil, aerosol and vaporizer (mat, electric liquid, lotion) mosquito repellent. [3]

Basically, a lot of people are fooled by the fragrance of the insect repellent and think that this is the safest option. In fact, the perfume in insect repellent contains active ingredient of d-allethrin. This compound acts as a trigger of some kinds of respiratory problems, such as cough and asthma. [4]

D-allethrin is an organic material that is used as a catalyst combustion processes other insecticides, such as dichlorovynil dimethyl phosphate (DDVP), propoxur (carbamate), and Diethyltoluamide. Therefore, it is not common residue because the burning of these substances will be stuck to the skin. If it happens, it can lead to irritation and redness of the skin rash. [1]

In the study conducted by Prastiwi [3], mosquito coils contain the active ingredients of d-allethrin and transflutrin, respectively by 0.1% and 0.25%. While the same brand, in liquid

and aerosol sipermetrin form, contain 0.4 g / l and 0.10% that causes hypoxia experienced by over-treated mice. Hypoxia is caused due to decreased oxygen levels in the air around the mice room due to the presence of smoke or an electric mosquito coil. Smoke-repellent categorized as one of the sources of indoor air pollution.

According to the results of Wahyono and Oktarinda [5] about the use of mosquito in Jakarta and Depok, from 83 people who responded to the questionnaire (consisted of 65% women, 45% had high school education and 11% one family member had suffered from dengue fever), found that the type of repellent that is often used is an insect repellent lotion (32.5%), spray (26.5%), spray or liquid (18.1%), electrical (15.7%) and fuel (1.2%).

Gradually, the use of the chemical insecticides is now beginning to switch to a plant-based insecticide. Vegetable insecticides are safer to human health, do not leave residue in nature, and reduce pollution. Some examples of plant-based insecticide which contain lime leaf extract can be used as a repellent / mosquito repellent and citronella leaves are also not spared from research on the extract of the power to kill mosquito larvae.

Lime leaves Limonoida contain substances that are considered toxic to mosquito larvae. [6] Limonoida absorbed through the skin (by osmosis) which is permeable larvae will inhibit the metabolic processes in the body of the mosquito. Epidermal cells in the skin that are conducting the current division of skin turn-over process will experience paralysis (paralysis) which in turn can lead to death on the larvae. [7]

Citronella plants contain chemicals that are found in lemongrass stalks and leaves. They contain the most substantial, including sitronela geraniol 35% and amounted to 35-40%, two important chemical compounds that can be used to kill mosquito larvae. [8] Citronellal and geraniol compounds have the characteristic of a contact poison that can cause loss of Aedes Aegypti fluids continuously and eventually death due to dehydration. [9]

Results of a preliminary study that was conducted on October 15, 2018, shows that extracts of lime leaf wetness can cause death of the adult mosquito. It is caused by spray that has been in the extract. Adult mosquitoes are got from the Campus Biomedical Laboratory STIKes Surya Mitra Husada Kediri.

The purpose of this study was to determine the effect of extracts of lime leaves, lemon grass leaf extract, mixture of lime leaves and lemongrass on the level of adult mortality; and to investigate differences in the effectiveness of extracts of lime leaves and lemongrass as a natural mosquito insecticide.

Based on the problem above, the proposed hypothesis is extract mixture of lime leaves and lemongrass provide a greater influence on the level of adult mortality, compared with individual extract lime leaves and lemongrass.

METHODS

This is an experimental type of study to determine the effectiveness of extracts of lime leaves and lemongrass as a natural mosquito insecticide according to time and concentration that have been applied. The study design is the posttest-only control design, which is an experimental design that consists of two groups, each of which is selected randomly, namely the control group and the experimental group. Treatment (X) is given only in the experimental group. [25]

The dependent variable in this study is adult mortality. While the independent variable is the extract and lime and lemongrass.

1. **Test Preparation Materials**
   a. Provide a container or bucket.
   b. The bucket filled with water.
   c. The bucket filled with mosquitoes.
   d. Take the leaves (don’t be too ripe).

2. **Mosquitoes Breeding**
   a. Mosquito larvaes move the dirt and microorganisms along with black on the bottom of the mosquitoes tank into the jar sized 10L.
   b. Cover the top of the jar with gauze.
3. **Preparation of Extract**
   a. Wash the leaves (lime and lemongrass) by using clean water.
   b. Dry the leaves for ± 7 to 10 days to dry completely and easily destroyed when squeezed.
   c. Blend the leaves into fine powder.
   d. Measure each leaf as 20gr.
   e. Pour the powder into a vessel merasi, then add 96% of ethanol liquid (each leaf is carried out separately with a ratio of 240 ml 96% ethanol: 10 g ml leaves)
   f. Especially for the making of a mixture of lime leaves and lemongrass extracts, mix with 10 grams of lime leaves and lemongrass leaves 10 grams, 96% ethanol is then added 480 ml.
   g. Let it stand for 2 x 24 hours
   h. At the time of immersion, stir it every 2 hours for 5 minutes, then three hours later for 5 minutes, and so on up to 2x24 hours.
   i. Always close the merasi vessel when we do not stir it so that the liquid does not evaporate.
   j. Wrap extracts with a glass funnel and filter paper.
4. **Making the preparations**
   a. Set up a spray bottle and then supply a name label extract.
   b. Fill it with lime leaves extracts, lemongrass leaf extract, a mixture of lime leaves extract and lemongrass (each 10 ml).
5. **Stage Research**
   a. Comparison of extract in spray and mosquito living space volume is 1 ml of extract: 1 L volume of the room.
   b. Squirt of lime leaf extract in the jar containing the first group of adult mosquitoes.
   c. Squirt of lemongrass leaf extract in the jar containing the second group of adult mosquitoes.
   d. Squirt a mixture of lime leaves extract and lemongrass in a jar containing the third group of adult mosquitoes.
   e. Observe changes of them after 10 minutes and after 20 minutes.
   f. Repeate 6 times.

RESULTS

1. **Variable Characteristics**
   1) The Death of Mosquitoes in the 20th Minutes with Lime Leaf Extract

Table 1. Number of the Death of Mosquitoes in the 20th Minutes with Lime Leaf Extract

Repetition	Control			Experiment		
				Lime Leaf Extract		
	10 minutes	20 minutes	total	10 minutes	20 minutes	total
1	0	0	0	0	2	2
2	0	0	0	1	1	2
3	0	0	0	1	1	2
4	0	0	0	2	1	3
5	0	0	0	1	1	2

6	0	0	0	2	0	2
Total	0	0	0	7	6	13

Table 1 shows the number of deaths of the mosquitos span of 20 minutes, with 6 repetitions and there are five mosquitoes live in each repetition. There is no mosquito dies until 6 repetitions in the control group. Meanwhile, in experimental group, the mosquito dies in minute 10 with two repetitions, and then there are an increased number of dead mosquitoes in minute 20. But in next repetitions, there is no gain of the number of dead mosquitoes in the 20th minute.

2) The Death of Mosquitoes in the 20th Minutes with Serai Leaf Extract

Table 2. Number of the Death of Mosquitoes in the 20th Minutes with Serai Leaf Extract

Repetition	Control			Experiment		
				Serai Leaf Extract		
	10 minutes	20 minutes	total	10 minutes	20 minutes	total
1	0	0	0	0	2	2
2	0	0	0	1	1	2
3	0	0	0	1	2	3
4	0	0	0	2	2	4
5	0	0	0	2	2	4
6	0	0	0	1	2	3
Total	0	0	0	7	11	18

Table 2 shows the number of deaths mosquito span of 20 minutes, with 6 repetitions and there are five mosquitos live at each repetition. There is no mosquito dies until the 6th repetition in the control group. However, in experimental group, the mosquito dies in minute 10 with two repetitions, and then there are an increased number of dead mosquitoes in minute 20.

3) The Death of Mosquitoes in the 20th Minutes with Mixed of Lime Leaf Extract and Lemongrass

Table 3. Number of the Death of Mosquitoes in the 20th Minutes with Mixed of Lime Leaf Extract and Lemongrass

Repetition	Control			Experiment		
				Mixed of Lime Leaf Extract and Lemongrass		
	10 minutes	20 minutes	total	10 minutes	20 minutes	total
1	0	0	0	2	1	3
2	0	0	0	1	3	4
3	0	0	0	2	3	5
4	0	0	0	2	2	4
5	0	0	0	1	2	3
6	0	0	0	2	1	3
Total	0	0	0	10	12	22

Table 3 shows the number of deaths of the mosquitos span of 20 minutes, with 6 repetitions and there are five mosquitoes live in each repetition. There is no mosquito dies

until 6 repetitions in the control group. Meanwhile, in experimental group, the mosquito dies in minute 10 with two repetitions, and then there are an increased number of dead mosquitoes in minute 20. In the third repetition, the extract mixture and citronella causing the death of all samples.

2. Statistic Test

1) Paired t Analysis

Table 4 Results of Analysis of Effect of Long Time T Couple Adult Mosquitoes' Death for 10 Minutes and 20 Minutes by Granting Extract

Variables Independent	Paired Sample Test	
	T	Sig.
Lime Leaf Extract	0.307	.771
Serai Leaf Extract	-2.000	0.102
Lime Leaf and Lemongrass Extract	-0.675	.530

Based on the table above, it is shown that paired T test results shows no significant effect in mortality of mosquitoes between 10 minutes and 20 minutes with the provision of each extract (sig.> 0,05).

2) Anova Analysis

Table 5 Results of ANOVA Analysis

Group	Test of Homogeneity Variances		
	Sig.		
Lavene Statistics			.007
	ANOVA Sig.		
Death Mosquitoes			.000
	Games-Howell		
Extract treatment	Extract treatment	Mean Difference	Sig.
- Lime Leaf Extract	Serai Leaf Extract	-.833	.249
	Lime Leaf Extract + Serai	-1.500 *	.019
	Control	-2.167 *	.000
- Serai Leaf Extract	Lime Leaf Extract + Serai	-.667	.556
	Control	3,000 *	.002

- Lime Leaf Extract + lemongrass	Control	3,667 *	,000

Based on the table above, it is shown that significant value test Lavene 0.007 < 0.05 (α) so it can be interpreted that the distribution of the data is not homogeneous. ANOVA shows a significant value 0.000 < 0.05 (α), so it can be concluded that there is a significant effect, there is at least one treatment which gives a different effect in the extract lime leaves and lemongrass on adult mortality. Based on Games-Howell output sig. which shows a significant difference is kontrok with all treatment groups extract (0.000 / 0.002 / 0.000 < 0.005 (α)), as well as lime leaf extract with an extract mixture of lime leaves and lemongrass amounted to 0.019 < 0.05 (α).

DISCUSSION

Effect of Lime Leaf Extract against Mosquitoes Adult Mortality

In this study, extract of lime leaves to the survival of adult mosquitoes in the container experiments. Lime leaf extract obtained from the extraction by maceration method using ethanol and 96%. The extraction itself is a process in obtaining a chemical compound (extract) by separating one or more components with the appropriate solvent. Maceration using solvent extraction technique is soaked with some time shaking or stirring at room temperature. [10]

This is in line with research of Prijadi [7] and Liana [11] conducted for Aedes aegypti larvae that the extraction method used is macerated with 96% ethanol. Immersion in research that has been conducted by researchers for 2 x 24 hours in line with research from Liana [11]. This process is different from the soaking process in Prijadi [7] that is for 1 week.

The extract conducted by researchers have differences with Liana's research [11]. The extract conducted by researchers of 10 ml in each experimental group and performed at baseline before long computation time of dead mosquitoes, while Liana's study [11] given the number of different extracts of each treatment is 2 ml; 4 ml; 6 ml; and 8 ml of extract.

The number of deaths of mosquitoes by the extract can be seen in table 1. Table 1 indicates that there is a difference between the number of adult mortality in the control group and the experimental group. In the control group there was no adult mortality until the 20th minute, while the experimental group showed the presence of adult mortality for 20 minutes until repetition to 6. The test results in Table 5 shows the effect of extract in the rate of adult mortality. But there is no big difference (relatively similar) on the number of adult mortality between minute 10 and minute 20 in the experimental group. This is consistent with paired T test results in Table 4.

This is in line with research Murdani [12] that the leaf extract of lime can affect mosquito death (in this case the mosquito larvae). Abilitylime leaf extract in killing mosquitoes due limonoida compounds contained therein. Limonoida will enter the body through the skin permeable mosquito went into the digestive organs and are absorbed by the intestinal wall and then inhibits the metabolic processes so that mosquitoes lack of energy. [13] In addition to affecting the metabolism process, limonoid that spreads to the nervous tissue will affect the function - the function of other neurons and cause seizures larvae which will result in the sudden activity in the central nervous system. [7] Adrianto research results

[14] to strengthen the research conducted by the researchers conclude lime leaf extract led to the death of a mosquito that increase as an increase in the concentration of the extract.

Adult mosquitoes will react in the extracts of lime that has been on his body. In a study conducted by researchers showed that adult mosquitoes have been exposed to the extract gives a mixed reaction. Mosquito artifacts were silent after being given treatment and there is also a mosquito that fell into the water in the container experiment.

Limonoid compounds in the extract into the mosquito's body and damage body tissues mosquito itself causes the organ is not functioning as it should. Mosquitoes will lose consciousness and lime leaf extract has worked effectively in killing adult mosquitoes.

Effect of Leaf Extract Serai against Mosquitoes Adult Mortality

In research that has been conducted by researchers used a solvent extraction method maserai with 96% ethanol. To obtain an extract of leaves of lemongrass, soaking 2 X 24 hours and then filtering the results maserai. The filtered extract is then given to each group of experiments as much as 10 ml. Extract given at the beginning before calculating the time for 20 minutes.

This is supported by research Nisak and Amilah [9] that the immersion time lime leaves and lemongrass for 2-3 days. The amount of the extract on studies conducted by different researchers about 5 ml of research Yulianis et al. [15] Yulianis et al [15] give 5 ml of extract of leaves of lemongrass kitchen in the experimental group.

Results of extract of lemongrass can be seen in Table 2 that in the control group there were dead mosquitos and the movement was clearly active. For the experimental group there were dead mosquitos began minute to 10 and increase in minute 20, but a repetition of the first to demonstrate the mosquito mortality occurred at minute 20. The test results in Table 5 shows the effect of extract in the rate of adult mortality. Based on Table 4 the number of mosquito mortality difference between minute 10 and minute 20 in the experimental group was relatively similar and there are no significant effects.

This is in line with research Yulianis et al [15] that the essential oils of lemongrass leaves the kitchen to have activity in killing mosquitoes. Variations in the concentration of essential oils of lemongrass leaves the kitchen affect the level of adult mortality. The higher level of mortality of mosquitoes, the greater the concentration of essential oils of lemongrass leaves the kitchen. This is reinforced by research daughter [16] that mosquito mortality is influenced by the essence of lemongrass leaves.

The leaves of lemongrass contains the active substance in the form of citronellal and geraniol which can serve as a natural insecticide mosquito. [24] The content of the active substance in lemongrass plant is located in lemongrass and citronella leaves. Moreover, the content in the leaves of lemongrass essential oil by 85%. [17] According Hidayati, et al [18] The essential oil has toxic properties. Essential oils get into the mosquito's body through the cuticle, trachea and mouth mosquitoes. Mosquitoes will die if direct contact with the active substance. Toxic compounds in the leaves of lemongrass that enter the mosquito's body will cause mosquito dehydrate continue - constantly. So mosquitoes will always feel dehydrated and soon will experience death. [19]

In the research that has been done, the mosquitoes are exposed to extracts of leaves of lemongrass react falling on the water container where it grows on the experiment and did not react at all. This is consistent with research Saleh et al [20] that the mosquitoes that death has a sign such as mosquitoes do not move at all and shows stiffness despite having received the stimulation of touch.

Based on the review showed that the lemongrass leaf extract may affect adult mortality. This is because the active substance in the form of essential oils, stronelal, and geraniol

which serves as toxins in the bodies of mosquitoes. Mosquitoes will experience signs of death in the form of stiff and does not move at all after contact with lemongrass leaf extract.

Effect of Mixed Leaf Extract Lime and Lemongrass against mosquitoes Adult Mortality

Based on table 3 it can be seen that compared with the control, experimental extract lime leaves and lemongrass mixture can cause death in mosquitoes since the first repetition. Even granting that the whole sample extract can kill mosquitoes. This happens on repetition to 3. The number of dead mosquitoes were big enough to mean the death of mosquitoes each repetition of four mosquito (3.67).

Extract mix lime leaves and lemongrass provide a significant impact on adult mortality rates nyaumuk based on test results in Table 5. Length of time of death mosquitos 10 minutes and 20 minutes did not significantly affect the number of adult mortality. This is consistent with the test results in Table 4.

Essential oil is a natural ingredient insecticide mosquito. Essential oils are volatile by nature and are secondary metabolites of plants, making this oil is called oil fly. Essential oils can be found in all parts of the plant. [21] Research that has been conducted by researchers supported by the concept of Millati and Sofian [22], that the plant lemongrass, zodia, basil, rosemary and grapefruit contain essential oils with the most potential as an alternative mosquito repellent that is the main compound sitronela, geraniol, linalool and limonene.

Geraniol and citronellal compound is a compound contained in the leaves of lime [23], in addition to compounds that are limonoida Juvenile hormone analogues in insects and nature as a stomach poison on mosquitoes. [7] Geraniol and citronellal compounds are also present in the leaves and lemongrass. Two of these compounds function as mosquito and is a contact poison that can kill mosquito larvae. [15]

Based on research that has been conducted by researchers, the extract mixture of lime leaves and lemongrass effect on the rate of adult mortality. This is caused by the compound limonoida, citronellal, and geraniol contained in each leaf.

Difference Effect of Leaf Extract Lime and Lemongrass against mosquitoes Adult Mortality

Based on Table 1 to Table 3 it can be seen that the highest total mortality of mosquitoes in the experimental group happens to extract a mixture of lime leaves and lemongrass, and total mortality was lowest in the mosquito extract lime leaves. Yet not discovered the dead mosquitoes in the control group in each repetition, because there is no treatment of the extract. So it can be seen that the extract to give effect to the mosquito mortality when compared with the control group (Table 5 ANOVA 0.00 <0.05 (α)).

Prijadi research results et al [7] explains that lime leaves to give effect to the mosquito larvae mortality. Death mosquito counted within 24 hours after treatment, while research has been done taking the time counting in 20 minutes after treatment. This is because the sample of adult mosquitoes death total in the last 40 minutes.

The ability to kill the larvae extracts due lime leaves contain compounds that are limonoida Juvenile hormone analogues in insects and nature as a stomach poison in mosquitoes. Lime leaves also contain compounds geraniol, and citronellal which are toxic to mosquitoes. [23]

In Table 5 indicate that the lemongrass extract did not affect effective than lime leaf extract, and mix extract lime leaves and lemongrass more effectively influence diandingkan with lime leaf extract. But this extract did not affect effective than citronella extract.

This is consistent with research Nisak and Amilah [9] that there is no significant difference between the extracts of lime and lemongrass leaf extract is based on the analysis of

independent t test (p> 0.05 (α)). But the lethal concentration 50 leaves of lemongrass extract higher 0.01 points from lime leaf extract (1.59> 1.57 g / l).

Mixing of the two leaf extract causes an increase in the number of citronellal and geraniol compounds that exist in the lime leaves and lemongrass, thus providing them with a large adult mortality (22 in number). In addition to the increase in the number of such compounds, compounds limonoida also instrumental in helping to influence adult mortality.

Based on the above review, it is known that the extract mixture of lime leaves and lemongrass extract the most influential is effective against adult mortality levels. This is because the mosquitoes toxic compound including, the compound is more than other extracts.

CONCLUSION

Based on the research findings, lime leaf extract, lemongrass leaf extract, and extract mix lime leaves and lemongrass effect on the rate of adult mortality. Meanwhile, to extract the most influential effectively to the level of adult mortality is an extract of a mixture of lime leaves and lemongrass.

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