

THE EFFECT OF VARIOUS DOSAGES OF CLOVE ESSENTIAL OIL (*Syzygium aromaticum*) AS AROMATHERAPY CANDLES ON *Aedes aegypti* MOSQUITO PERCHING POWER AND PROTECTION POWER

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Abstract

[The Effect of Various Dosage of Clove Essential Oil (Syzygium aromaticum) as Aromatherapy Candles on Aedes aegypti Mosquito Perching Power and Protection Power] **Introduction:** Dengue hemorrhagic fever vector control can use natural insecticides such as cloves that containing Eugenol. Eugenol gives a distinctive smell and aroma, has a spicy taste and evaporates easily when left in the open air so that the compound can be used as a repellent against *Aedes aegypti* mosquitoes. The research objective was to determine the effect of various dosages of clove essential oil as aromatherapy candle on the perching power and protective power of *Aedes aegypti* mosquitoes. **Methods:** This study used a true experiment with a post-test only control group using variations in the dosage of clove essential oil 0%, 12%, 13%, and 14%. Perching power and protective power were observed every 15 minutes for six hours of treatment. **Result:** The perching power of *Aedes aegypti* mosquitoes on the use of clove essential oil dosage of 0%, 12%, 13% and 14% respectively was 140%, 30%, 15% and 10% with protection power was 0%, 78.57%, 89.29% and 92.85%. Clove essential oil dosage variation had no effect on perch ($p=0.098$); but the protective power has an effect ($p=0.000$). **Conclusion:** The higher the dose of clove essential oil, the higher the protective power, while the most effective dose was 13%, because statistically it was not different from the 14% dose. The protection power reaches 89.29%

Keywords: Clove Essential Oil, Perching Power, Protection Power, *Aedes aegypti* Mosquitoes

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1. Introduction

The dengue virus, which is spread by the *Aedes* sp., causes Dengue Hemorrhagic Fever (DHF). Mosquitoes are one of Indonesia's most serious public health issues. Over the last five decades, the incidence of Dengue Hemorrhagic Fever has increased 30 times and continues to rise in tandem with population migration (1). Every day, 100 to 500 cases of Dengue Hemorrhagic Fever are reported in Indonesia, with a total of 71,633 thousand cases expected by July 2020 (2). Physical, biological, chemical, and environmental management strategies have been used to prevent dengue hemorrhagic fever through the control of *Aedes* sp. mosquito vectors (3). Chemical agents in the form of insecticides are the most commonly used vector control method of all.

Chemical pesticides are the most used method for controlling vectors, despite the fact that long-term use can lead to resistance and have harmful environmental consequences (4). Insecticide resistance can also lead to a failure of vector control (5). As a result, effort should be made to find alternative insecticides that can repel or even kill target insects while having no negative consequences for human health or the environment. There have been numerous other control mechanisms established. Natural pesticides including lemongrass, cloves, tobacco, zodia, and lavender are one of them (6).

Clove is a tropical plant native to Indonesia that can be found in a variety of habitats throughout the country, including the plains, near the coast, and mountainous places up to 900 meters above sea

level. Clove essential oil is one of the clove products, and it contains chemicals that have natural repellent characteristics. Clove essential oil is mostly consists of eugenol, eugenol acetate, and caryophyllene molecules (7). The eugenol concentration of clove flower essential oil ranges from 78 to 95 percent (8). Moreover, clove essential oil includes numerous flavonoids (9) as well as sesquiterpenols, naphthalenes (10), tannins, and saponins (11).

Eugenol is the main component in clove essential oil. Eugenol has a distinct odor and flavor, a spicy taste, and is volatile in the open air, making it suitable for use as a mosquito repellent against *Aedes aegypti* (12). Eugenol is a neurotoxic substance that can make the *Aedes aegypti* mosquito dormant. The *Aedes aegypti* mosquito's neurological system is suppressed by neurotoxic, which is characterized by the insect's body feeling mushy and feeble when handled (13). Clove essential oil is also a vegetable pesticide, larvicide, fungicide, antioxidant, and antibacterial, according to BALITTRO.

The goal of utilizing repellents is to keep infections from spreading from vectors to humans. Spray, cream, and patches are the most common types of repellents used by the general public (14). Although repellent compounds in the form of aromatherapy candles have good potential to prevent contact between humans and *Aedes aegypti* mosquitoes, they have not been widely used by the public. This is because essential oil aromatherapy candles repel the *Aedes aegypti* mosquito by releasing micro-particles that float in the air through the trachea. If enough *Aedes aegypti* mosquitoes ingest these microparticles, they will avoid them and possibly die (15).

Candles are increasingly being used by the community as a fly and mite deterrent (16). While there hasn't been any evidence of clove essential oil aromatherapy candle (*Syzygium aromaticum*) as a mosquito repellent *Aedes aegypti*. The goal of this study is to find a new way to reduce mosquito bites and hence control dengue disease.

2. Material and Method

A true experiment with a post-test only-control group design was used in this study. The independent variables in this study were 0%, 12%, 13%, and 14% clove essential oil (*Syzygium*

aromaticum) as aromatherapy candles. The perch and protection power of clove essential oil aromatherapy candle (*Syzygium aromaticum*) against the *Aedes aegypti* mosquito were the dependent variables in this study. The condition of the bait body, the condition of the mosquito, the size of the test room, and the exposure time of clove essential oil aromatherapy candles were all used as control variables in this study.

The *Aedes aegypti* mosquito used in this study was hatched from *Aedes aegypti* mosquito eggs obtained from Banjarnegara's Class I Health Research and Development Center. The manufacture of clove essential oil aromatherapy candles is done by melting 50 grams of soy wax which is then mixed and stirred until evenly distributed with clove essential oil at a dose of 0%, 12%, 13% and 14%. The soy wax and clove essential oil mixture was then poured into a glass glass with a wax wick in the center and left to harden at room temperature.

The treatment for each dose variation of clove essential oil was repeated six times, by preparing 20 female *Aedes aegypti* mosquitoes in each experiment so that the total sample required was 480 female *Aedes aegypti* mosquitoes. Prior to the experiment, female *Aedes aegypti* mosquitoes were fasted overnight or at least 12 hours. Clove essential oil in the form of aromatherapy candles was exposed to bait in the form of white rats in a three mm thick glass chamber with a length of 0.7 meters, a width of 0.7 meters and a height of 0.7 meters.

The researcher observed the *Aedes aegypti* mosquito's perching capacity and the protective power of clove essential oil as an aromatherapy candle preparation by counting the number of mosquitoes that landed on the bait body. Observations were made every 15 minutes for six hours of exposure. In addition, researchers also measured temperature and humidity in the glass chamber. Statistical analysis used is One-Way Anova with Post Hoc test as a further test (17).

3. Result and Discussion

The exposure of clove essential oil aromatherapy candles was carried out on the results of measuring the average initial and final temperatures in the glasschamber during the test respectively 26.7°C and 27.7°C with initial and

final humidity of the glasschamber of 79.1% and 72.7%, respectively. The average perching power and protection power of clove essential oil in the form of aromatherapy candle preparations against the *Aedes aegypti* mosquito as *repellent* are presented in Table 1.

Table 1. *Aedes aegypti* Mosquito Perch and Protection Power of Clove Essential Oil as Aromatherapy Candle for 6 Hours of Exposure

Dose	Replication	\bar{x} Perching Power	\bar{x} Protection Power
0%	1-6	140%	0%
12%	1-6	30%	78,57%
13%	1-6	15%	89,29%
14%	1-6	10%	92,85%

1. *Aedes aegypti* Perching Power

In the test of clove essential oil repellent as aromatherapy candle, the dosage variations used were 0%, 12%, 13% and 14%. The number of female *Aedes aegypti* mosquitoes used in each test was 20 tails. The test was carried out for six hours and then the *Aedes aegypti* mosquito perched power was calculated using the following formula:

$$\text{Perching Power} = \frac{\sum \text{mosquitoes perched}}{\sum \text{mosquitoes tested}} \times 100\%$$

The results of the average perching power are shown in table 1 where the highest mosquito perch is at a dose of 0% and the lowest mosquito perch is at a dose of 14%. So it can be seen that each dose of clove essential oil used has a different landing power, where the lower the dose of clove essential oil used, the higher the *Aedes aegypti* mosquito's landing capacity. The higher the capacity of the *Aedes aegypti* mosquito to land, the higher the number of mosquitoes that perch. The more mosquitoes that land, the greater the chance of mosquitoes biting and spreading disease through their bites (18).

Mosquitoes perch because of the odor produced by the bait's metabolic processes. Mosquitoes prefer the smell produced by the body's metabolic functions, which draws them to perch. The use of clove essential oil as an aromatherapy candle can affect the *Aedes aegypti* mosquito alighting, where mosquitoes do not like the smell of clove essential oil. Clove essential oil contains

several active components such as eugenol, eugenol acetate, caryophyllene, flavonoids, sesquiterpenol, tannins and saponins. The components of this active ingredient emit an odor which then mixes with other gases in the air and is detected by the mosquito's olfactory receptor (olfactory receptor), which then converts the odor into impulses that are transmitted by sensory nerve axons to the nerve center (brain) and will respond to mosquitoes. avoid the source of the odor (19) which causes mosquitoes to not perch. The lower the dose of clove essential oil used, the higher the mosquito perching power. This is due to the lack of active components of clove essential oil in aromatherapy candles that compete with odors caused by metabolic processes.

Aedes aegypti mosquito perch is influenced by the number of *Aedes aegypti* mosquitoes that perch. In this study, there are several factors that affect the number of *Aedes aegypti* mosquitoes that perch, including the content of clove essential oil in candles, temperature and humidity, where the number of mosquitoes that perch will increase at high temperatures with low environmental humidity (20) and the condition of the mosquitoes being full because they had sucked the blood of the bait at the beginning of the candle exposure. In addition, the number of *Aedes aegypti* mosquitoes perched was also influenced by the order of candle exposure which was carried out randomly, where a higher dose of clove essential oil in aromatherapy candles was exposed before a lower dose of clove essential oil, so there is a risk that the residue of clove essential oil content which is more concentrated on the walls of the test chamber and is still contained in the air around the test chamber which causes a higher effect of clove essential oil residue on the previous exposure on the results of exposure to a lower dose of clove essential oil on aromatherapy candles.

2. The Protection Power of Clove Essential Oil as Aromatherapy Candle

Table 1 shows the average results of the protection power of clove essential oil as aromatherapy candles after exposure for six hours with the repetition of each dose treatment six times calculated using the formula:

$$\text{Protection Power} = \frac{K-R}{K} \times 100\%$$

Explanation :

$K = \Sigma$ mosquitoes perched on the control

$R = \Sigma$ mosquitoes perched on the treatment

Comparison between the concentration of 0% with a concentration of 12%, 13% and 14% showed that there was a comparison of the protection power between the treatment groups given clove essential oil with a concentration of 12%, 13% and 14% with the highest protection power results being at a dose of 14 % with 92.85% protection power. In table 1, it can be seen that the level of protection tends to increase, which means that the higher the dose of clove essential oil in aromatherapy candles, the higher the protection power against *Aedes aegypti* mosquitoes.

The mechanism of repelling mosquitoes using clove essential oil as aromatherapy candle is by evaporating the active compounds contained in clove essential oil in the candle. The higher the dose mixed with aromatherapy candles, the higher the active compound of clove essential oil which is evaporated and mixed with gas in the air. Therefore, the higher the dose of clove essential oil used, the higher the protection power of aromatherapy candles using clove essential oil as a mixture. The active compounds contained in clove essential oil are eugenol, eugenol acetate, caryophyllene, flavonoids, sesquiterpenol, tannins and saponins.

Eugenol and eugenol acetate give a distinctive odor and aroma, have a spicy taste and have neurotoxic properties that cause insects to become inactive. The content of eugenol in clove essential oil is between 75-95%. Caryophyllene has a very pungent aroma and is not liked by insects. Flavonoids, which are active ingredients in the manufacture of vegetable insecticides, have an effect as an antifeedant, namely as a food inhibitor. The chemical compound sesquiterpenol can be used as a contact poison and stomach poison that damages the larval tissues and organs. Tannins can reduce the activity of protease and amylase enzymes and interfere with the activity of intestinal proteins so that mosquitoes eat less. Saponins are detergent-like substances whose insecticidal activity is by damaging membranes.

The active compound content of clove essential oil in the wax evaporates due to the combustion process. Through their antennae,

mosquitoes detect the aroma of attractants (in the form of white mouse bait in this study) and active compounds (eugenol, eugenol acetate, caryophyllene, flavonoids, sesquiterpenols, tannins and saponins) contained in clove essential oil. The active compound in clove essential oil then binds (as a competitive inhibitor of attractants) with Odorant Binding Proteins (OBP) which functions to bind the aroma and pass it on to the Olfactory Receptor Neurons (ORN) olfactory receptors. As a result, aroma attractants cannot bind to Odorant Binding Proteins. Because Odorant Binding Proteins do not bind to attractants, but instead bind to the aroma of the active compound of clove essential oil, Olfactory Receptor Neurons which have a function to recognize aroma cannot recognize the aroma of the attractant, but the aroma of the active compound of clove essential oil. This process is then continued by sending signals to the brain. The result is that the mosquito brain assumes that there is no attractant in that place, so that mosquitoes do not perch (21).

Repellent or mosquito repellent can be said to be successful if it has a protection power of 80% for up to 6 hours (22). Based on the research that has been done, the use of clove essential oil as aromatherapy candle with a dose of 13% and 14% can be said to be effective as a repellent against the *Aedes aegypti* mosquito. At a dose of 13% the protection power for six hours given was 89.29% and at a dose of 14% the protection power of clove essential oil as aromatherapy candle was 92.85%. This is in line with the research conducted by Kardinan (23), where the higher the concentration of basil essential oil, which has eugenol, such as clove essential oil, the better the protection power it provides. A similar study was conducted by Mulyani (24) with the use of clove essential oil in the form of incense combustible (incense) as a repellent at a level of 20% able to repel mosquitoes 100% at 45 minutes and a level of 10% is an effective level. Research conducted by (25) proves that clove leaf extract with a concentration of 35% can be used as a repellent because every hour of observation the protection power is more than 90%. The use of clove leaf extract lotion was also effectively used as a repellent for four hours, ie a dose of 100% was able to resist 93.5% mosquito bites for 1 hour; 86.9% for 2 hours; 83.7% for 3

hours; 81.7% for 4 hours, and at the 5th and 6th hours the repulsion was 76.7% and 51.9% (6).

The big difference in protection power in each study could be caused by the dosage form and the form of the clove plant product used, even at the time of the study, there were mosquitoes that also died. This is caused by the smoke generated from aromatherapy candles entering the insect's respiratory tract through the trachea in the form of microparticles that float in the air. Insects will avoid and even die if they inhale these microparticles in sufficient quantities (15).

3. Analysis of the Effect of Various Dosage of Clove Essential Oil as Aromatherapy Candle on Perching Power and Protection Power of *Aedes aegypti* Mosquitoes

Table 2 shows the results of statistical analysis of the influence of variations in the dose of clove essential oil on the perching power of the *Aedes aegypti* mosquito and the protective power of clove essential oil as aromatherapy candles using One Way Anova (17). Using the Post Hoc test with the test results presented in Table 3, calculate the most effective dose to lower the *Aedes aegypti* mosquito perch and boost the protective efficacy of clove essential oil as an aromatherapy candle.

Tabel 2. Results of Statistical Analysis of the Effect of Variations in Dose of Clove Essential Oil on Mosquito Perchance and Protection Power of Clove Essential Oil

Dependent Variable	F	p	Explanation
Perching Power	2,347	0,098	Not Significant
Protection Power	9,535	0,000	Significant

Because the results of the statistical analysis of protective power are substantial, more tests are needed to determine the dose's effectiveness, while the following are the results:

Tabel 3. Advanced Test Results for Protection Power

Dose	Dose	Mean Difference	p	Explanation
0%	12%	-58,2473	0,004	Significant
	13%	-82,3980	0,000	Significant

	14%	-83,5024	0,000	Significant
12%	13%			Not
		-24,1506	0,195	Significant
	14%			Not
		-25,2550	0,176	Significant
13%	14%			Not
		-1,1044	0,952	Significant

There is a substantial difference in the protective potential of clove essential oil with a dose of 0% when compared to all other dose variations of clove essential oil. However, there was no significant difference in protective power between a dose of 12% and doses of 13% and 14%, or between doses of 13% and 14%. Statistically, the effective dose is at 12%, but because the protective power of 78.57% has not reached 80% at that dose (see table 1), the most effective dose is at 13%, because it has reached the WHO-recommended parameter value and is relatively the same as a dose of 14%.

4. Conclusion

With an effective dose of 13 percent clove essential oil when aromatherapy candles are manufactured with a protective power of 89.29 percent, which has exceeded the WHO 80 percent limit, the conclusion that can be derived is that the larger the amount taken, the greater the protective power.

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