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## Mosquito repellent activities of citrus fruit peels

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**Abstract**

Mosquitoes are accountable for the spread of numerous diseases like malaria, Dengue, Zika, Yellow fever, Chikungunya etc. among human beings. The best way to limit mosquito contact is to use personal protection and insect repellents. The majority of chemical repellents however, is unhealthy for people and can have negative effects on their skin in addition to other health-related issues. As a remedy, mosquito repellents from natural sources may not create any such kind of harm and could be a better alternative to synthetic repellents. Hence, natural repellents are preferable to chemical ones. Therefore, this study was designed to utilize an olfactometer to examine mosquito vector behaviour in response to fresh citrus fruit peel extracts. The statistical analysis showed that there were significant differences in mosquito repellency of three citrus peel extracts against the four mosquito species tested ( $F = 3.1668$ ,  $df = 3$ ,  $p < 0.05$ ). The study result proved that the *Citrus sinensis* (Sweet orange), *Citrus limetta* (Sathukudi), and *Citrus Limon* (Lemon) tend to repel the mosquito vectors such as *Aedes aegypti*, *Culex tritaeniorhynchus*, *Culex quinquefasciatus* and *Anopheles stephensi*.

**Keywords:** Mosquito repellents, citrus peel extracts, y-tube olfactometer, mosquito repellency

**1. Introduction**

Vector borne diseases like Dengue (DEN), Malaria, Lymphatic filariasis (LF), Chikungunya (CHIK), Japanese encephalitis (JE), Zika, West Nile virus fever (WNVF) and Yellow fever (YF) are spread by the bite of infected female mosquitoes [1]. Most vector mosquitoes belong to the genus *Aedes*, *Culex*, and *Anopheles* are common all over the world and dominate in the tropical regions of Africa, Asia, Europe and South America [2, 3]. Globally around seven hundred million people are suffering from mosquito-borne illnesses, resulting in nearly one million deaths [4]. 17.0% of the total global infectious disease burden is mosquito-borne diseases [5] and half of the world's population is threatened by mosquito-borne diseases [6]. Integrated mosquito control methods and human personal protection may reduce human-vector contact, controlling vector mosquitoes and mosquito-borne disease transmission. In the integrated mosquito control methods, mosquito repellents are being used to reduce human-vector contact [7]. The most commonly used mosquito repellents obliterate the human aroma, making them efficient insect repellents [8] and many mosquito repellents are commercially available [9].

All over the world, particularly in India, mosquito repellents are available in various forms, like coils, vaporizers, mats, and creams. They are used to reduce the human-mosquito interaction by inhibiting the sensory receptors of mosquitoes [10, 11]. These chemical agents that are utilized in the commercial production of mosquito repellents are synthetic and non-biodegradable [12]. Being exposed to these chemical agents continuously causes contamination of environment and many health problems in human beings [13].

In recent years many efforts have been made to find mosquito repellents that will have low toxicity and not be harmful to the ecosystem. [14, 15]. There are 2000 plant species from various families that have been used to control the mosquito at their various life stages [16]. Citrus fruits are an integral part of the human diet due to their nutritional and therapeutic value. Meanwhile, the peels generated through the consumption of these fruits would pollute the ecosystem. So, in this present work, an attempt has been made to study the mosquito repellent activity of citrus fruit peel extracts against adult mosquitoes using Y shape olfactometer.

## 2. Materials and methods

### 2.1 Procurement of Citrus fruit peels

Fresh citrus fruits, *Citrus sinensis* (Sweet orange), *Citrus limetta* (Mousami), and *Citrus limon* (Lemon) were purchased from the local market. The fruits were peeled and stored in the refrigerator before usage.

### 2.2 Extraction of citrus fruit peel<sup>[17-18]</sup>

10 gm. of citrus fruit peel was macerated with 100 ml of distilled water. Maceration released the compounds present in citrus peels. Water is the universal solvent that is used to extract polar compounds. Then the samples were centrifuged to get a clear solution and the residue was discarded. The clear aqueous extract was collected in 250 ml flask separately and stored at 4 °C for further use.

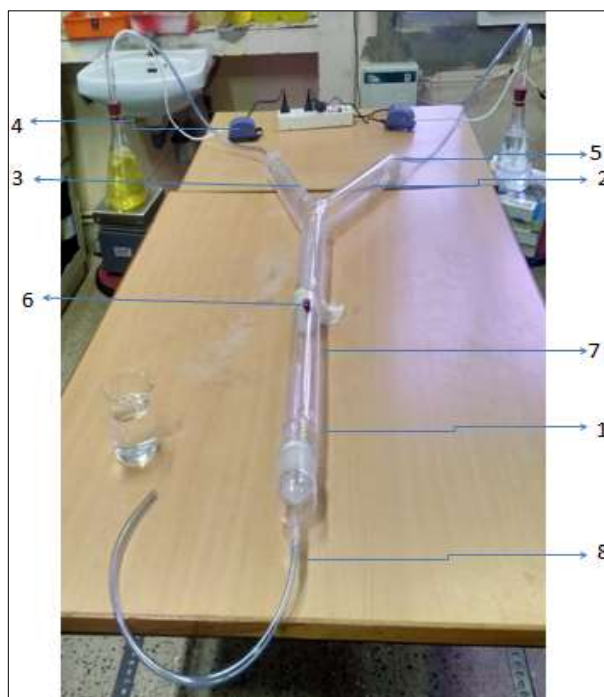
### 2.3 Collection of Mosquitoes<sup>[19]</sup>

Female adult mosquitoes such as *Aedes aegypti*, *Culex tritaeniorhynchus*, *Cx. quinquefasciatus* and *Anopheles stephensi* were used for the repellency tests against citrus fruit peel extract using a Y-tube olfactometer. All these mosquito species were collected from the laboratory reared mosquito

colony. Separated mosquito species were further transferred to one-foot mosquito cages with the facility of air entry and fed with a 5% glucose solution feeding for mosquitoes. Glucose feeding was stopped for four hours before release into a Y-tube olfactometer to test the repellency of citrus peel extracts.

### 2.4 Assay with Y shape olfactometer

The repellent behaviour of adult mosquitoes exposed to citrus fruit peel extract was studied using a Y-shape olfactometer (Fig. 1). The olfactometer with a diameter of 19.5 cm and a length of 2 feet was made up of glass material. This assembly contains three parts such as the release part, the flight part and the olfactometer arms. A portable air outlet was connected at the end of the release part. The removable shutter which is made up of a fabric mesh screen was placed between the release part and the flight. It allows the mosquitoes to acclimatize in the release part before each test. The angle of the two olfactometer arms was 75 °C. The control flask and the sample flask were connected respectively at the end of each arm.



**Fig 1:** Olfactometer connected with a conical flask contains citrus peel extracts in one arm and distilled water in the other arm. 1. Main arm 2. Control arm 3. Test arm 4. Air pump 5. Odor inlet 6. Mosquito released part 7. Mosquito resting chamber 8. Air outlet

A behaviour study of non-haematophagous freshly emerged female adult mosquitoes was tested using a Y-tube Olfactometer<sup>[20]</sup>. Three replicates were used for each species of the mosquitoes and in each replicate twenty-five unfed female mosquitoes were introduced. The duration of this experiment is 3 minutes. The mosquitoes released from the released part and entered into the direction of the Y arms that they preferred. The number of mosquitoes in the control and test arms was counted manually. After testing each of the extracts the Olfactometer was cleaned thoroughly.

The following formula was used to determine the preference index (PI) of the mosquitoes to the citrus peel extracts<sup>[20]</sup>.

$$\text{Preference index (PI)} = (T_n - C_n) / (T_n + C_n) \quad (1)$$

TN = Number of mosquitoes in test arm;

CN = Number of mosquitoes in the control arm

The preference index value -1 represents repellency; 0 denotes neutral response; +1 indicates attractant.

The experiment was repeated three times for each extract to find the mean percentage of mosquito repellent activity. The mosquito-repellent activity was calculated by the following formula<sup>[21]</sup>.

$$\% \text{ Repellent activity} = \frac{\text{No. of mosquitoes in the control arm} - \text{No. of mosquitoes in treatment arm}}{\text{No. mosquitoes in the control tube}} \times 100$$

**2.5. Data analysis**

All the data were analyzed using SPSS Ver 15 software (SPSS Inc., USA).

**3. Results**

The yield of peels from citrus fruits varies with the species. About 160-230 grams of fresh peels were obtained from each fresh citrus fruit's one-kilogram peel (Table 1).

**Table 1:** Yield of Peels from Citrus fruits

Name of citrus fruit	Yield of peels per kg
<i>Citrus sinensis</i> (Sweet orange)	226.6667±3.9 g
<i>Citrus limetta</i> (Sathukudi)	167.3333±3.3 g
<i>Citrus limon</i> (Lemon)	208.6667±4.0 g

Among the three peels, *C. sinensis* showed the highest yield (226.6667±3.9 g) and *C. limetta* showed the lowest yield of (167.3333±3.3 g) peels.

The response of female adult mosquitoes *Aedes aegypti*, *Culex tritaeniorhynchus*, *Culex quinquefasciatus* and *Anopheles stephensi* towards the citrus fruit peel extracts were examined in a modified olfactometer. In this olfactometer assay, the mosquitoes were exposed to both control and citrus fruit peel extracts at the same time. The results regarding the preference of mosquitoes towards the citrus fruit peel extracts are given in Table 2.

**Table 2:** Preference Index for adult mosquitoes towards the Fresh Citrus peel extracts

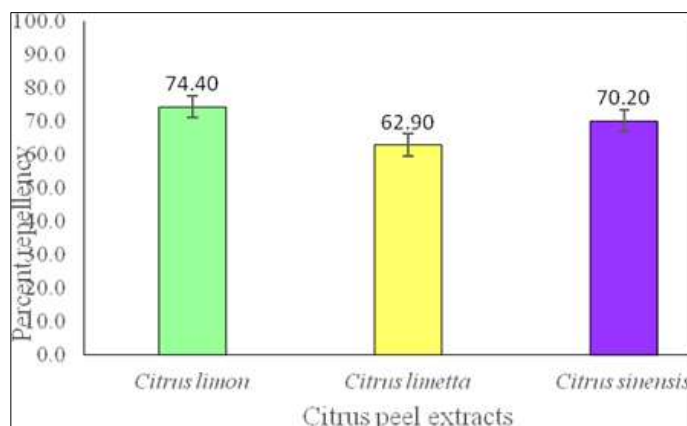
Name of the sample	Name of the mosquito species			
	<i>Aedes aegypti</i>	<i>Culex quinquefasciatus</i>	<i>Culex tritaeniorhynchus</i>	<i>Anopheles stephensi</i>
<i>Citrus limon</i>	-0.744±0.032	-0.292±0.046	-0.759±0.0073	-0.530±0.013
<i>Citrus limetta</i>	-0.629±0.028	-0.568±0.055	-0.540±0.0127	-0.690±0.028
<i>Citrus sinensis</i>	-0.702±0.020	-0.710±0.032	-0.880±0.0171	-0.520±0.017

The Preference Index (PI) was used in orientation tests to show how mosquitoes reacted to various repellents in the olfactometer. From the above result it indicated that citrus fruit peel extracts were shown to have a negative PI, proving that the compounds in the extracts were effective at repelling the mosquito species that were tested.

In the present study, the mosquito-repellent activity of *C.*

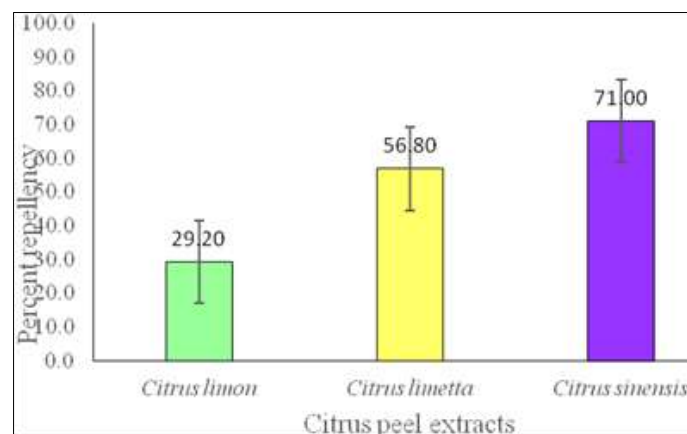
*sinensis*, *C. limetta* and *C. Limon* against *Ae. aegypti*, *Cx. tritaeniorhynchus*, *Cx. quinquefasciatus* and *An. stephensi* were evaluated and the results are given in figures 2-5.

The repellency levels of citrus fruits peel aqueous extracts against starved *Ae. Aegypti* females varied according to citrus peel species (Figure 2)



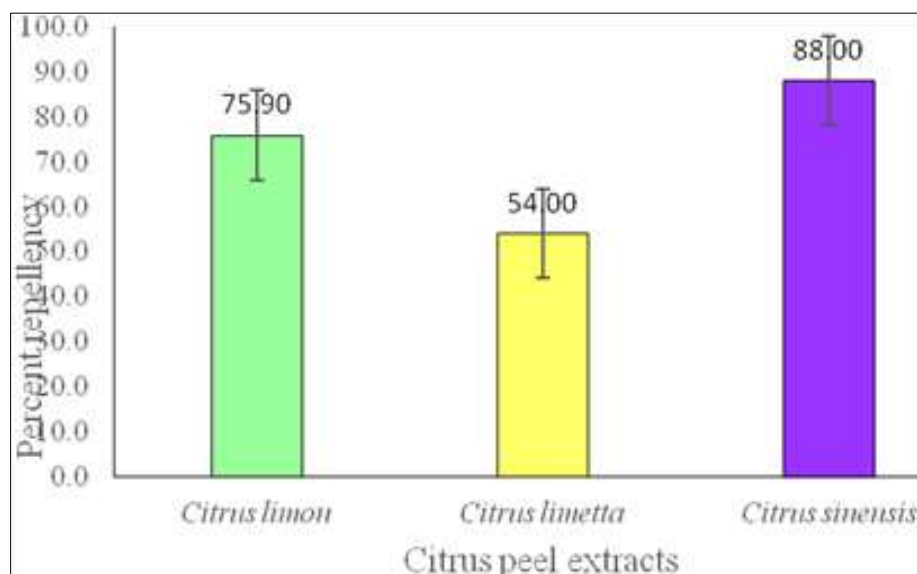
**Fig 2:** Repellent potentials of citrus fruits peel against *Aedes aegypti*

The results showed that *C. Limon* (74.40%), *C. limetta* (62.90%) and *C. sinensis* (70.20%) showcased repellency against *Ae. aegypti* mosquitoes. The mosquito repellency of citrus fruits peel extract against *A. aegypti* in the order *C. Limon* > *C. sinensis* > *C. limetta*.



**Fig 3:** Repellent activity of citrus fruits peel against *Culex quinquefasciatus* Results of mosquito repellent activity of citrus peel extract *Cx.*

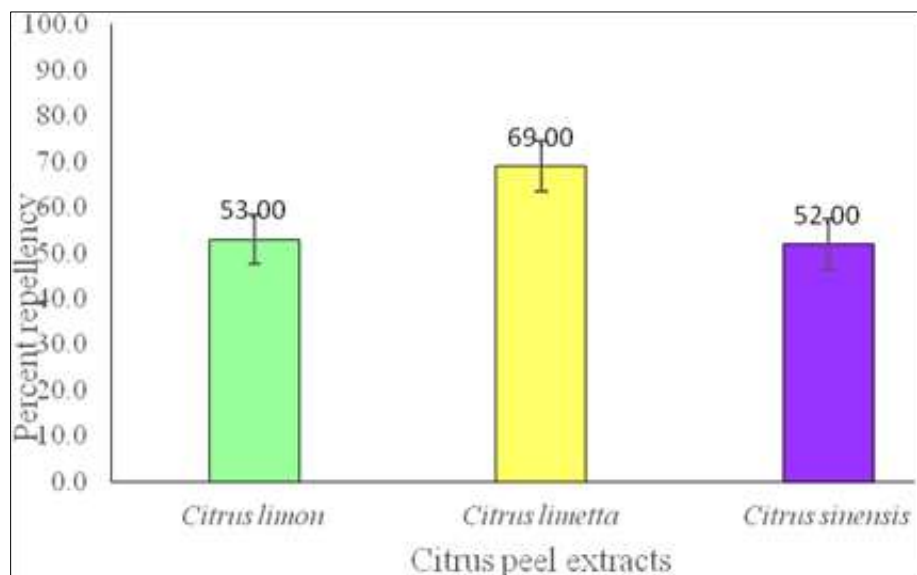
*quinquefasciatus* are illustrated in Figure 2. Results showed that among three citrus peels the highest repellent activity was observed with *C. sinensis* (71%) followed by *C. limetta* (56.80%) and *C. Limon* (29.20%) against *Cx. quinquefasciatus*.



**Fig 4:** Repellent activity of citrus fruits peel against *Culex tritaeniorhynchus*

The repellency of citrus peel extracts was tested against the mosquito vector *Culex tritaeniorhynchus* is depicted in Figure

4. Among all the extracts, *C. sinensis* expressed the highest repellent activity (88%) against *Cxs. tritaeniorhynchus*



**Fig 5:** Repellent activity of citrus fruit peel against *Anopheles stephensi*

From the result (figure 5) it was found that mosquito repellent activity against *Anopheles stephensi* was highest in *C. limetta* (69%) and lowest in *C. sinensis* (52%) peel extract. The *C. Limon* extract also repelled the *An. stephensi*. The results were similar to Oshaghi *et al* [22] who found that lemon has the potential to prevent bites of *A. stephensi*.

The repellent activities of Citrus fruit peel extracts varied according to the mosquito species. From the above findings, it was revealed that the mosquito repellency of citrus fruits peel extract against mosquito species in the following order.

*C. sinensis* - *Cx. tritaeniorhynchus* > *Cx. quinquefasciatus* > *Ae. Aegypti* > *An. stephensi*

*C. limetta* - *An. Stephensi* > *Ae. Aegypti* > *Cx.*

*quinquefasciatus* > *Cx. Tritaeniorhynchus*

*C. Limon* - *Cx. tritaeniorhynchus* > *Ae. Aegypti* > *An. stephensi* > *Cx. quinquefasciatus*

There was a significant difference in percent mosquito repellency among the four mosquito species tested against three citrus peel extracts ( $F= 3.1668$ ,  $DF= 3$ ,  $P<0.05$ ). Therefore, it was concluded that the mosquito repellent activity of the three extracts differs against the adult mosquitoes from each other. From the study results, it was revealed that fresh citrus peels have good repellence against adult vector mosquitoes.

#### 4. Discussion

Mosquitoes pose a severe threat to public health because they spread several serious and well-known diseases like malaria, dengue fever, arboviral encephalitis, West Nile virus, chikungunya fever and yellow fever. They are the main reason for the enormous amount of illness and fatality among human beings. Nowadays new synthetic repellents have been produced as well as made commercially available. However, using these repellents frequently and recklessly can have negative effects on the user<sup>[23]</sup>. In the present investigation result proved that aqueous extracts of citrus fruit peel have a significant mosquito repellent activity against *Ae. Aegypti*, *Cx. tritaeniorhynchus*, *Cx. quinquefasciatus* and *An. stephensi*. *C. sinensis* recorded the highest repellent activity against *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* while this extract exhibited the least repellent activity against *An. stephensi*. The results also coincide with Murugan *et al*<sup>[24]</sup> who identified the significance of *C. sinensis* orange peel extracts in the mosquito control strategy. *C. limetta* extract exhibited the highest repellent activity against *Anopheles stephensi*. *Citrus Limon* has maximum mosquito repellent activity against *Culex tritaeniorhynchus* and minimum mosquito repellent activity against *Anopheles stephensi*. There was remarkable repellent activity shown by the aqueous extract of citrus fruit peel against adult mosquitoes. These study results concurred with the research report of Effiom *et al*<sup>[25]</sup> who found that the photochemical of citrus fruit peel extracts have good mosquito-repellent activity. Further studies need to be done to identify the individual mosquito-repellent compounds present in citrus fruit peels.

#### 5. Conclusion

According to observations, citrus fruit peels perform well as repellents and can be used to replace commercial repellents. The plant-based repellent poses no danger to the health of humans, animals, or the environment. Additional research on citrus fruit peel extracts may aid in discovering suitable repellent compounds in the citrus species for the commercial synthesis of natural mosquito repellents.

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