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## *Evodia suaveolense*'s Repellent Action against *Aedes aegypti* from Entering a Room

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# *Evodia suaveolense*'s Repellent Action against *Aedes aegypti* from Entering a Room

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**Abstract.** *Evodia suaveolense* have been extensively studied to repel dengue's mosquitos' vectors that still become health problem in Indonesia. However there has not been any research yet that shows number of *E. suaveolens* plants needed to repel mosquitoes from the room. This study aims to find the minimum number of *E. suaveolens* plants required per cubic meter to make room free from *Aedes aegypti* mosquitoes. This was an experimental study using two rooms size 36 m<sup>3</sup> each connected by a door. The first room contained *Ae. aegypti* while another room contained rabbit lived in large-sized cages with 4 CCTVs installed in every corner. *E. suaveolens* plants placed in the rabbit's room. The door connecting those rooms was opened so that *Ae. aegypti* entered the rabbit's room. We placed *E. suaveolens* plants in the rabbit's room and measured how many *E. suaveolens* plants needed to avoid *Ae. aegypti* from biting the rabbit. The result of research using regression analysis showed  $Y = 67.321 - 3.411X$ . Thus, it takes at least 20 *E. suaveolens* plants per 36 m<sup>3</sup> or 0.55 *E. suaveolens* plant per m<sup>3</sup> to repel *Ae. aegypti* mosquitoes entering a room.

## 1. Introduction

*Aedes aegypti* mosquito is the main vector of Dengue Haemorrhagic Fever disease (DHF). Various attempts have been made to control these mosquitoes by using insecticides from various classes, but cases of DHF are reported to remain high in various countries around the world.

Long term insecticide use will cause resistance. *Ae. Aegypti* resistances against insecticides have been reported in various countries such as Thailand [1], Saudi Arabia [2], Viet Nam [3], Mexico [4,5], Pakistan [6], Sri Lanka [7], India [8], Latin America [9], Europe [10], Malaysia [11-13], and Indonesia [14,15].

Along with that, studies on the use of natural-based repellent which is safer for ecosystem are much developed. Various plants have been studied to produce odours / scents that can repel mosquitoes [16-34]

Many industries that operate in this field produce various repellent in the form of lotion and spray to be applied on exposed skin in order to avoid the mosquito bites. The weakness of research results on the use of lotion or spray repellents is that they easily fade with sweat or water thereby decreasing the effectiveness and efficiency of its use. It turns out to be a problem coupled with the habit of *Ae. Aegypti* to lay eggs around humans [35] and the habit of this mosquito to suck the blood intermittently (intermittent feeding) [36]. For that reason, it is required to conduct research on the method that can keep *Ae. Aegypti* out of a room with human and also environment-friendly.

One of the plants that thrives in Indonesia and has been researched to have the ability to repellent *Ae. Aegypti* is *Evodia suaveolense* which is known as Zodia [37]. *E. suaveolense* leaf extract that is applied



on the hands is reported capable to protect the hands from mosquitoes. Aside from being a mosquito repellent, *E. suaveolense* has fresh green leaves, a fresh and sweet aroma, and a medium size to be conveniently placed in a vase as a decorative plant. This study aims to find the repellent power of *E. suaveolense* to keep *Ae. Aegypti* out of the room.

## 2. Methods

### 2.1. Research Design

This research is an experimental research with time series design.

### 2.2. Objects and Materials Research

The object of this research is *Ae. Aegypti* as the main disseminator of Dengue Hemorrhagic Fever in Indonesia. About 200 adult *Ae. Aegypti* mosquitoes aged 5-7 days were bred from larvae *Ae. Aegypti* from Bandung. Identification of mosquito larvae and adult stage refers to Atlas of tropical medicine and parasitology [38]. To count the mosquito bites, two shaved rabbits were used. The research material is a 6-8-week *E. suaveolens* planted in pots.

### 2.3. Research Procedure

The study used two closed rooms with a connecting door. Each room's size is 3x4x3 m<sup>3</sup> and has 2 x 2, 6 m<sup>2</sup> transparent glass on two walls. The walls of the room were covered with gauze for extra precaution to prevent the possibility of escaped mosquitoes. The first room (Room A) was filled with *Ae. Aegypti* in mosquito coops. The second room (Room B) contains rabbits in a full condition, already shaved, and located in a hutch with a 2x3 cm<sup>2</sup> hole. The rabbit hutch was placed in the middle of the room B. In the four corners of the rabbit hutch, CCTV was installed with enlargement capability up to 640x and has auto infra-red so it can record clearly even in dark room. No lights were used in the room during the experiment.

The mosquito coop in room A was opened then the door between room A and room B was opened so that the mosquito entered room B containing the rabbits. CCTV in rabbit hutch in room B monitored the number of mosquitoes for 24 hours. This procedure is aimed at obtaining the initial condition (control) of the study. Furthermore, 8 pots of *E. suaveolens* were spread in room B. The CCTV in rabbit hutch in room B monitored the number of mosquitoes on the first rabbit for 24 hours. Then *E. suaveolens* in room B was added to 10 pots and performed the same procedure. And so on with the number of *E. suaveolens* pots of 12,14,16,18 and 20 fills in the room B. In the condition of the room with 20 pots, *E. suaveolens* was not added because there were no mosquitoes bit the rabbit. The same steps were performed on the second rabbit with a 3x 24-hour observation time gap of the first rabbit.

### 2.4. Statistical Analysis

The data obtained from this study were analysed using linear regression analysis using SPSS program.

## 3. Results and discussion

The results of linear regression analysis are shown in Table 1.

**Table 1.** Linear Regression Analysis of the Effect of *E. suaveolens* against *Aedes aegypti*

Coefficients <sup>a</sup>						
Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	67.321	1.855		36.286	.000
	Evodia suaveolens	-3.411	.127	-.992	-26.767	.000

a. Dependent Variable: Number of insect bite 24 hours

Linear regression equation of research:

$$Y = 67.321 - 3.411X$$

This means to produce insect bite 0, it takes 19.7 pots of *Evodia suaveolens* aged 6-8 weeks for a 36m<sup>3</sup> room. As a result, 0.55 pots of *Evodia suaveolens* are required per m<sup>3</sup>.

The results of this study indicate that by putting twenty pots of *E. suaveolens* in a 36m<sup>3</sup> room can keep out *Ae. aegypti* of the room. In other words, it takes at least 2 pots of *E. suaveolens* per m<sup>3</sup> to protect the room from *Ae. aegypti*. In getting food, the female *Ae. Aegypti* relies heavily on the clues obtained from smell to find the source of blood as food. In this case the role of olfactory and sensilla organs are very important. Sensory distributions are widely reported in antenna, labial palp, maxillary palp, tarsus of the feet, and mosquito ovipositor. Antenna is the principal place of chemoreceptor that serves to detect and distinguish various airborne odor stimuli and guide mosquitoes to blood food sources. The sensilla antenna is assumed to be the main living guide for finding food sources [39].

Odorants (smells) that is the guiding force of the mosquito are hydrophobic and must pass through the lymph glands before they are linked with their trans-membrane receptors. Olfaction is mediated by specific receptor proteins that are expressed on the sensory neuron membrane. The dendrites of these neurons are immersed in sensory lymph nodes containing odorant-binding proteins (OBPs). OBPs play an important role in odor acceptance for binding and dissolving odorants around the sensillum [40]. Recent studies have provided compelling evidence that OBP insects are required for proper olfactory performance. OBPs are an attractive target strategy to disrupt the chemoreception system in insects. The complex odor of OBP moves to the membrane-bound receptor, where the OBP will release its ligand. Experiments on the *Drosophila* gene, by changing one of the OBPs result in changes in insect behavior. The same results are also obtained by diluting specific olfactory receptors. This indicates that both the OBP and the receptor are required for the olfaction [41].

In the mosquito antenna lobe, there is an olfactory receptor neurons / ORNs tasked with synapses to a small number of secondary neurons called projection neurons (PN). Changes in the physiological ability of the source of blood supply cause changes in female *Ae. Aegypti* behavior. This is consistent with the study of Siju et al that alters the olfactory receptor neurons (ORNs) that control sensitivity over the lactic acid produced by hospes and the study by Davis that alters the peripheral ORN sensitivity in mosquito antennas [35].

Carbon dioxide, lactic acid, and ammonia in sweat and other excretory products in warm-blooded animals (in this study is rabbit) act as odorants of interest to female mosquitoes [31,42]. The perception of the odor is delivered through the chemoreceptor available in the mosquito antenna. However, the odor generated by two pots of *E. suaveolens* per m<sup>3</sup> is able to block the OBP and ORN in catching the odor produced by the rabbit, consequently the mosquito lost contact with its host. Thus *Ae. aegypti* do not know the existence of the source of blood food and do not touch hospes, in this case the rabbit as the object of research.

## 5. Conclusion

*E. suaveolens* has the ability to repellent against *Ae. aegypti*. It takes at least 0.55 pots containing *E. suaveolens* aged 6-8 weeks per m<sup>3</sup> room to keep *Ae. aegypti* out of the room containing the host.

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