

## THE EFFECTIVENESS OF VARIOUS ESSENTIAL OILS ON THE MARKET AS AEDES AEGYPTI MOSQUITO REPELLENT

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### Article Info

Submitted : 1 July 2023  
In reviewed : 10 September 2023  
Accepted : 2 January 2024  
Available Online : 31 January 2024

**Keywords :** *Aedes aegypti*, Essential oil, Mosquito repellent

Published by Faculty of Public Health  
Universitas Airlangga

### Abstract

**Introduction:** In Indonesia, the average dengue cases are 121,191 each year with a Case Fatality Rate (CFR) of 0.69%. Mosquito control with common chemical methods will negatively impact the environment and human health and can cause mosquitoes to develop resistance. Environmentally-friendly replacement chemicals derived from plants such as citronella contains 16.27% citronella and 19.88% geraniol. In addition, lavender plants also contain 35% alcohol comprising linalool and geraniol as well as other chemicals such as borneol. Furthermore, 8-20% of camphor and borneol in rosemary can also be a natural mosquito repellent ingredient. **Methods:** This study is a True Experiment with The Posttest-Only Controlled Group Design. The number of *Aedes aegypti* mosquitoes used for each treatment was 20. The study subjects were divided into 4 groups comprising 0.4 ppm of citronella, lavender, and rosemary essential oils and aquades as control. Replication was carried out 3 times and observations were conducted for 20 minutes. **Results and Discussion:** The Anova Factorial test resulted in a p-value of  $\leq 0.05$ . In addition, there was a significant difference in the number of *Aedes aegypti* mosquitoes expelled from humidifier vapor. Furthermore, the Least Significant Difference (LSD) test showed that citronella essential oil was most effective in repelling mosquitoes because it has a p-value of  $\leq 0.05$  and largest average difference between other essential oils. **Conclusion:** Essential oils from citronella are most effective and stable for 30 seconds to 20 minutes to repel mosquitoes compared to lavender and rosemary essential oils.

## INTRODUCTION

Dengue Haemorrhagic Fever (DHF) disease in Indonesia has increased every year (1). Based on data from the Directorate General of Disease Prevention and Control of the Ministry of Health of the Republic of Indonesia, dengue cases have increased on average every  $\pm 10$  years. In the last five years, as many as 121,191 dengue cases were found each year (2). Although the occurrence of dengue cases tends to increase, the Case Fatality Rate (CFR) has decreased from time to time, reaching 0.69% in 2020 (3).

The significant reduction in mortality is certainly supported by various promotive, preventive, and curative actions. Preventive measures or efforts to prevent dengue disease can be done by controlling the

vector that causes the disease, namely *Aedes aegypti* (4-5). *Aedes aegypti* vector control consists of several methods such as physical, biological, environmental management, and chemical (6). Control using chemical techniques is generally carried out by the public using synthetic insecticides (7). Currently, there are many insecticide products on the market such as aerosols, electric mats, and lotions, which contain chemicals belonging to the organophosphate, organochlorines, pyrethroids, carbamates, and Diethyltoluamide (DEET) compounds. Improper use of these compounds can cause headaches, seizures and even paralysis. Besides, it can irritate the skin and result in skin disorders (8). In addition to having an impact on human health, these chemicals, if continuously used, have a negative impact

### Cite this as :

Mitasari A, Santjaka A, Ardiansyah I. The Effectiveness of Various Essential Oils on the Market as *Aedes Aegypti* Mosquito Repellent. *Jurnal Kesehatan Lingkungan*. 2024;16(1):1-9. <https://doi.org/10.20473/jkl.v16i1.2024.1-9>



on the environment and can cause mosquitoes to become resistant to insecticides (9). This is reinforced by the results of a susceptibility resistance test conducted in Papakelan Village using *Aedes* sp. Mosquitoes, which shows that the *Aedes* sp. population was already resistant to 0.8% malathion insect venom, Furthermore, the percentage of mosquito mortality was 70% (10). This study is another alternative to minimize the use of these chemicals by replacing them with environmentally friendly chemicals in plants .

Chemicals in plants act as natural insecticides that can decompose in the environment and do not cause deposits in the air, water, or soil, and are relatively safe to use (11). Citronella plants have chemical compound components that can be used as mosquito repellent because they contain 16.27% citronella and 19.88% geraniol (12). In addition, lavender plants also contain 35% alcohol comprising linalool and geraniol. Other chemicals such as 0.8-6% esters and 8-20% alcohols along with the main content of 1.8-cineol, borneol, camphor, and bornyl acetate in rosemary can also be natural ingredients in mosquito repellent (13).

### METHODS

This study is an experimental research in the laboratory using the True Experiment research design and The Posttest – Only Controlled Group Design (14). The research location is at the Center for the Research and Development of Disease Vector and Reservoir Salatiga, Central Java. The independent variable in this study was the various types of citronella, lavender, and rosemary essential oils on the market and the dependent variable was the number of mosquitoes evicted from the humidifier vapor.

The samples in this study were adult female *Aedes aegypti* mosquitoes aged 3-5 days obtained from rearing at the Center for the Research and Development of Disease and Reservoir Salatiga, Central Java (15). 20 mosquito samples per cage and 2 guinea pigs as bait (16–18) were used. 4 cages with a size of 110 cm x 25 cm x 25 cm were used for the treatment of each variant of citronella (*Cymbopogon nardus*), lavender (*Lavandula angustifolia* mill), and rosemary (*Rosmarinus officinalis* L.) essential oils with a dose of 0.4 ppm water and aquades as a control evaporated using a humidifier, as can be seen in figure 1. Replication was carried out 3 times. The observation time for 20 minutes starts from the 30th second, the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup>, 7<sup>th</sup>, 8<sup>th</sup>, 9<sup>th</sup>, 10<sup>th</sup>, 15<sup>th</sup> and 20<sup>th</sup> minutes in each time period on the observation sheet (19).

The Anova Factorial statistical test was used to analyze the data (20). Then, the Least Significant

Difference (LSD) test was conducted to see the difference in the number of *Aedes aegypti* mosquitoes expelled from the humidifier steam that had been added to each variant of citronella, lavender, and rosemary essential oils as *Aedes aegypti* mosquito repellent.

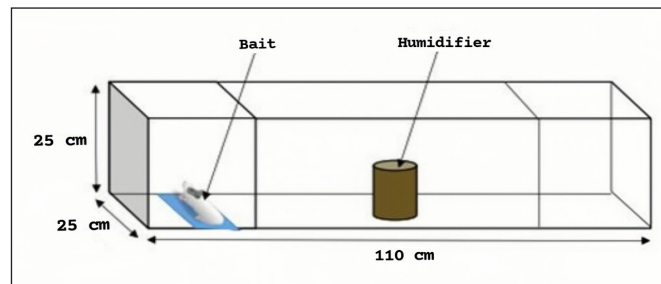


Figure 1. Test Cage (Glass Tunnel)

### RESULTS

Essential oil testing performed on mosquitoes was prepared as in figure 2. Testing of citronella, lavender, and rosemary essential oils was carried out alternately. In the preparation process, there was no significant color difference between the three essential oils but all three had distinctive aromas. As the observation progressed, essential oil vapors from the humidifier filled the enclosure space as can be seen in figure 3.

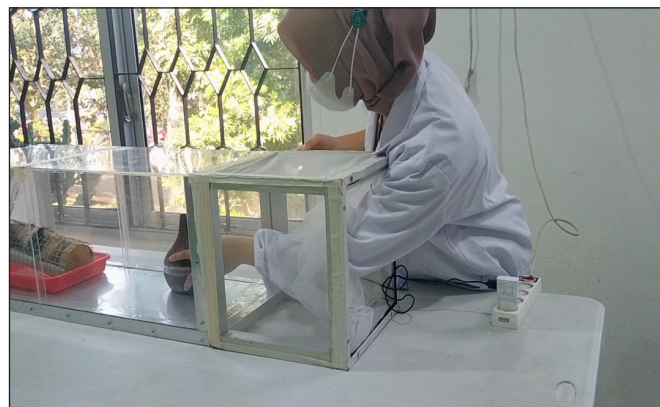


Figure 2. Test Cage Layout for Observation



Figure 3. The Condition of the Cage During the Observation

The results of the study were analyzed using the Anova Factorial statistical test to compare different types of essential oils, time, and the interaction between essential oils and time.

Table 1 shows that all variables, i.e. types of essential oils, time, and interaction between both, have p-values  $\leq 0.05$ , which constitute significant. Therefore,

further tests of Least Significant Difference (LSD) were carried out.

**Table 1. Factorial Anova Test Results**

Variable	df	Mean Square	f	p-value
Types of Essential Oils	3	130.470	27.542	0.000
Time	12	12.710	2.683	0.003
Types of Essential Oils x Time	36	8.854	1.869	0.008

The results of further LSD tests on the different types of essential oils in table 2 show that citronella essential oil has a p-value of  $\leq 0.05$  and has the largest average difference among other essential oils. On the other hand, lavender and rosemary essential oils have a p-value of  $> 0.05$  and have an average difference that is not that far apart, meaning that their mosquito repellent power is relatively the same. This means that the most effective essential oil in repelling *Aedes aegypti* mosquitoes is citronella essential oil.

**Table 2. Results of LSD Analysis (Least Significant Difference) Types of Essential Oils**

Various Essential Oil	Mean Difference	p-value
<b>Citronella</b>		
Lavender	3.3077*	0.000
Rosemary	3.4872*	0.000
Control	4.0256*	0.000
<b>Lavender</b>		
Rosemary	0.1795	0.716
Control	0.7179	0.148
<b>Rosemary</b>		
Control	0.5385	0.277

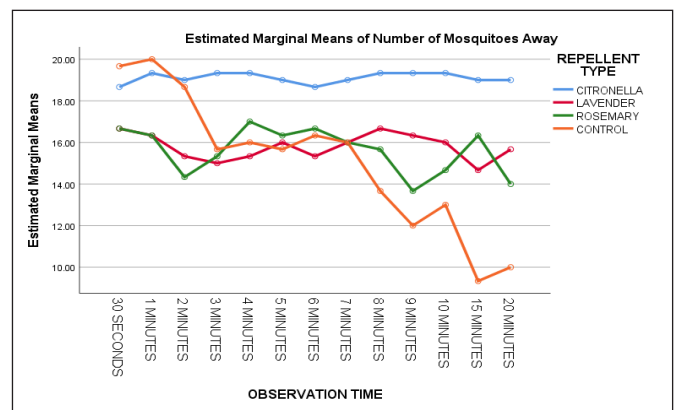
The observation time correlates with the number of mosquitoes displaced because it produces p-values of  $0.003 \leq 0.05$  as in table 1. This can be seen in the results of further LSD tests in table 3, where the mosquitoes did not approach the bait after being observed for 20 minutes.

**Table 3. LSD Analysis Results (Least Significant Difference) Time**

Observation Time	Mean Difference	p-value
<b>30 seconds</b>		
9 minutes	2.5833*	0.004
10 minutes	2.1667*	0.016
15 minutes	3.0833*	0.001
20 minutes	3.2500*	0.000
<b>1 minutes</b>		
9 minutes	2.6667*	0.003
10 minutes	2.2500*	0.013
15 minutes	3.1667*	0.001
20 minutes	3.3333*	0.000
<b>2 minutes</b>		
15 minutes	2.0000*	0.026
20 minutes	2.1667*	0.016
<b>3 minutes</b>		
8 minutes	0.0000	1.000

Observation Time	Mean Difference	p-value
<b>4 minutes</b>		
15 minutes	2.0833*	0.021
20 minutes	2.2500*	0.013
<b>5 minutes</b>		
15 minutes	1.9167*	0.033
20 minutes	2.0833*	0.021
<b>6 minutes</b>		
15 minutes	1.9167*	0.033
20 minutes	2.0833*	0.021
<b>7 minutes</b>		
15 minutes	1.9167*	0.033
20 minutes	2.0833*	0.021
<b>15 minutes</b>		
20 minutes	0.1667	0.852

Indications that there is an interaction between essential oils and observation time can be seen in figure 5. It clearly shows that essential oils and time correlate with the number of *Aedes aegypti* mosquitoes expelled from humidifier vapors.



**Figure 5. Graph of the Number of Mosquitoes Expelled from Humidifier Vapors Compared to Essential Oil Type and Time**

**DISCUSSION**

Citronella's ability to repel mosquitoes is attributed to its active ingredients: citronellal, citronellol, and geraniol (21). Citronellal is the main constituent of citronella oil (22). Citronellal compounds are monoterpene compounds with a distinctive aroma, containing high antioxidant, antibacterial, and antifungal activity. This compound has the potential to be leveraged as a burn therapy, considering its capacity to inhibit bacteria and fungi that may be present in wounds. In addition, its antioxidant properties contribute to the accelerated regeneration of skin cells, making it a viable option as a remedy for burns. Until now, citronellal compounds have been isolated and used for its antibacterial properties, establishing citronella oil as one of the alternatives for medicines and household essentials (23-24). An active ingredient called Diethyltoluamide (DEET) is still found in some repellent products available in the market.



However, many mosquito repellent lotions for babies do not contain such compound, switching to the use of active natural ingredients such as citronella, eucalyptus, and other ingredients.

It is reported that after undergoing a three-hour maceration process, kaffir lime (*Citrus hystrix* DC) leaves yield 63.66% citronellal (25). Lime (*Citrus aurantifolia*) peel also contains citronellal which can be used as an ingredient in making insecticides (26). There are several chemical processes involving citronellal (27).

By the influence of acid, citronellal can be converted into isopulegol which will generate menthol after undergoing hydrogenation, serving useful functions for medicines and having the potential to be an additional ingredient in toothpaste, beverages, and food. When reduced, citronellal can be converted into citronellol which emits a rose-like scent and is used as a component of perfume, making it become one of the expensive fragrances. The reaction of this compound with Grignard reagent results in the formation of an alcohol derivative called alkyl citronellol in a liquid form, emitting a very fragrant aroma, thus widely used in perfume and cosmetic products. Furthermore, citronellal can also be converted into hydroxy citronellal compounds which are often labeled as King of Perfume. This compound takes on a yellowish liquid form with a fragrant aroma reminiscent of lily flowers and comes with a considerably high price.

Geraniol is an acyclic alcohol monoterpene, a compound consisting of two isoprene molecules and one water molecule. Pure geraniol is an oily, colorless liquid with a characteristic aroma. When oxidized, it will transform into geranial or citral (28). Geraniol is also one of the main components of citronella essential oil (29). As one of the most important molecules in the perfume industry, geraniol is used as a raw material in the production process. Beyond its pleasant odor, insecticidal and repellent properties are also commonly known to be found in geraniol, explaining its ability and prevalence as a natural pest control with low toxicity (30). Given this fact, it is not surprising to find geraniol contained in several mosquito repellent products to various repellents packaged in the form of sprays, topical oils, and lotions available in the market, which remain in demand among the public. It has been reported that the content of geraniol is also found in yellow, white, and red frangipani flowers with a percentage of 2.64%. Geraniol attributed in frangipani is usually employed as aromatherapy for relaxation to relieve stress and repel mosquitoes (31).

The active ingredient linalool constitutes 27-48% of lavender's composition. Linalool, characterized by its liquid and colorless appearance, is a terpenoid alcohol

compound with the empirical formula  $C_{10}H_{18}O$  and the structural formula 3,7 dimethyl-1,6 octadiene-3-ol. Linalool is a straight-chain alcohol compound (32) and is widely recognized as one of the active compounds in essential oils possessing sedative effects. Linalool is a volatile compound found in many plant tissues such as leaves, fruits, and, generally, flowers. Linalool or 3,7 dimethyl-1,6 octadiene-3-ol, with the empirical formula  $C_{10}H_{18}O$  which includes terpenoid alcohol compounds, is a liquid substance that is devoid of color and gives off a fragrant scent. It is also worth noting that linalool is a straight-chain alcohol compound (33). The most common linalool compound is primarily sourced from the lavender plant. It has also been largely marketed in the form of essential oils, a common and popular choice among the general public as aromatherapy for relaxation. It is noted that linalool contained in basil leaves (*Ocimum basilicum*) exhibits toxic effects on the tracheal and digestive systems of *Aedes aegypti* mosquito larvae. This shows that basil leaf extract possesses a biolarvicide effect on instar IV larvae, thus with an increase in the amount of extract concentration used, it has the potential to further increase the number of dead larvae (34). Apart from its significant role as essential oils, this compound also finds its use as an active ingredient for air fresheners, electric liquid drugs, and lotions because of its distinctive fragrant aroma.

In addition, rosemary essential oil comprises esters ranging from 0.8-6% and 8-20% of alcohols with the main content of 1.8-cineol, borneol, camphor, and bornyl acetate (13). Esters derived from carboxylic acids containing  $-COOH$  groups and hydrogens in these groups are replaced by hydrocarbon groups. These can be either alkyl groups, such as ethyl or methyl, or those containing benzene rings, such as phenyl or benzyl groups, and, the most commonly discussed compound, ethyl ethanoate (35). Ester compounds are distinguished by their distinct fragrance according to the types found in nature and obtained from the reaction of alcohol and carboxylic acids, explaining their prominent use as synthetic fragrances in various products, such as perfumes and cosmetics (36). In addition to being utilized as a component of both products, people also benefit from ester compounds to enhance flavors in food (37). Alcohol compounds contain hydroxyl functional groups that are the derivatives of alkanes with hydroxyl groups ( $-OH$ ) bonded to carbon atoms,  $R-OH$  (38). The compound 1,8-cineol is a compound belonging to the oxygenated hydrocarbon group. It embodies refreshing properties as well as a pungent aroma and taste, providing abundant uses, such as external medicine, nasal spray, disinfectant, pain reliever or food flavoring,

and cosmetics (39). Essential oils with 1,8-cineol content are usually found in the Indonesian community in the form of eucalyptus oils, with a wide range of needs from infants to adults (40). Meanwhile, borneol is a terpene and bicyclic organic compound that can be obtained by extracting from plants or through a chemical synthesis process (41). This compound can be found in various plant extracts, such as pine sap stems (*Pinus merkusii Jungh*), sembung leaves (*Blumea balsamifera*), lilies (*Lilium auratum*), curcuma (*Curcuma xanthorrhiza Roxb*), cardamom (*Elletaria cardamomum*), and many other plants (42–45). Borneol offers many benefits in the health sector, such as serving as a therapy for cancer patients, to prevent blood clots, as a therapy to overcome anxiety and fear disorders, and as a chronic pain therapy where this compound works as a novel agent that can improve the level of human health (43).

Camphor is a dextrorotary ketone compound appearing as small transparent crystals with the molecular formula  $C_{10}H_{16}O$ , generating a spicy taste and a specific aromatic scent that induces a cold sensation and evaporates at ordinary temperatures (13). Despite being widely used as an anti-inflammatory and analgesic, camphor compounds possess other valuable functions, such as aromatherapy oil which has a relaxing effect and has the potential to improve the respiratory system by cleansing the lungs to improve blood circulation and the immune system (46). Bornyl acetate is known as a natural aromatic monoterpene ester with a wide range of pharmacological and biological activities. In addition, it has analgesic, antioxidant, bleach, anticancer, antitumor, antiabortion, and anti-anxiety effects, and possesses antibacterial, insecticide, and anesthetic effects that are symbiotic with other aromatic compounds in essential oils (47).

The process of how vegetable insecticides eliminate insects is divided into three groups, namely stomach poisons, gas poisons, and contact poisons (48). As the name suggests, insects are killed by stomach poison after insecticides enter their mouth and are absorbed into the body through the digestive tract. Gas poisons, also called as fumigants, are used on a limited basis for all types of insects in enclosed rooms, emitting toxic gas that will enter through breathing and insect surfaces. On the other hand, contact poison kills insects by entering their bodies through the skin, penetrating the blood vessels, or going through the respiratory tract. This type of poison usually comes in the form of liquid or powder (49). The active ingredient contains toxic properties. This poison works the same as contact poison which causes mosquitoes to lack fluids for a long time, making mosquitoes dehydrated (50). In addition to

contact poison due to these active ingredients, the use of essential oils with a humidifier is also capable of making these materials become gas poisons as a result of the evaporation of essential oils produced.

The active ingredient has the same ability to repel mosquitoes and as an insecticide. The way insecticides work in the insects' bodies is known as the mode of action and mode of entry. Mode of action refers to how insecticides affect the target site in the insect's body. The catch point in insects is usually an enzyme or protein. Some types of insecticides can affect more than one point of capture in insects (51). Meanwhile, essential oils' ability to repel mosquito bites is related to the chemical components that act as repellents, as well as the aroma produced by the essential oils. *Aedes aegypti* mosquito has anthropophilic properties which means it prefers to suck human blood rather than animal blood (52). Human skin secretes lactic acid and excretory products, such as carbon dioxide that mosquitoes can use to detect human odors and presence (53). To avoid getting bitten, carbon dioxide and body odor are eliminated by using the aroma of evaporated essential oils through a humidifier. A humidifier is a tool to increase the air humidity within a room, some of which are equipped with essential oil diffusion features. A humidifier's working system converts water into water vapor and spreads it into the air (54). With added essential oils, this tool will then spread the aroma all over the room. The pungent fragrance that spreads throughout the experimental box room will interfere with mosquitoes' respiratory system. After undergoing a distillation process, citronella produces geraniol and citronellal whose aroma is useful to repel mosquitoes as it causes mosquitoes to lose contact with the host or humans (55). In addition to the use of humidifiers as a means of repelling mosquitoes, electric mosquito repellent heater, which are simply made as a place to heat mosquito repellent, are now largely available in the market. People usually use this tool along with mosquito repellent in the form of magic sand with lavender variants (56).

In another research, male palm flower extract lotion or *Elaeis guineensis Jacq* is mentioned. It serves as a mosquito repellent with the most effective concentration formula of 5% male oil palm flower extract generating a 92% protection rate (57). The active ingredients such as citronellal, citronellol, and geraniol create a concentrated aroma that mosquitoes find less attractive compared to the active ingredients of lavender and rosemary essential oils.

For 30 seconds to 20 minutes, various types of essential oils were relatively stable in repelling *Aedes aegypti* mosquitoes. Meanwhile, there was a significant

decrease from 30 seconds to 20 minutes during the control experiment, thus it is safe to say that mosquitoes preferred this more. The ability of citronella essential oils in Figure 2 shows that it is more stable in repelling mosquitoes. This finding is in line with previous research which suggests that a 20% concentration of citronella lotion does not show any significant effects from the first until the sixth hour. However, at a concentration of 40% for 6 hours, citronella extract lotion provides stable protection. Similarly, at a concentration of 80% for 6 hours, the percentage of citronella lotion's ability to protect reaches 86.4%. This figure shows that there is an interaction between concentration and observation time (58). While adult human rest time ranges from 7 to 8 hours a day, the number is different for children as their need for rest reaches about 16 hours per day, with adequate rest time believed to provide a refreshing effect (59). The observation time carried out in this research is only 20 minutes which is barely sufficient to represent human rest time. However, essential oils assisted by a humidifier for evaporation can repel mosquitoes well within 20 minutes. This finding can serve as the basis for further research to increase observation time adjusted to human rest time in general.

The abilities of lavender and rosemary essential oils are relatively similar. At concentrations of 15%, 20%, and 25% for a 2-hour observation time, the lavender extract was declared able to kill mosquitoes. The observation results highlight a concentration of 25% as the most effective concentration where 96% of mosquitoes died (60). It has been previously reported that pandan leaves contain linalool and essential oil, thus generating a fragrant aroma, with an effectiveness of 93.55% in repelling mosquitoes in the first hour (61). At concentrations of 6%, 12%, and 24%, essential oils sourced from rosemary extract actively act as a repellent against *Aedes aegypti* mosquitoes for 4 hours of observation, with the greatest protection power of 92.15% at the largest concentration (62). Another research suggested that zodia (*Evodia Suaveolens*) essential oil lotion also has a protective power for six hours at concentrations of 1%, 1.5%, and 2%. The higher the concentration, the better the protection against mosquito bites (63). This proves that the higher the concentration is used, the greater the effect has on mosquitoes. In this research, only one concentration is used to allow changes in the results of effectiveness when a certain concentration is added to each type of essential oil. Therefore, it is arguable that the weakness of this research lies in its limited ability to consider the concentration of each variant of essential oils.

## ACKNOWLEDGMENTS

The author would like to thank the Lecturers of the Department of Environmental Health Purwokerto and the Center for Research and Development of Disease Vectors and Reservoirs Salatiga for giving endless support and assistance during the course of this research.

## CONCLUSION

Essential oils sourced from citronella are more effective at repelling mosquitoes than lavender and rosemary essential oils. Citronella essential oil is the most stable one in repelling mosquitoes for 30 seconds to 20 minutes. It is highly recommended as a mosquito repellent compared to lavender and rosemary essential oils. Future research may explore further possibilities by extending the observation time or using different research designs.

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