

Mosquito Menace

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Aim: Observing the life cycle of *Aedes aegypti* mosquito and understanding its behavior towards different natural oils for encouraging natural methods of repellence

1. Introduction

India being a sub-tropical country has an optimum temperature and humidity conditions for mosquito breeding and thus is affected by repeated epidemic like conditions created due to mosquito-borne diseases. A major chunk of these diseases namely dengue, chikungunya, yellow fever etc is spread by the species *Aedes aegypti*. The key identifying features of this species are: a smallish, dark mosquito with conspicuous white markings and banded legs, the proboscis is all black although the palps are white tipped, wings are dark scaled, hind legs with femur pale scaled for basal three-quarters with dark scales. With the large scale increase in mosquito-borne diseases, it becomes imperative to work for better control methods and repellents. Most repellents today aiming to increase the duration of action are using chemicals like DEET (N, N-Diethyl-meta-toluamide) which stand effectively for longer durations. However, it becomes a matter of rising concern of the serious drawbacks of long term exposure to such chemicals. The experiment, therefore, aims to identify natural repellents in the form of **natural oils** namely citronella oil, lemongrass oil, celery seed oil, lavender oil, and cinnamon oil and then comparing their effect with the chemical DEET using **Contact Irritancy Assay** on laboratory-reared mosquitoes. Further, the oil was tested for its duration of repellency using **Human Bait Technique**.

2. Materials and Method

[1] Rearing of mosquitoes for the experiment: [1.1] For the process of rearing the following conditions were administered: temperature maintained at $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$, ~80% humidity, a 12 hour day and light cycle. [1.2] 3-5 day old caged adult females were blood fed on mice for a duration of 30 minutes. Following this, a small bowl lined with a strip of filter paper with water up to a depth of 2.5 cm was placed in the cage of adults. This bowl was collected after 48 hours. [1.2] The collected eggs were transferred to rearing cage where they were placed in a rearing tray containing 150 ml of distilled water with a small amount of fish food. [1.3] The eggs hatched after two days from a collection and larval stages were allowed to develop and fed with crushed dog biscuits. [1.4] 4-5 days old larvae, that is a stage observed after a week of initiation of the process, the larvae are divided into several trays for the development of pupae. The water in all the trays was changed at day 10. The pupal stages could be seen around Day 12 and the adults emerged out at Day 16. Post the pupal stage the net has to be strictly administered. [1.5] The trays are gradually removed and the adults are no fed with 10% sucrose diet for initial 3-4 Days. [2] Contact Irritancy Assay:

[2.1] The contact irritancy assay was performed on 3-day-old nonblood fed females to check the repellence caused by different natural oils and then comparing it with DEET. Oil-impregnated papers were prepared with Whatman filter paper no. 1, which were cut out in circles of 8 cm in diameter. The papers were impregnated with 5% and 1% concentration of the sample and then allowed to shade-dry. [2.2] The completely dried paper was placed on a glass plate, and a funnel with a hole on the top was kept inverted over the impregnated paper. A single female adult was released in the funnel with an aspirator and preconditioned for 3 min. Thereafter, the time at which first flight was taken was recorded. The experiment was continued for 15 min and the total number of flights undertaken by each female adult was scored. Parallel control tests were performed with papers impregnated with acetone. [2.3] The relative irritability caused by different oils was calculated with respect to control by the following formula: Relative Irritability = Mean number of take-offs stimulated by the sample/ Mean number of take-offs by control. [2.4] The experiment was performed twice for 5% and 1% concentration for each natural oil. The same experiment was performed by 5% and 1% concentration of DEET and then the result was compared with that of natural oils. [3] Celery seed oil which gave the best results was then studied to check the duration of repellency using human bait technique in which a marked area with applied oil was introduced in the cage of 3 days, 3-day look fed females and the duration of repellency was recorded. A control test was performed using ethanol.

3. Observation

While rearing the mosquitoes, the larval stage was observed on Day 7 while the pupal stage was observed around Day 12 with the emergence of adults at Day 15. Out of the five oils, celery seed oil gave the best results showing maximum relative irritability. Figure 1 shows the observation for contact irritancy assay for 5% and 1% concentration of the sample and Figure 2 shows a comparative study of the relative irritability observed. Since celery seed oil gave the best results out of the chosen oils it was further studied to check for the duration of repellency (Figure 3). The natural oil exhibited 100% repellency for 150 minutes which reduced to 96% in 165th min and 92% by 180 minutes.

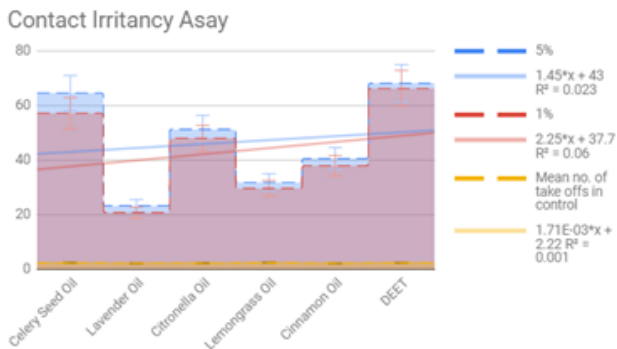


Figure 1

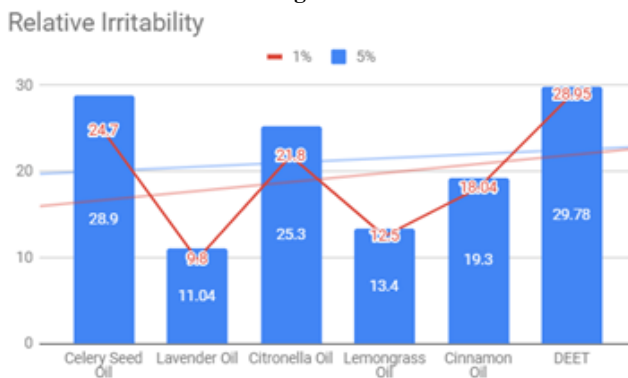


Figure 2

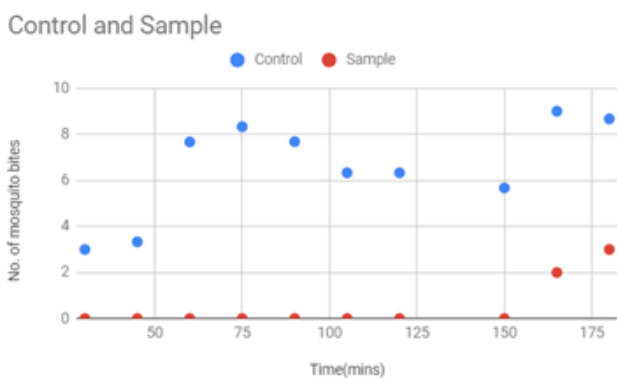


Figure 3

References

- [1] Lee H-S. Mosquito activity of aromatic medicinal plant oils against *Aedes aegypti*
- [2] World Health Organisation (2011) World dengue report 2011. WHO Global Dengue Programme. Geneva.
- [3] Warikoo R, Wahab N, Kumar S. potential of commercially available pine (*Pinus longifolia*) and cinnamon
- [4] (*Cinnamomum zeylanicum*) oils against an Indian strain of dengue fever mosquito, *Aedes aegypti* L.
- [5] (Diptera: Culicidae). *Acta Entomol Sin* (2011)
- [6] https://en.wikipedia.org/wiki/Aedes_aegypti

4. Result and Conclusion

Celery seed oil showed the highest relative irritability with a maximum number of take-offs observed both in 5% and 1% sample concentration thereby proving to be the most effective out of the chosen natural oils. Further, when the test with DEET is compared with celery seed oil, it is seen that it shows close results and the relative irritability for both is also very close. Therefore it can be interpreted that celery seed oil is a very effective natural repellent. For a repellent to be effective, the duration of repellency should be considerably large. On studying the duration of repellency of celery seed oil, it becomes evident that it works for a considerably high duration since it exhibited 100% repellence up till 150 minutes and up to 92% repellency for three hours. Therefore, high relative irritability and considerably longer duration of repellency make celery seed oil an effective choice for developing a natural mosquito repellent.