

Dosage Effectiveness of Kaffir Lime Leaf Powder (*Citrus Hystrix*) in Aromatic Wax Preparations as a Natural Repellent, House Flies (*Musca Domestica*)**Nirwana**

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ABSTRACT

*House flies (*Musca domestica*) are mechanical vectors of various diseases that endanger human health. Chemical fly control has risks to the environment, so a more environmentally friendly natural alternative is needed. This study aims to determine the effectiveness of kaffir lime leaf powder (*Citrus hystrix*) in aromatic wax preparations as a house fly repellent. The method used is quasi experimental with a post-test only control group design. Subjects were divided into five treatment groups (doses of 0, 10, 20, and 30 grams and white candles as positive controls), each with five replicates and 20 flies per test. The results showed an increase in fly repellent power as the dose increased. The average number of flies expelled: 4 (10 g), 9 (20 g), and 14 (30 g). The ANOVA test showed a significant difference between treatments ($p < 0.05$), and the probit test showed an ED_{50} value of 22.667 grams. In conclusion, kaffir lime leaf powder aromatic wax is effective as a house fly repellent, especially at a dose of ≥ 30 grams, and has the potential to be an environmentally friendly vector control alternative.*

Keywords : *Citrus hystrix*, aromatic wax, house fly, natural repellent, *Musca domestica***INTRODUCTION**

According to the Minister of Health Regulation No. 2 of 2023 concerning the Implementation Regulation of Government Regulation No. 66 of 2014 concerning Environmental Health, vectors are arthropods that can transmit, move and/or be a source of disease transmission. Therefore, in order to achieve environmental health quality standards and health requirements, it is necessary to make efforts in vector control.¹

A healthy environment needs to pay attention to the vectors that have an important role in the spread of disease to humans. One of the insects that acts as a vector of disease is flies. The existence of flies is affected by supporting factors such as food sources, temperature or temperature conditions and humidity. This makes the perspective that the existence of flies is an indicator of good and bad sanitation in a place.²

Flies act as intermediate vectors in the spread of diseases due to their ability to carry pathogens on their bodies. Flies have a body surface filled with a fine hairy structure, which can serve as a medium or a place for pathogens such as bacteria or viruses to attach. Flies tend to land on feces, garbage and carcasses, places where pathogens can attach to their bodies. When flies then land on unprotected food or food surfaces, they can transfer the pathogen. Thus, the consumption of food contaminated by bacteria carried by flies can cause foodborne diseases such as diarrhea.³

There are two types of flies that often cause harm to humans, namely the house fly species (*Musca domestica*) and the green fly (*Lucilia sericata*).⁴ House flies (*Musca domestica*) are one of the types of flies that are known to be carriers of disease and are often found in places where there is wet garbage from household waste, especially organic matter that is decaying and is known to be able to transmit around 100 types of disease-causing organisms to humans and animals.⁵

House flies (*Musca domestica*) are known as mechanical vectors of various diseases that are harmful to human health, such as cholera, thypoid, diarrhea, and dysentery by carrying several types of bacteria such as *Salmonella*, *Shigella*, *Escheriscia coli*, and *Staphylococcus* which are widely found in the human digestive tract.⁶ Therefore, in order to meet the standards of healthy environmental quality and established health requirements, it is important to carry out these fly vector control efforts. Thus, the risk of spreading diseases caused by flies can be significantly reduced.

One of the efforts to control flies that can be done is to repel the existence of fly vectors. The common way people use to repel flies is to spray air fresheners and use adhesive traps, but this method is considered less effective in controlling flies. Therefore, there is a need for other alternative formulations that are useful for controlling fly vectors.⁷ The community also controls by using chemicals to reduce the fly population immediately, but the use of chemicals is not safe enough if used excessively because it can leave residues that reduce the quality of the environment. Therefore, efforts are needed to minimize the use of chemicals

through the use of alternative materials that are more environmentally friendly, such as the use of natural materials from plants that function as insect repellents.⁸

One of the plants that has the potential to be an insect repellent is kaffir lime leaves (*Citrus hystrix*), this is because kaffir lime leaves have bioactive and positive content containing flavonoid compounds, saponins, tannins, alkaloids and essential oils.⁹ The compound that plays a major role in repelling/repel insects is citronellal compounds contained in the composition of kaffir lime leaf essential oil, this is evidenced by the results of previous research which states that citronellal compounds in the essential oil content of kaffir lime leaf extract are able to reject the number of *S. oryzae* insects present.¹⁰ The effect of repellent is suspected to be due to the active effect of citronellal compounds in the essential oil content of kaffir lime leaves causing insects to lose their appetite or to act as an antifeedant. This causes insects to be reluctant to approach the surrounding environment of substances containing citronellal compounds even though they have been given bait.¹¹

The method of making plant-based repellent (natural insect repellent) can be done in various ways, one of which is by drying plant material and grinding it into powder or flour so that it is more practical to use. This is proven from previous research, which obtained results that kaffir lime leaf powder is effective in repelling American cockroaches (*Periplaneta americana*). The effectiveness of leaf powder is known because of its citronellal content which is insect-repellent.¹²

In addition to the use of air fresheners in the form of sprays and adhesive traps, another alternative that can be applied in an effort to control house flies is to use aromatic candles. Candles that were initially only used as a lighting device, but now have another function, namely as aromatic candles that are used as a form of relaxation. Aromatic wax is also known to have a function as a fly repellent, this is because the aroma produced by the wax comes from the content of plant essential oils that have the potential to be insect repellent.⁷ The active force of this aromatic wax is as a repulsion force in insects, the essential oil content in the burnt plant will float in the air in the form of micro particles, then enter the respiratory tract of insects and cause disturbances in the sensory nervous system of insects. Thus, insects will quickly dodge and be reluctant to approach even if they have been given a bait.¹¹

Aromatic candles as a fly repellent can be used by every group of people because of their environmentally friendly nature, besides that they can also be applied in public places, especially restaurants. Restaurants are often an attractive location for flies to forage for food and shelter. This is because restaurants usually have abundant food sources, both provided to visitors and from organic waste resulting from the food processing process. Flies are attracted to the aroma of the food available and also to the humid environmental conditions inside the restaurant. Therefore, aromatic candles are very suitable for application in restaurants to repel the presence of fly vectors.¹³

Based on the above background, it can be seen that insects are rejected because of the compounds contained in the essential oil of kaffir lime leaves (*Citrus hystrix*), then kaffir lime leaves (*Citrus hystrix*) can be formulated in powder form and mixed in wax preparations as an alternative to other preparations to disperse the presence of vector insects. In this case, the researcher is interested in researching and wants to find out "The effectiveness of the dosage of kaffir lime leaf powder (*Citrus hystrix*) in aromatic wax preparations as a natural repellent for house flies (*Musca domestica*)".

RESEARCH METHODS

This study is a *quasi experimental* research with a Post-Test Only Control Group Design. Subjects were randomly divided into two groups, namely the experimental group treated with kaffir lime leaf aromatic wax (*Citrus hystrix*), and the control group with paraffin and white candle wax. Effectiveness was tested based on the results of observation after treatment (post-test).

The object of the study was an adult house fly (*Musca domestica*) aged 2–5 days obtained through breeding with wet rice and fresh shrimp media, from flies caught at TPS Pasar Lambaro, Aceh Besar. The test subject was the effect of fly repellent by aromatic wax.

The study used 5 replications, according to the calculation of the degree of freedom $(t-1)(r-1) \geq 15(t-1)(r-1) \geq 15$. Each group consisted of 20 flies, so the total sample was 500. The aromatic wax is made with doses of kaffir lime leaf powder of 10 g, 20 g, and 30 g per treatment, with a total requirement of 300 g for five replications.

The research was conducted in February-March 2024 at the Pharmacy Laboratory of the Ministry of Health of the Ministry of Health of Aceh for the manufacture of candles, and in Lueng Bata, Banda Aceh for fly breeding and experiments.

RESULTS AND DISCUSSION

Result

1. Univariate Analysis

Table 1
Average Number of House Flies Expelled After Being Given Kaffir Lime Leaf Aromatic Candle

Powder Dosage	Number of Flies	Number of Flies That Land and Be Driven Away for 60 Minutes										Average Fly Squirt
		P1		P2		P3		P4		P5		
		H	T	H	T	H	T	H	T	H	T	
K (-)	20	19	1	20	0	20	0	20	0	21	0	0
K (+)	20	18	2	17	3	17	3	17	3	18	2	3
10 gr	20	15	5	17	3	16	4	17	3	17	3	4
20 gr	20	9	11	10	10	13	7	12	8	12	8	9
30 gr	20	9	11	5	15	5	15	5	15	7	13	14

Source : Primary Data, 2024

Information:

K(-) = negative control (0 grams of powder)

K (+) = positive control (white candle)

P(1,2,3,4,5) = repetition

H = perch (number of flies perched)

T = expelled (number of flies expelled)

Table 1 above shows the average number of house flies (*Musca domestica*) that were expelled after being given an aromatic wax of kaffir lime leaves (*Citrus hystrix*). The flies that were expelled at a dose of 10 grams were 4 heads, at a dose of 20 grams as many as 9, and at a dose of 30 grams as many as 14 heads.

Furthermore, to find out the average percentage of house flies that are expelled after being given kaffir lime leaf aromatic wax, a calculation of their repellent power (DT) is carried out. The repulsion force is calculated based on the number of flies that perch on the bait, where the difference between the initial number of flies and the number of flies that catch on the bait is divided by the initial number of flies and then percentaged. The results of data processing from the research that has been carried out can be seen in the presentation of the following table.

Table 2
Repellent Power (%) of Kaffir Lime Leaf Aromatic Wax (*Citrus hystrix*) Against Flies Rumah (*Musca domestica*)

Replication	DT (%)				
	K (-)	K (+)	10 gram	20 gram	30 gram
1	5	10	25	55	55
2	0	15	15	50	75
3	0	15	20	35	75
4	0	15	15	40	75
5	-5	10	15	40	65
Average	0	13	18	44	69

Source : Primary Data, 2024

Information:

DT = Repellent Power (%)

K(-) = negative control (0 grams of powder)

K (+) = positive control (white candle)

Table 2 above shows that the highest percentage of repulsion is found at a dose of 30 grams with a percentage of repulsion of 69%, which is as many as 14 flies that are expelled, while the lowest repulsion is shown at a dose of 10 grams with a percentage of repulsion of 18%, which is as many as 4 flies that are expelled.

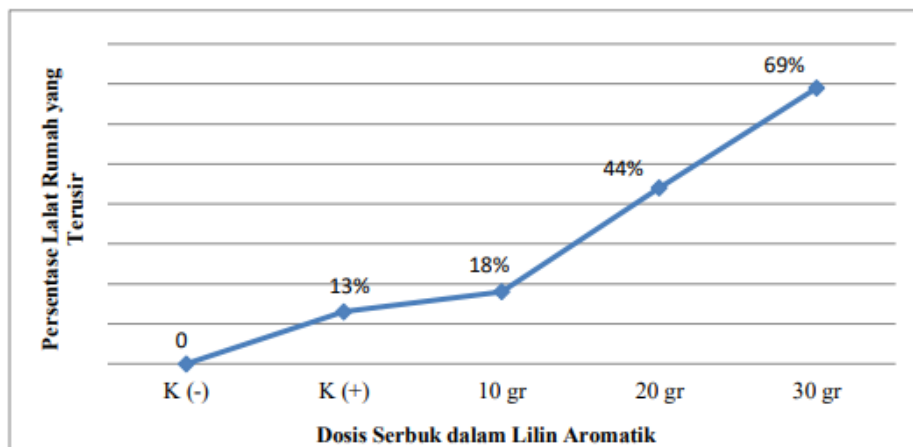


Figure 1 Graph of the Percentage of Repellent Resistance of Kaffir Lime Leaves (Source: Primary Data, 2024)

The figure above shows that there is a direct relationship between the repulsion and the dose of kaffir lime leaf powder in aromatic wax preparations, meaning that the higher the dose given, the stronger the repulsion produced. The most expelled houseflies and show the average difference with other doses is at a dose of 30 grams. As for the dose of 10 grams and positive control, there is only a slight difference/difference in the average of flies that are expelled, meaning that the aromatic candle of kaffir leaves at a dose of 10 grams with positive control has the same repulsion ability.

2. Bivariate Analysis

Table 3
Anova One Way Test Results of Kaffir Lime Leaf Aromatic Wax (*Citrus hystrix*) as an Repellent Natural House Fly (*Musca domestica*)

Treatment Dosage	Average	Std. Deviation	95% Confidence Interval		P
			Lower Limit	Upper Limit	
K (-)	20,00	0,707	19,12	20,88	0,000
K (+)	17,40	0,548	16,72	18,08	
10 gram	16,40	0,894	15,29	17,51	
20 gram	11,20	1,643	9,16	13,24	
30 gram	6,20	1,789	3,98	8,42	
Total	14,24	5,158	12,11	16,37	

Source : Primary Data, 2024

Information:

K(-) = negative control (0 grams of powder)

K (+) = positive control (white candle)

Based on table 3, it is known that the results of the one way anova test show a significant value of 0.000 ($p < 0.05$) meaning that there is an effect, this can be seen from the average comparison of each dose of kaffir lime leaf powder (*Citrus hystrix*) in aromatic wax preparation.

Furthermore, to find out which treatment is significantly different between the variations of treatment given, it is necessary to carry out a follow-up test, namely LSD (Least Significant Difference) testing. The test results can be seen in the following table.

Table 4
LSD Test Results of Kaffir Lime Leaf Aromatic Wax (*Citrus hystrix*) as an Repellent Natural House Fly (*Musca domestica*)

(I) Dosage	(J) Dosage	Meaningful Difference (I-J)	P	95% Confidence Interval	
				Lower Limit	Upper Limit
K (-)	K(+)	2,600*	0,003	0,98	4,22
	10 gram	3,600*	0,000	1,98	5,22
	20 gram	8,800*	0,000	7,18	10,42
	30 gram	13,800*	0,000	12,18	15,42
K (+)	K(-)	-2,600*	0,003	-4,22	-0,98

	10 gram	1,000	0,211	-0,62	2,62
	20 gram	6,200*	0,000	4,58	7,82
	30 gram	11,200*	0,000	9,58	12,82
10 gram	20 gram	5,200*	0,000	3,58	6,82
	30 gram	10,200*	0,000	8,58	11,82
20 gram	10 gram	-5,200*	0,000	-6,82	-3,58
	30 gram	5,000*	0,000	3,38	6,62
30 gram	10 gram	-10,200*	0,000	-11,82	-8,58
	20 gram	-5,000*	0,000	-6,62	-3,38

Source : Primary Data, 2024

Information:

(*) = there is a difference

K(-) = negative control (0 grams of powder)

K(+) = positive control (white candle)

Based on table 5.4 of the LSD (Least Significant Difference) test, differences were found in each dose variation marked by a significant value of $P < 0.05$. In the negative control (0 grams) it was significantly different from the positive control, the doses were 10 grams, 20 grams and 30 grams. In positive control and 10 gram doses were significantly different from 20 gram and 30 gram doses. At a dose of 20 grams it is significantly different from the dose of 10 grams and 30 grams. At the 30-gram dose it was significantly different from the 10-gram and 20-gram doses, except for the 10-gram dose with positive control (P -value > 0.05).

Next, a probit regression test was carried out to determine the effective dose that could cause the most effective expulsion of flies in the test cage for 60 minutes. The test results can be seen in the following table.

Table 5
Probit Test Results of Kaffir Lime Leaf Aromatic Wax (*Citrus hystrix*) as an Repellent
Natural House Fly (*Musca domestica*)

PROBIT	Powder dosage (grams)	95% Dose Limit	
		Lower Limit	Upper Limit
ED50	22,667	20,517	25,143
ED90	40,890	36,145	48,890

Source : Primary Data, 2024

Table 5 above shows the probability of the relationship between the percentage level of house fly repellency (*Musca domestica*) and the dose of kaffir lime leaf powder (*Citrus hystrix*). In aromatic wax preparations as a natural repellent for house flies, the ED50 (*Musca domestica*) value is found at a dose of 22,667 grams while the ED90 value is found at a dose of 40,890 grams.

Discussion

This study was conducted to determine the effect of the dosage of kaffir lime leaf powder (*Citrus hystrix*) in aromatic wax preparations as a house fly repellent (*Musca domestica*). The test was carried out with 3 doses of treatment and 5 repetitions with each dose of kaffir lime leaf powder (*Citrus hystrix*) used, namely 10 grams, 20 grams, 30 grams, and using 2 controls as a comparison, namely negative control (0 grams) and positive control (white candle).

House flies in this study were caught or taken directly at the Lambaroe Market TPS, Aceh Besar. The house flies used for one treatment were 20 and were carried out 5 times. Kaffir lime leaves were obtained from the researcher's yard which was dried and used as a powder for experiments, namely 300 grams of dark kaffir lime leaf powder which will be mixed in an aromatic wax preparation.

The testing stage was carried out on the research sample. Previously, a room temperature and humidity test was carried out first to see whether there was an influence of environmental factors on the power to repel house flies. The average room temperature measurement was 29°C while the temperature range of activity and life of flies was between 22 – 35°C.¹⁴ The average room humidity measurement results are 80% while the optimum humidity of active flies ranges from 45 – 90%.¹⁴ Based on the measurement results, it shows that temperature and humidity do not affect flies because they are still in the range of temperature and humidity of activity and life of flies. Each test cage was inserted 20 house flies for the aromatic wax of kaffir lime leaves with a dose of 10 grams, 20 grams, 30 grams while the control treatment was carried out in a different room, then the number of house flies that perched on the bait was observed for 60 minutes of exposure.

The results of observations showed that in the control treatment, flies were very active in the shrimp skin bait due to the pungent aroma of shrimp skin and was preferred by house flies (*Musca domestica*). On the other hand, in the treatment of doses of 10 grams, 20 grams, and 30 grams, the number of flies that perched on the bait decreased, and the flies tended to stay longer on the cage wall. This is due to the aroma produced from the aromatic wax of kaffir lime leaves (*Citrus hystrix*).

The test results of the number of house flies (*Musca domestica*) that perched on the bait after being given aromatic wax showed that the higher the dose, the less number of flies landed. The dose of 30 grams is the best dose of treatment, this is because the least perch is found in aromatic wax with a mixture of 30 grams of kaffir lime leaf powder with a percentage of expellency of 69%. Meanwhile, the effective dose of treatment was tested using probit analysis. It is known that ED50 is 22,667 grams, which means that the dose has effectively expelled 50% or half of the population of house flies tested for 60 minutes.

Based on the results of the repellent test, kaffir lime leaf aromatic wax (*Citrus hystrix*) has a medium repellent scale, this is based on the results of the calculation of the repellent power of house flies (*Musca domestica*) which is 69%. The repulsion scale is at a moderate level, this is not like previous research related to the use of papaya leaf powder in paraffin wax preparations against the presence of flies. A dose of 30 grams has been effective in repelling flies because only 1 fly has landed on the test medium. This is due to possible obstacles during the study, namely the unstable flame of the candle when burned so that the aroma that comes out is not too strong. Among the possible causes are a wick that is too large or there are air bubbles inside the candle. Based on the research that has been conducted, the cause of the flame that is too large is due to the size of the wick that is too large, as a result of which it will produce an unstable flame and burn fuel faster. The unstable flame may also affect the repulsion of house flies because if the fire grows, the aroma that comes out is not strong, but this has been anticipated by cutting the wick until it leaves a little and touches the surface of the liin so that when it burns it can immediately emit an aroma and let the fire burn for ± 5 minutes outside the cage until the fire becomes stable and then testing the test sample.

From the research that has been conducted, there is an increase in the percentage of the number of house flies (*Musca domestica*) that are expelled along with the increase in the dose of kaffir lime leaf powder (*Citrus hystrix*) in aromatic wax preparations, this means that the higher the dose of powder, the more house flies are expelled. This is in line with the research of Rika Wulandari (2018), who explains that flies do not like the smell emitted from the aromatic wax of lime leaf extract (*Citrus aurantifolia*). The number of house flies that are expelled varies depending on the concentration of lime leaf extract given. The higher the extract given, the higher the number of flies that are expelled, because the active ingredients in plant ingredients will increase along with increasing concentration.¹¹

The results of the data analysis of the number of flies expelled in this study obtained a P value of 0.000, meaning that H_a was accepted and H_o was rejected so that it was known that there was an effect of aromatic wax of kaffir lime leaves (*Citrus hystrix*) with doses of 10 g, 20 g, and 30 g on the number of house flies (*Musca domestica*) that were expelled.

Sitronellal is one of the compounds in the essential oil content of kaffir lime leaves that effectively works as an insect repellent. The characteristic property of these compounds is that they emit odors or aromas that flies do not like. The way citronellal compounds work is antifeedant, this is evidenced by the results of research by Amad and Ulfiani (2018) who stated that citronellal compounds in the essential oil content of kaffir lime leaf extract are able to reject the number of *P. americana* insects present, where the effect of repellent is suspected to cause insects to lose appetite or is an antifeedant.¹² The Gospel of Jesus Christ

The mechanism of how the aromatic wax of kaffir leaf (*Citrus hystrix*) works in repelling house flies (*Musca domestica*) is to disrupt the respiratory system and nervous system of flies, where the chemical compound content of kaffir leaf powder will burn and float in the air and then enter the respiratory tract of house flies. The resulting scent will make the fly lose its appetite, so the fly will avoid the area around the candle even though it has been bait.

Kaffir lime leaves (*Citrus hystrix*) in addition to having benefits to be used as a medicine can also be used as an alternative to vector control, namely as an insecticide or repellent, as in this study. Therefore, the use of kaffir lime leaf powder (*Citrus hystrix*) in aromatic wax preparations is one of the options to minimize the use of chemicals that are harmful to humans and the surrounding environment.

CONCLUSIONS AND SUGGESTIONS

Conclusion

1. The average number of house flies (*Musca domestica*) that are expelled on kaffir leaf aromatic wax (*Citrus hystrix*) at a dose of 10 grams is 4.
2. The average number of house flies (*Musca domestica*) that are expelled on kaffir lime leaf aromatic wax (*Citrus hystrix*) at a dose of 20 grams is 9.
3. The average number of house flies (*Musca domestica*) that are expelled from the aromatic wax of

kaffir lime leaves (*Citrus hystrix*) at a dose of 30 grams is 14.

4. There was a difference between the variation in the dosage of kaffir lime leaf powder (*Citrus hystrix*) in aromatic wax preparations and the number of houseflies (*Musca domestica*) expelled ($P < 0.05$).
5. The results of the study showed that kaffir lime leaf aromatic wax (*Citrus hystrix*) has activity as a repellent or repellent against house flies (*Musca domestica*) with a dose of 22,667 grams has been effective in repelling 50% of the existence of house flies (*Musca domestica*).

Suggestions

1. Suggestions for the public
It is hoped that the community can use kaffir lime leaves (*Citrus hystrix*) as a natural repellent for insects, especially flies. People can apply it in the form of aromatic waxes for use in daily activities, such as putting it on the dining table as a substitute for chemicals to repel flies.
2. Suggestions for Institutions
It is hoped that educational institutions can use this research as information and reference material for the development of knowledge related to vector control efforts.
3. Suggestions for future researchers
 - a. It is necessary to conduct further research on the type of wick used in the manufacture of aromatic candles and the length of time to burn aromatic candles.
 - b. It is hoped that it will be able to conduct further research on the trial of kaffir lime leaf aromatic wax (*Citrus hystrix*) on house flies (*Musca domestica*) by applying it to open spaces, for example in the dining room and researching how much it affects the food nearby.

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