

# Enhanced Efficacy of Kaffir Lime (*Citrus hystrix*) Essential Oil Spray Gel Against *Aedes aegypti* Mosquitoes

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**ABSTRACT:** The primary mode of dengue disease transmission is by the *Aedes aegypti* mosquito. The essential oil derived from Kaffir lime (*Citrus hystrix*) leaves comprises 66.85% beta-citronellal, 6.59% beta-citronellol, 3.90% linalol, and 1.76% citronellol. These constituents exhibit repelling properties. The objective of this study was to develop a spray gel formulation using kaffir lime leaf essential oil to create an efficient repellent. The process involves tearing fresh kaffir lime leaves and subsequently distilling them with distilled water. The distillation procedure yields an essential oil that is subsequently incorporated into a spray gel formulation, with varying concentrations of 1%, 5%, and 10%. Based on the findings of the efficacy test from 3 spray gel formulas through mosquito repelling activity conducted over 6 hours, it can be concluded that all tested formulas exhibited variation in effectiveness in repelling mosquito bites. The efficacy of the protection index against *Aedes aegypti* mosquito bites is contingent upon the concentration of kaffir lime essential oil in the formulation. There is a positive correlation between concentration and the duration of protection.

**KEYWORDS:** repellent; vector; dengue fever; malaria; formulation.

## 1. INTRODUCTION

Mosquitoes, belonging to the arthropod phylum, serve as vectors for numerous diseases, including malaria, dengue hemorrhagic fever (DHF), filariasis, chikungunya, yellow fever, and West Nile virus, hence posing a significant global health threat to millions of individuals. Efforts aimed at managing mosquito populations can be effectively carried out through the use of Integrated Mosquito Management (IMM) or integrated mosquito control strategies. Among the several methodologies employed in Integrated Mosquito Management (IMM), the utilization of Mosquitocide has been found to exhibit the highest level of efficacy(1). An additional technique that enhances the efficacy of the Mosquitocide (mosquito medication) approach involves the utilization of personal protective equipment, such as repellents.

Synthetic repellents, namely those derived from N,N-diethyl-meta-toluamide (DEET), are prevalent throughout the population. The efficacy of DEET as a repellent is well acknowledged; yet, its usage remains a subject of ongoing discussion due to its documented potential to produce varying degrees of toxicity in humans, including irritation of the mucous membranes [2]. An alternate approach that has been devised to mitigate these adverse consequences is the utilization of active chemicals derived from natural sources, specifically plants, as repellents for plants.

The plant species that exhibit considerable promise for utilization in pest control belong to the botanical groups Meliaceae, Rutaceae, Annonaceae, Labiatae, and Zingiberaceae. Numerous plant species belonging to this botanical family have been documented to exhibit repellent properties against mosquitoes. The kaffir lime (*Citrus hystrix* D.C), a member of the Rutaceae family, has been found to possess a protection index of 100% against *Aedes aegypti* and *Culex quinquefasciatus* for durations of 3 hours and 1.5 hours, respectively [3][4]. Based on the provided description, the study aimed to assess the efficacy of kaffir lime leaf essential oil, specifically in its spray gel formulations.

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## 2. MATERIALS AND METHODS

### 2.1. Isolation of kaffir lime essential oil

A quantity of 200 grams of kaffir lime mature leaves (determination certificate by Indonesian Institute of Science, Research Center for Biology) is finely chopped and placed within a distillation flask. Distilled water is added to the flask until it reaches half of its total capacity. The flask is then subjected to heat using an air bath, employing the method of distillation with water. The distillation process is allowed to continue for a duration of around 3.5 to 4 hours. Gather all of the distillate and consolidate it. The process of separating oil and water is accomplished through the utilization of a separatory funnel, followed by the addition of anhydrous sodium sulfate to achieve desiccation.

### 2.2. Formulation of Spray Gel

The extracted essential oil was then made into a spray gel preparation (**Table 1**) with concentrations of 0% oil (Formula 1, as a negative control), 1% oil (formula 2), 5% oil (formula 3), and 10% oil (formula 4), using carbomer 934 and HPMC as a combination of gelling agents, TEA as a neutralizer, propylene glycol as a humectant and cosolvent, 96% ethanol as a solvent for essential oils, and BHT as an antioxidant. For positive control, using commercial gel containing *N,N*-diethyl-*meta*-toluamide.

Table .1 Various concentration of kaffir lime essential oil in spray gel

Composition	F.1	F.2	F.3	F.4
KL Oil	-	1%	5%	10%
Carbomer 934	0,4%	0,1%	0,1%	0,1%
HPMC	0,4%	0,1%	0,1%	0,1%
NaCl	+ NaCl	- NaCl	- NaCl	- NaCl
Propilen glikol	15%	15%	15%	15%
TEA	0,2%	0,2%	0,2%	0,2%
Etanol 96%	15%	15%	15%	15%
BHT	0,05%	0,05%	0,05%	0,05%
Aquadest	ad 100%	ad 100%	ad 100%	ad 100%

### 2.3. Test the effectiveness of spray gel preparations using *Aedes aegypti* mosquitos

Kaffir lime leaves were tested for their ability to repel insects from the wrist to the tips of latex-gloved hands. Apply each formula equally to the left arm from the wrist to the elbow, following the dose instructions, and let it sit for five minutes. The examiner is not permitted to do anything during the five minutes of waiting. The tip of the right arm was covered with a latex glove, but the rest was left untreated. Through the right hole, insert the right arm for 5 minutes into the test cage, which is 40-50 cm x 40-50 cm x 40-50 cm and already contains 25 female mosquitoes. Female mosquitoes selected from breeding cage through physical appearance difference from male mosquitoes. Inclusion criteria included, female mosquitoes had not been bitten and exposed for 1 day before treatment and exposed for 1 day before treatment [6]. After counting the number of mosquitoes that landed on the right arm, replace the treated left arm through the left opening into the test cage for 5 minutes. For a minimum of six hours, the test is administered once per hour. From the first hour (immediately following exposure) to the sixth hour, the number of mosquitoes perched on the arm without treatment or control (right) and the number perched on the arm receiving treatment (left) were counted.

#### 2.3.1. Efficacy Standards [6]

Based on the protection index generated by the following formula, the effectiveness of the tested repellent is determined.

$$\text{Protection Index (PI)} = \frac{K-R}{K} \times 100\% \quad (\text{equation 1})$$

Information:

K = how many perches are on the control arm.

R = the quantity of perches on the treatment arm.

If the protection index is above 90% (PI > 90%) up to the sixth hour, repellent is deemed effective.

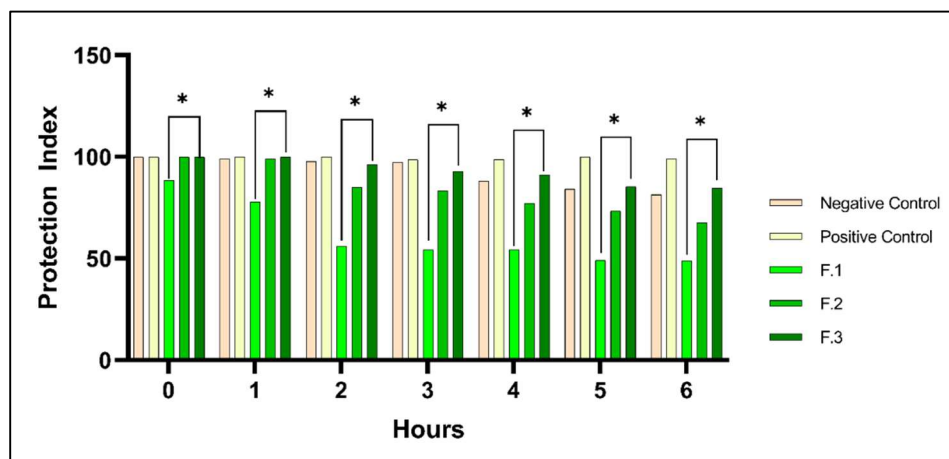
## 2.4. Statistical Analysis

Analysis of variance and Tukey post hoc test were used to identify significant differences among the groups. Results are presented as the mean  $\pm$  SD, and  $p < 0.05$  denoted statistical significance.

## 3. RESULTS

The findings from the computation of the protection index over a 6-hour period indicate that the spray gel formulations in formula 1, formula 2, and formula 3 exhibit insufficient efficacy as repellents, as the protection index falls below 90% by the 6th hour. Specifically, formula 1 demonstrates no effectiveness, formula 2 provides effectiveness for only 1 hour, and formula 3 offers effectiveness for 4 hours as a repellent.

The findings indicated a negative correlation between the concentration of kaffir lime leaf essential oil and the number of mosquitoes that made contact with the probandus' hands, therefore demonstrating an increase in the protection index as the concentration of the oil increased. A positive correlation exists between the duration of time and the frequency of mosquito perching. Figure 1 displays the mean protection index during the initial 0 to 6 hours of observation for each therapy.



**Figure 1.** Protection index from 0-6 hours. There are significant different between formula 1 and formula 3. This significant different due to difference in kaffir lime essential oil concentration in formula. \* $p < 0.05$

## 4. DISCUSSION

Female mosquitoes were selected as the subjects for this experiment because to their biological requirement for blood in order to facilitate egg production and the continuation of their species. Although female mosquitoes initially consume nectar or plant juices to replenish their energy and engage in mating, they afterwards require protein, which is found abundantly in blood. Male mosquitoes exclusively consume nectar or plant juice as their primary source of sustenance. The female mosquitoes included in the experiments underwent a period of fasting lasting four days. According to Agus Kardinan (2010) [5], this indicated that the mosquitoes had reached sexual maturity and started to necessitate blood in order to facilitate the development and maturation of their eggs. Additionally, it was ensured during the experiment that the female mosquitoes were drawn to the hands of the probandus. To ensure the validity of the results, the mosquitoes were subjected to a fasting period before to the experiment. This precautionary measure aimed to mitigate the possibility of the mosquitoes refraining from landing on the probandus' hands due to satiety rather than the actual efficacy of the tested mixture.

Based on the obtained test results, it is evident that the positive control exhibits efficacy for a duration of 6 hours. This efficacy can be attributed to the presence of *N,N*-diethyl-meta-toluamide (DEET) as the active ingredient in the positive control. Thus far, no alternative substance has been identified that can rival the effectiveness of DEET as a long-lasting repellent, capable of providing protection for a duration of 8 hours at a concentration of 30%. The established reference standard formula for linalool exhibits efficacy for a duration of solely three hours. The inclusion of kaffir lime leaf essential oil is significant, accounting for a substantial

proportion of 10%. In contrast to the limited effectiveness of formula 2, which lasts for only one hour, the efficacy of the bp formula containing linalool is comparatively higher, despite both formulas having the same concentration of 5%. Formula 2 incorporates 5% kaffir lime leaf essential oil, whereas the bp formula employs 5% linalool. This observation suggests that linalool, serving as a marker compound in kaffir lime leaf essential oil, exhibits greater repellent properties than the essential oil derived solely from kaffir lime leaves. In contrast, the utilization of 1% kaffir lime leaf essential oil in formula 1 of the experiment yielded little repelling effects, even at the onset of the experiment (0 o'clock), due to the overshadowing of the odor emitted by the active constituents by supplementary components incorporated into the formula. The results indicate that the gel formulation of kaffir lime leaves essential oil spray exhibits limited efficacy. The potential inefficacy of kaffir lime leaf essential oil spray gel formulations as a repellent against *Aedes aegypti* mosquitoes may be attributed to human factors, specifically the physiological secretion of sweat. This sweat can potentially interact with the active ingredients in the applied substance, leading to alterations in its chemical structure [6].

## 5. CONCLUSION

The efficacy of the protection index against *Aedes aegypti* mosquito bites is contingent upon the concentration of kaffir lime essential oil present in the formulation. There is a positive correlation between concentration and the duration of protection.

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