



The Effectiveness of *Citrus Hystrix* Leaf Extracts on the Application of Electrical Mat in Killing Mosquito *Culex sp.*

Authors

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Abstract

Background: *The most effective and accessible vector control in the community is the use of insecticides. The purpose of pesticides aims to kill vectors to break the chain of dengue fever transmission. However, poisons circulating in the community are still chemical insecticides. Chemical vector control is more widely used for easiness and practical reasons. The content of chemicals contained in synthetic chemical pesticides may interfere human health, contaminate the environment, and have a carcinogenic effect. Efforts to avoid the negative impact of the use of anti-mosquito material can be made by using natural insecticide materials that are relatively environmentally friendly, i.e., the leaves of Citrus hytrix.*

The Objective: *This study aimed to determine the effectiveness of various concentration of Citrus hytrix leaf extract on electrical mat applications in killing mosquito Culex sp.*

Method: *The type of the research was experimental with one group post-test design with control where the treatment group was Culex sp mosquitos exposed to Citrus hytrix leaf extract on the application of electric mat with different concentration variations. The deadly effects on Culex sp adult mosquito were observed and compared to the control group afterward.*

Results: *The results of the test showed there was a significant difference of mosquito mortality rate in each concentration of Citrus hytrix leaf extract. The result of the post hoc test showed that the difference of tested concentrations levels gave significant mean difference effect to the mortality of mosquito.*

Conclusion: *It was obtained that the average mortality of mosquitoes increased by the increased concentration given and there was a significant difference of mosquito mortality in each level of Citrus hytrix leaf extract.*

Keywords: *Citrus hystrix, Culex sp., Electrical mat, Leaf extract, Mosquito.*

Introduction

The most effective and accessible vector control in the community is the use of insecticides. The

method of insecticides aims to kill vectors to break the chain of dengue fever transmission. However, insecticides circulating in the

community are mostly chemical insecticides. Chemical vector control is more widely used for easiness and practical reasons, for example, the use of electrical mat anti-mosquitoes (Kardinan, 2004).

Recently, anti-mosquito material that uses synthetic chemicals which can interfere human health is widely used in Indonesia. Chemical compounds in insecticides are harmful to human health, and it is contained in all anti-mosquito insecticides circulating in the domestic market in Indonesia (Indonesian Pharmaceutical Watch, 2001). Whether in the form of a spray, electric, or liquid, there are dangerous chemical compounds in anti-mosquito insecticides, i.e., dichlorvos, propoxur, and some pyrethroid species such as d-allethrin, transfluthrin, bioallethrin, prallethrin, d-phenothrin, and esbiothrin. These chemical compounds are proven to damage the nervous system and even carcinogenic (Wiwiek, 2010).

Efforts to avoid the negative impact of the use of the anti-mosquito material is the use of plants as the natural insecticide materials, which are relatively environmentally friendly. The primary requirement of the use of plants for anti-mosquito insecticides is that it should contain various compounds functioned as insecticides such as cyanide, saponin, tannin, flavonoids, alkaloids, steroids and essential oils (Susana et al., 2010). One type of plant that has anti-mosquito compounds is lime (*Citrus hystrix*). Based on previous research, results reported that the leaves of *Citrus hystrix* contain flavonoids and essential oil (Qorih, 2010).

Citrus hystrix leaves can potentially be natural insecticides that are not harmful to health because they contain essential oils with limonene, mirsen, linalool, octanal, decanal, citronellol, nerol, geraniol, valensen, sinnsial and sinential components. Linalool, citronellal, and geraniol are including insecticidal compounds capable of killing arthropods. *Citrus hystrix* leaves can be easily applied by extraction with aquades and filtration (Susana et al., 2010; Qorih, 2010).

In the current era where everything is developing, researchers are required to innovate in obtaining environmental friendly goods. Based on above explanations, researchers made the new electrical mat natural mosquito-repellant. The general purpose of this research was to determine the effectiveness of *Citrus hystrix* leaf extract on the application of electrical mat in killing mosquito *Culex sp.*

Materials and Method

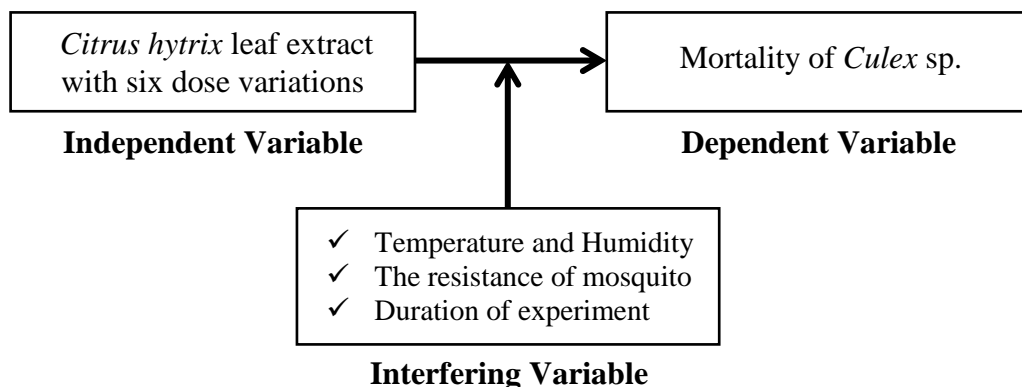
The type of the research was experimental with one group post-test design with control where the treatment group was *Culex sp* mosquitos exposed to *Citrus hystrix* leaf extract on the application of electric mat with different concentration variations. The deadly effects on *Culex sp* adult mosquito were observed and compared to the control group afterward.

The research design used in this research was Completely Randomized Design. In general, this design was used for similar environmental conditions, tools, materials, and media. This experiment was conducted using six different variations of a dose of *Citrus hystrix* leaf extract, including control variations with four repetitions. This research was held at the Parasitology Laboratory of Minister of Health Polytechnic Banten, from March to June 2017.

The population was larvae of *Culex sp.* which had been colonized into an adult mosquito in the Tangerang area, Banten Province, Indonesia. The samples used were 15 *Culex sp.* in each container, treated with *Citrus hystrix* leaf extract with six dose variations.

Primary data from the observation of the number of the dead *Culex sp.* mosquitoes were analyzed using statistical tests to see the difference (difference test). The data obtained from the observation results were first tested using normality and Levene statistic test. Normal and similar data were tested using One Direction Anova (One Way ANOVA). Ho was rejected if $p < \alpha (0,05)$. Unnormal and heterogeneous data were tested using Kruskal-Wallis.

Research Concept Framework



Results

During the experiment, both temperature and humidity were monitored continuously in the morning, afternoon, and evening. The average temperature of the study room was 27.33°C, with the highest temperature was 30.2°C and the lowest temperature was 25.1°C. The average humidity of the study room was about 65.83%, with the highest humidity was 70%, and the lowest was 47%.

Table 1 shows that there was an increase in mortality from each dosage of *Citrus hytrix* leaf

extract electric mat for every 6 hours of observation. The highest mortality occurred at the 24 hours observation with the dosage of 1 mL, which was 15 mosquitoes in the second and third repetition. The percentage of death at 0.5 mL dosage at 24 hours was 64.74%, and it was concluded that *Citrus hytrix* leaf extract was effective in killing *Culex sp.* with a percentage of 100% at a one mL dose during 24 hours of observation.

Table 1 Mortality of *Culex sp.* Mosquito after The Exposure of Electric Mat with *Citrus hytrix* Leaf Extract Active Ingredient

Replication	Dosage			Control
	0,5 mL	0,75 mL	1 mL	
1	10	15	15	1
2	10	14	15	1
3	11	14	15	2
4	10	14	15	1
5	10	15	15	1
6	10	15	15	2
7	11	14	15	2
8	9	14	15	1
9	10	15	15	1
The average mortality of mosquitoes	10,11	14,44	15,00	1,33
% Mortality	67%	96%	100%	9%

Further, the data of the research were tested by using ANOVA to check the difference between the treatment of each concentration during 1-hour

exposure to *Citrus hytrix* leaf extract electric mat and 24 hours observation. Anova results are given in Table 2.

Table 2 Average Mortality of Mosquitos Based on Insecticide Dosage and Mean Difference Test Value

NO	Insecticide Dosage	Average Mortality		Results of Post Hoc Test	
				Test Between Concentrations	p Value
1	Control	1.33	0.000	Control * 0.5 ml	0.000
2	0.5 ml	10.11		Control * 0.75 ml	0.000
3	0.75 ml	14.33		Control * 1.0 ml	0.000
4	1.0 ml	15.00		0.5 ml * 0.75 ml	0.000
				0.5 ml * 1.0 ml	0.000
				0.75 ml * 1.0 ml	0.000

Table 2 shows that the average mortality of mosquitoes was higher by the increasing concentration of insecticide given; with the most top mosquito death rate (15.00) found at 1.0 mL dosage. Bivariate analysis obtained p-value = 0.000 ($p < \alpha$), which means that H_0 was rejected; in other words that there was a significant difference of mosquito mortality rate in each concentration of *Citrus hytrix* leaf extract given.

The results of the follow-up test (post hoc test) obtained a p-value of 0.000 ($p < \alpha$), which means that H_0 was rejected on all test results between concentrations; in other words that the difference of tested concentrations levels gave significant mean difference effect to the mortality of mosquito.

Discussions

Room temperature was an important variable that influenced this research. Room temperature was measured using a thermometer. According to Susanna and Sembiring (2011) and Direktorat Jenderal Pengendalian Penyakit dan Penyehatan Lingkungan (2008), the average temperature of mosquito's optimum growth is 25-27°C. Temperatures above 35°C do not support the growth of mosquito because of it subject to change and slow down the physiological processes, and the growth of mosquitoes stops at temperatures of less than 10°C or above 40°C. Based on these explanations, it can be stated that the temperature in this study still supported the growth and development of *Culex sp.*

Humidity was one of the variables measured in this study. Humidity was measured using a hygrometer. According to Jumar (2000), the humidity that supports the growth and

development of mosquitoes is 60% -89%. For above reason, it concluded that the humidity of the room at the time of the experiment did not interfere the growth of mosquitoes. The lifespan of mosquito will be short if the humidity is less than 60%. Further, it cannot become vector and cannot transfer the virus from the stomach to the salivary gland (Yudhastuti et al., 2005). Adaptation to high humidity lead the mosquitoes to feel tired (Susanna et al., 2011).

Table 1 shows that at a weight of 0 mg mat containing tissue paper which just dropped with water found only 1 to 2 dead mosquitoes. This happened because of the tiredness of mosquitoes when observed for 24 hours. However, on the mat filled with *Citrus hytrix* leaf extract resulted in the death of *Culex sp.* The essential oil of *Citrus hytrix* leaf extract contains volatile compounds and allelochemical compounds such as terpenoids, tannins which will cause the mosquito's metabolism rate to decline (Titik, 2016).

The results of the study showed that with 100 mg weight, 50% mortality of *Culex sp* mosquito had not been achieved. LD₅₀ was obtained at a dose of 200 mg at 24 hours of 53.33% mortality of mosquito. The lethal dose is a precise dose of a material capable of killing 50% of experimental animals (Kardinan, 2004). It was concluded that 24 hours of exposure with one mL volume of *Citrus hytrix* leaf extract was effective in killing *Culex sp.* This means that *Citrus hytrix* plants can be utilized as electric mats that capable to kill *Culex sp.*, because of the chemical compounds content of *Citrus hytrix* leaf extract, i.e., essential oils, limonene, mirsen, linalool, octanal, decanal, Citronellol, neral, geraniol, valensen, sinnsial and sinential. Linalool, citronellal and geraniol

components are insecticides to arthropods (Titik, 2016).

Citrus hytrix leaf extract is effective in killing *Culex sp.* with the mortality percentage of 71.11% at a one mL dosage during 24 hours of observation. This was supported by some research from Agustin Andriana et al. (2012), Umiyan Kamarullah (2015), and Malvin Abidatun Istianah (2013) that *Citrus hystrix* is efficiently used as biolarvicide and bioinsecticide.^{21,22,23} Hebert Adrianto et al. (2014) suggested that *Citrus hytrix* leaf extract has the highest toxicity compared to other citrus species, so that lime leaves are the best formula to make an effective insecticide.

The decrease in the average mosquito mortality occurred at a weight of 400 mg, which was nine mosquitos of 3 repetitions. This decrease was due to lack of evaporation from the *Citrus hytrix* leaf extract, thus affecting the amount of odor emitted from the anti-mosquito electrical mat. The higher the weight of the *Citrus hytrix* leaf extract used, the more moisture required for the evaporation process and the higher solidity of the mat needed. Moisture content that was not suitable with *Citrus hytrix* leaf extract affected the evaporation of the aroma of *Citrus hytrix* leaves. This causes not optimal dehydration of the *Citrus hytrix* leaves. Optimal drying is also supported by the amount of heat coming from the electric engine. The different weight of *Citrus hytrix* leaf extract was assumed to require varying amounts of heat as well. The heavier the *Citrus hytrix* leaf extract, the higher the amount of heat needed.

Based on the way it works, lime leaves can also be classified as a nerve insecticide because *Culex sp.* experienced changes before and after exposure to electric mat containing *Citrus hytrix* leaf extract. Mosquito *Culex sp.* moved very actively trying to get out (due to the aroma of *Citrus hytrix* leaf extract) until it eventually became sluggish and limp and then paralyzes and dies. Saponin compounds in *Citrus hytrix* leaf extract are cytotoxic and hemolytic to insects (Geyter et al., 2007). These compounds lead to death, decrement in appetite, weight, and the reproductive capacity

of insects. Saponins can also be repellent, cause digestive problems, insect defects or cause toxic effects. The bitter taste of saponins makes these insects dislike their food (Direktorat Jenderal Pengendalian Penyakit dan Penyehatan Lingkungan, 2008). This means that aside from being an insecticide, lime leaves can also be used as mosquito repellent plants.

Conclusion

Based on data analysis and discussion, it can be summarized as follows:

- 1) The higher death rate of mosquitoes by the increased concentration of *Citrus hytrix* leaf extract given; with the most senior mosquito death rate (15) found at one mL concentration.
- 2) There was a significant difference in mosquito mortality rate in each concentration of *Citrus hytrix* leaf extract given.
- 3) The difference of tested concentrations levels gave significant mean difference effect to the mortality of mosquito.

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