



A Preliminary Study on *Ocimum basilicum* Essential Oil as a Repellent against *Aedes aegypti* in Surabaya

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Abstract

Introduction: *Aedes aegypti* is the main dengue vector in Indonesia. One of the dengue controls is through insecticides use. The plant-based insecticide is one alternative that can be used to minimize the impact of chemical insecticides. *Ocimum basilicum* is one of the plants that have the potency to be used as insecticides. The aims of this study were to analyze the insecticide activity of *Ocimum basilicum* essential oil against *Aedes aegypti* as well as its Lethal Concentration (LC₅₀ and LC₉₀) and Knockdown Time (KT₅₀ and KT₉₀).

Methods: Treatment groups were given *Ocimum basilicum* essential oil of 100 ppm, 50 ppm, and 25 ppm. The tests were performed four times with 10 mosquitoes per test.

Results: The mortality rate of the mosquito on the extract with a concentration of 100 ppm was 100%, 50 ppm was 10% and 25 ppm was 0% and the value of LC₅₀ = 62.9 ppm and LC₉₀ = 78.7 ppm. KT₅₀ of this study was 19.4 minutes and KT₉₀ was 8.07 minutes.

Conclusion: *Ocimum basilicum* essential oil has a mosquitocidal effect on *Aedes aegypti*. Further studies to investigate the substances of *Ocimum basilicum* against *Aedes aegypti* are needed.

Keywords: *Aedes aegypti*, mosquito, essential oil, insecticide, *Ocimum basilicum*

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Introduction

Aedes aegypti mosquito is the vector of Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF) that has been distributed in tropical countries. Indonesia is one of the tropical countries in the world with high humidity that triggers the breeding of mosquitoes such as *Aedes aegypti* so that DHF is easily transmitted through the bite of *Aedes aegypti* mosquitoes. This causes health problems because there are many endemic areas so that the number of sufferers is increasing and the spread is expanding to other regions with increased mobility and population density.

Dengue fever is a major public health problem throughout the tropics and subtropics. The spread of the disease is rapid with a 30-fold increase in incidence over the past 50 years. The World Health Organization (WHO) estimates 50-100 million cases of infection occur each year and nearly half of the world's population is in endemic countries. Currently, about 75% of the global population at risk of the dengue virus is in the Asia-Pacific region. The number of DHF cases has increased dramatically worldwide in recent years. More than 2.5 billion people worldwide, more than 40% are at risk of DHF. An

estimated 50-100 million people worldwide are infected with dengue fever each year.

Surabaya is an endemic area of DHF disease because every year there must be cases that occur in high numbers. In 2021 to 2022 there was an increase in cases from 668 cases (CFR = 1.1 %) to 1.220 cases (CFR = 1.7%). While in 2002 there were 1913 cases with the death of 21 people (CFR = 0.6).¹

Study about mosquito repellent stated that the best way to avoid mosquitoes is to use mosquito repellent in the form of lotions, creams, or clothing that can protect the body from mosquito bites.² However, almost all mosquito repellent lotions circulating in Indonesia are actively made from DEET (Diethyl toluamide) which is a toxic synthetic chemical in concentrations of 10-15%.³ Based on study DEET is a dangerous toxic chemical especially for children and adults if inadvertent use.⁴ The use of dichlorvos in aerosol sprays has been banned by the Government of Indonesia because it endangers human health. The most frequent insecticide used is the chemical one (synthetic). Most of current study spotlight is to determine the resistance status of target organism, or insecticide potency concerning the effective concentration.⁵ Thus, it is necessary to make efforts to avoid mosquitoes by using plant-based insecticides.

In general, plant-based insecticides are defined as an insecticide that could be made with limited ability and knowledge with basic ingredients from plants. Plant-based insecticides can be made with simple technique or traditionally using chairmanship, growth, combustion, or pressing. Indonesia is a tropical country that has biodiversity-producing essential oils. Indonesia has about 40 types of 80 types of aromatic plants producing essential oils traded in the world. One of the aromatic plants producing essential oils is *Ocimum basilicum*.

The research about *Ocimum basilicum* explain that basil has bioactive compounds, eugenol, and methyl clavicle which has potential effects as an insecticide.⁶ Both are the chemical compounds that form essential oil extracted from basil. Another research on

Ocimum basilicum describes that saponin nature, act as an insecticide by affecting insects' dietary habit as it reduces the food uptake in the gastrointestinal tract and illustrates that saponin opiate destruct the cell membrane that promotes cytolysis.⁷ This disturbs cell components affecting cell metabolism in intake and outtake transport. Flavonoid contains neurotoxic effects that cause stretch of the body.⁸

Based on the previous studies mentioned, this study aimed to determine the ability of *Ocimum basilicum* essential oil in various concentrations to be used as a repellent against *Aedes aegypti*.

Method

Essential oil manufacture

The ingredient used in this preliminary study was the pure essential oil of *Ocimum basilicum*. Essential oils were obtained by distilling the leaves by steaming method. Furthermore, essential oils were analyzed with chromatography gas to determine the content of the active ingredient. Treatment in the form of essential oil concentrations *Ocimum basilicum* with concentrations of 100 ppm, 50 ppm, and 25 ppm. Dilution of essential oils using aquades. Aquades were used in dilution because this substance has no repellency towards *Aedes aegypti* mosquitoes.

Repellent Test

Protection power testing was conducted in the Laboratory of Entomology, Institute of Tropical Disease, Universitas Airlangga. A group of 14-day-old female *Aedes aegypti* mosquitoes was used in the test. The research was conducted with a completely random design. The test was conducted for 1 hour starting at 09.00 – 10.15 am. The potential of repellents tested is seen based on the protection power of each treatment. Protection power is calculated by the formula:

$$\frac{\Sigma \text{mosquitoes perched on tx} - \Sigma \text{mosquitoes on tx}}{\Sigma \text{mosquitoes on control}} \times 100\%$$

tx=treatment

Each treatment is tested by applying 1ml (result from the calibration)

essential oil to one of the arms. In turn, the arm is presented with a special bottle assay for repellent and mosquito testing used for protection power testing containing 10 female mosquitoes. The calculation of mosquitoes perched on treatment is based on the number of mosquitoes that come into contact with testers at each swipe. The number of swipes on each test hour is six strokes. The length of time from one swipe to another swipe is ten seconds. After the three cages were moved so that both the hands given treatment and control were in different test cages than before. One swipe is considered a replay. Body temperature, room temperature, and humidity are recorded before testing is performed on each hour of testing.

Data Analysis

Analysis of the data used to measure the Lethal Concentration (LC) and Knockdown Time (KT). Both Lethal Concentration and Knockdown Time values are calculated by using Probit analysis. Lethal Concentration is based on the amount concentration of essential oil needed to killed the mosquito, while the Knockdown Time is based on the length of time for the mosquitoes to die.

Results

In this study, it is known that *Ocimum basilicum* essential oil in liquid form can cause the death of *Aedes aegypti* mosquito. This can be seen from the percentage of *Ocimum basilicum* essential oil protection power against *Aedes aegypti* at different concentrations in **Table 1** and the graphics in **Figure 1**.

Table 1. *Ocimum basilicum* Essential Oil Protection Power against *Aedes aegypti*.

Essential Oil Concentration (ppm)	Power Protection (%)
100	100
50	10
25	0

One of the references used to determine the toxicity value of an insecticide is *Lethal Concentration* (LC). Based on the results of statistical analysis, this study obtained the value of *Lethal Concentration* 50 (LC₅₀) and *Lethal Concentration* 90 (LC₉₀). The LC value of 50 obtained is 62.9 ppm. This means that exposure to *Ocimum basilicum* essential oil with a concentration of 62.9 ppm can lead to death in *Aedes aegypti* mosquitoes as much as 50%. The value LC₉₀ obtained is 78.7 ppm. This means that exposure to

Ocimum basilicum essential oil with a concentration of 78.7 ppm can cause death in *Aedes aegypti* mosquitoes as much as 90%. In addition to *lethal concentration* (LC) analysis in this study also obtained *Knockdown Time* (KT) value. The KT values obtained are *Knockdown Time* 50 (KT₅₀) and *Knockdown Time* 90 (KT₉₀). The *Knockdown Time* calculations in this study were presented in minutes. The KT₅₀ value is 19.4 minutes. The value of *Knockdown Time* 90 (KT₉₀) is 8.07 minutes.

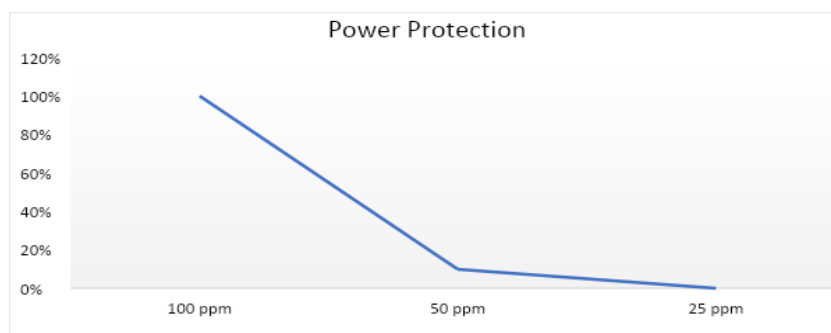


Figure 1. Graphic *Ocimum basilicum* Essential Oil Protection Power against *Aedes aegypti*

Discussion

Based on the results of statistical analysis it can be concluded that it takes 19.4 minutes for 50% of *Aedes aegypti* mosquitoes to experience knockdown due to exposure to *Ocimum basilicum* essential oil with a concentration of 62.9 ppm. The value of *Knockdown Time* 90 (KT₉₀) is 8.07 minutes. If the results of KT₉₀ are linked to the results of LC₉₀ then from the results of statistical analysis it can be concluded that it takes 8.07 minutes for 90% of *Aedes aegypti* mosquitoes to experience knockdown due to exposure to *Ocimum basilicum* essential oil with a concentration of 78.7 ppm. These results are classified either in insecticide testing, as knockdowns that occur in 90% of the *Aedes aegypti* mosquito samples tested still occur within minutes, or even under 10 minutes. These results indicate that the *Ocimum basilicum* has the potential to be developed as mosquito repellent because it is based on requiring that an anti-bacterial lotion mosquito can be effective if their protection at least 90% and able to last for 6 hours.⁹

Ocimum basilicum has some secondary metabolite content including alkaloids, saponins, tannins, and flavonoids. Alkaloids can inhibit the development of insects by disrupting three main hormones of insects, namely brain hormones, ecdysone hormones, and growth hormones. Besides, alkaloids act as anticholinesterases that cause decreased muscle coordination, resulting in death.¹⁰ Saponins can interfere with the respiratory system of insects. If it hits the surface of the insect's skin, the mucosa of the insect's skin will be damaged. Saponins will then enter the body and interfere with the work of insect respiratory enzymes. When the respiratory system is disturbed, the insect may experience death. Tannins act as an antifeedant for insects. Meanwhile, flavonoids work by disrupting the nervous and respiratory systems of insects. Therefore, tannins has potential as insecticides.¹¹ Flavonoids cause disturbances in the spiracles that disrupt respiration, resulting in the death of these insects.¹² Essential oil from *Ocimum basilicum* have been shown to repel insects and have larvicidal activity against

houseflies, blue bottle flies and mosquitoes.¹³

Chemical control of *Aedes aegypti* mosquitoes is still considered the most important in the eradication of DHF. Control that is often done today is chemical control because this way is considered to work more effectively and the results can be felt faster than other ways of control.¹⁴ Therefore, this research was conducted to examine the ability of natural ingredients, namely curdling leaves that are processed into extracts for liquid repellent. Liquid formulations have the advantage of good shelf life, good adhesion to fatty surfaces, are not electrical conductors, and have good penetration power into cracks and crevices, smoke-free, odourless, and able to intercept, paralyze, and kill targets. Also, liquid-repellent works by making mosquito breathing a target. When the repellent is heated, the secondary metabolite content contained in the extract will come out, so that the mosquito's respiratory system will be disturbed.¹¹

Ethics approval

Ethical clearance was obtained from the Ethics Committee for Health Research, Faculty of Medicine, Airlangga University (202/EC/KEPK/FKUA/2019). All subjects had signed informed consent before joining the study.

Availability of data and materials

The results of the data in this study were purely obtained by the author from the research and all of the material availability came from the Entomology laboratory, Institute Tropical Disease, Airlangga University.

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Author Contribution

All author conceived and designed the research. ADW conducted the experiments, analyzed the data and wrote the paper. All authors contributed to manuscript revisions. All authors approved the final version of the manuscript and agree to be held accountable for the content therein.

Conclusion

From the study that has been done, the essential oil of *Ocimum basilicum* has a great potential to be developed as an environmentally friendly insecticide and needs to investigate further on the substances of *Ocimum basilicum* against *Aedes aegypti*. With the $LC_{50} = 62.9$ ppm and $LC_{90} = 78.7$ ppm. KT_{50} of this study was 19.4 minutes and KT_{90} was 8.07 minutes.

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