REPELLENT EFFICACY OF SOME ESSENTIAL OILS AGAINST *AEDES ALBOPICTUS*

Varun Tyagi, Medical Entomology Division, Defence Research laboratory, Tezpur-784001, Assam, India

Johirul Islam, Division of Pharmaceutical Technology, Defence Research laboratory, Tezpur-784001, Assam, India

Amit Agnihotri, Medical Entomology Division, Defence Research laboratory, Tezpur-784001, Assam, India

Diganta Goswami, Medical Entomology Division, Defence Research laboratory, Tezpur-784001, Assam, India

Bipul Rabha, Medical Entomology Division, Defence Research laboratory, Tezpur-784001, Assam, India

PK Talukdar, Medical Entomology Division, Defence Research laboratory, Tezpur-784001, Assam, India, Division of Pharmaceutical Technology, Defence Research laboratory, Tezpur-784001, Assam, India

Sunil K Dhiman, Vector Management Division, Defence Research and Development Establishment, Gwalior-474002, Madhya Pradesh, India

Pronobesh Chattopadhyay, Medical Entomology Division, Defence Research laboratory, Tezpur-784001, Assam, India

Vijay Veer, Medical Entomology Division, Defence Research laboratory, Tezpur-784001, Assam, India

***Corresponding author:** Dr. Varun Tyagi, Medical Entomology Division, Defence Research laboratory, Tezpur-784001, Assam, India, Tel: 7086834166; E-mail: varundrl@rediffmail.com

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ABSTRACT

Background: Mosquito species are the most important among the insects; for the reason that they transmit major diseases viz. malaria, dengue, chikungunya, zika, filariasis and various forms of encephalitis; imposing enormous menace to human as well as animals. These diseases cause huge amount of mortality and morbidity across the world. The principal strategy for fighting these diseases is the vector control including the use of repellent against adult mosquitoes. Use of essential oils as mosquito repellent has been suggested by different studies. Keeping in view of the above, we have evaluated synergistic activity of five essential oils in different combinations against Aedes albopictus mosquito.

Methods: Five essential oils i.e., 1) Aniba rosaeodora (rosewood), 2) Cymbopogon citrates (lemongrass), 3) Eucalyptus citriodora (lemon scented), 4) Litsea cubeba (litsea), 5) Pelargonium graveolens (geranium), were used for study. The Klun and Debboun test module (K & D module) was used for this repellent testing.

Results: Among all combinations, the combination of Aniba rosaeodora (rosewood) and Litsea cubeba (litsea) oils showed 82% repellency against Aedes albopictus mosquito up to 4 h.

Conclusions: In conclusion, we extended the application of essential oils for medically important mosquito control management. The results of this study are useful for developing eco-friendly, efficient and secure mosquito repellent.

Keywords: Aedes albopictus; Klun and Debboun test module; essential oils

INTRODUCTION

Earlier, mosquitoes were known to cause malaria. Since the last few decades, it has been realized that mosquitoes not only transmit malaria but also a large number of other important pathological conditions such as dengue, chikungunya, yellow fever, Japanese Encephalitis (JE), filariasis and so on. Recently, Zika virus is creating havoc in Africa, Southeast Asia, Pacific Island and Brazil (NTG, 2016).

Dengue Fever and dengue hemorrhagic fever (DHF) caused by an *Arbovirus*, are the common and promising mosquito-borne viral diseases that affect a extensive range of people in the world (Wiwanitkit, 2014). It is caused due to the bite of infected *Aedes* mosquitoes particularly *Aedes albopictus* (Asian tiger mosquito) and *Aedes aegypti*. *A. albopictus* played a major role in transmission of chikungunya virus in 2006 in the southern states of India. After quiescence of three decades, chikungunya outbreak occurred again in India in 2006.

After three years the situation has not changed much, a long spell of rainfall and circulation of DEN-II have recorded an epidemic situation in Delhi and Mumbai. Nearly 4,000 dengue cases were reported from the national capital New Delhi in the month of September, 2013. Dengue cases are being reported at epidemic rates in the Capital with a whopping 395 cases reported in just 72 hours. In the recent years, the monsoon started from June, and rainfall has been reported even in October, November, December and January. This has lead to the accumulation of fresh water and resulted in potential mosquito breeding grounds which are causing an epidemic situation nationwide especially in metro cities (IANS, Times of India).

Recently, chikungunya outbreak in the national capital is the worst in the last six years. According to municipal data, over 1,000 people have been affected by this vector-borne disease till September 2016. The last time the number of affected people reached three-figure mark was in 2011.

Many approaches have been developed to control the mosquitoes. The current mosquito control methods are based on synthetic insecticides. Synthetic insecticides are the first line of action due to their quick action, but their continuous use may lead to the development of resistance and adverse effect on environment. These factors have created a need for search of easily biodegradable alternative insecticides. The use of plant extracts for vector control has several appealing features as they are biodegradable, less hazardous and rich stock house of chemicals of diverse biological activity.

Essential oils have been used as mosquito larvicides and for repelling insect pests by entomologist or many researchers are working in vector control programme. Vector species of mosquito play a vital role in transmission of fatal diseases i.e., malaria, dengue, chikungunya. So these oils may be used to prevent and control the outbreak of mosquito borne diseases. In our study we have tried to provide the information on the base of our result about repellency of effective essential oils in different combinations against *A. albopictus*.

MATERIALS AND METHODS

Culture of *Aedes albopictus* mosquitoes were maintained in our insectary. Adults were given 10% sugar solution and the female were offered rabbit blood once in a week. Tests were conducted in laboratory with the help of Klun and Debboun test module (K & D module) (Figure 1) during morning to evening period in the light room and the relative humidity (RH) of experimental room was 70 ± 5% and temperature was 27 ± 2˚C. Synergistic activity of five essential oils i.e., 1) *Aniba rosaeodora* (rosewood), 2) *Cymbopogon citrates* (lemongrass), 3) *Eucalyptus citriodora* (lemon scented), 4) *Litsea cubeba* (litsea), 5) *Pelargonium graveolens* (geranium), were studied on treated area. The combinations were made for 10% concentration of essential oils by mixing equal concentrations of oils in 1:1 (v/v) ratio. Twenty five µL test solution in Isopropanol was applied on marked area and after air drying five to six days old ten unfed *Aedes albopictus* female were randomly selected from the insectary and placed into three alternate cells in the K & D module for repellent test. The bottom shape of K & D module is slightly concave conformed to the curvature of a human thigh. Observations on the number of bites of *Aedes albopictus* female mosquito were recorded at 0, 1, 2, 4 and 6 hrs post treatment. We have used the procedure for this repellent testing as described by Uniyal et al. 2015; Klun & Debboun, 2000.

A. Statistical Analysis

Data generated in repellent activity experiments using K and D module were expressed as mean % repellency ± SD (Standard deviation). The data were then subjected to analysis by adopting analysis of variance (ANOVA) followed by Tukey's test of multiple comparisons. All Statistical analysis was performed by means of statistical software GraphPad Instat (Version 3.05) (Figure 1).

RESULTS AND DISCUSSION

In this present study, we have evaluated different combinations of essential oils against female *Aedes albopictus* mosquito using K and D module. Five replicates were performed for each combination of essential oils (litsea+rosewood, litsea+geranium, litsea+lemon scented, rosewood+geranium, rosewood+lemon scented and rosewood+lemongrass) in the ratio of 1:1 at 10% concentration (Table 1 and Figure 2). Combinations of all five essential oils showed full protection till 1 hr. Synergistic effect of litsea and rosewood showed 94% to 66% repellency for 2-6 hrs after application. While, litsea+geranium showed 86% to 54% repellency for 2-6 hrs, combinations of litsea+lemon scented, rosewood+geranium, rosewood+lemon scented showed 84% to 54%, 82% to 34%, 72% to 28% repellency for 2-6 h respectively. Last combinations rosewood+lemongrass showed 70% to 20% repellency for 2-6 hrs after application. Combination of litsea and rosewood showed higher repellency for up to 4 h and last synergistic effect of rosewood and lemongrass showed less repellency i.e., 70%, 46% and 20% after the end of different time interval i.e., 2, 4 and 6 h respectively. The values of repellency which are given in the Table 1 are not significantly different but synergistic effect of two different essential oils possess their unique property.

Plenty of different techniques for mosquito vector control such as attractants, insecticides, repellents, biological environmental and genetics control etc. are in use which has their own merits and demerits. Hence it is essentially important to develop safe and eco-friendly techniques for the control of vector borne diseases especially in the field of mosquito. In rural communities, Plant-based repellents are still extensively used (Moore, 2006). At the latest, in market many commercial repellent products containing plant-based ingredients have gained increasing prominence between consumers because of their safe and non-toxic nature in comparison to popular trend of using synthetic repellents and reliable means of mosquito bite prevention (Isman, 1995).

Figure 1: Repellent Activity of Different Essential Oils against *Aedes albopictus* Mosquito Using K & D Module.

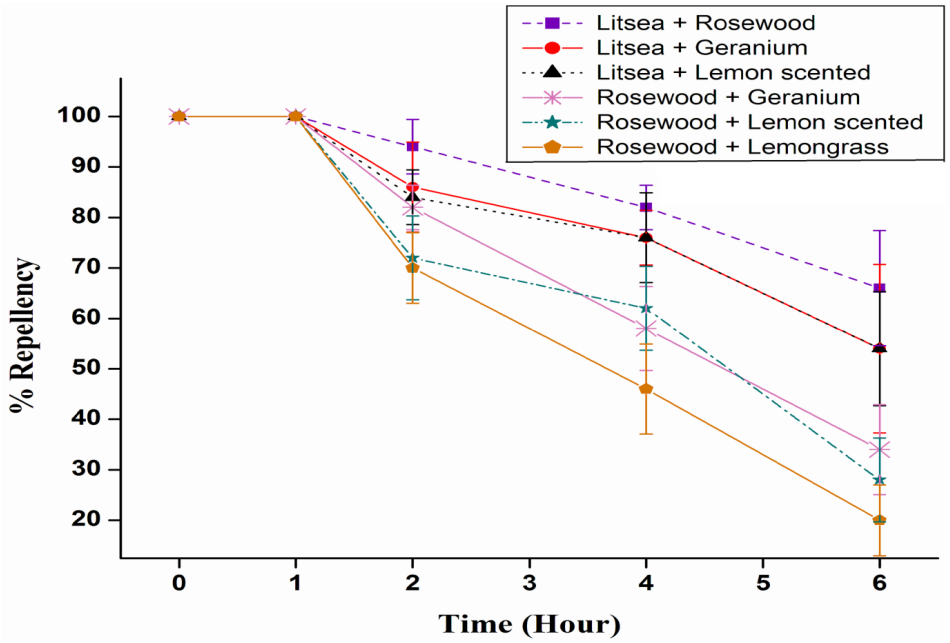

Figure 2: Synergistic Repellent Activities of Different Essential Oils against *Aedes albopictus* Mosquito.

Table 1
Synergistic Repellent Activity of Essential Oils Against *Aedes albopictus*

Mosquito	% Repellency						
Aedes albopictus	Combinations (Essential oils)	0.0 h	1.0 h	2.0 h	4.0 h	6.0 h	Significant
	Litsea + Rosewood	100.0±0.0	100.0±0.0	94.0±5.4	82.0±4.4	66.0±11.4	P>0.05
	Litsea + Geranium	100.0±0.0	100.0±0.0	86.0±8.9	76.0±5.4	54.0±16.7	
	Litsea + Lemon scented	100.0±0.0	100.0±0.0	84.0±5.4	76.0±8.9	54.0±11.3	
	Rosewood + Geranium	100.0±0.0	100.0±0.0	82.0±4.4	58.0±8.3	34.0±8.9	
	Rosewood + Lemon scented	100.0±0.0	100.0±0.0	72.0±8.3	62.0±8.3	28.0±8.3	
	Rosewood + Lemongrass	100.0±0.0	100.0±0.0	70.0±7.0	46.0±8.9	20.0±7.0	
	CONTROL	0	0	0	0	0	

In the column, Mean % repellency ± SD; ANOVA followed by Tukey's test of multiple comparison. The values in the column are not significantly different ($p > 0.05$).

Numerous scientist have also confirmed good quality plant based repellent efficacy against some mosquito species in bioassay tests as adulticidal activity (Manimaran et al. 2012), larvicidal activity (Choochote et al. 2004), repellent activity (Barnard, 1999) and adulticidal, repellent, larvicidal, oviposition activity (Traboulsi et al. 2005., Prajapati et al. 2005).

Plant chemicals are extremely volatile and different chemical constituent of essential oils having different repellent properties but in this context contact repellent testing & other bioassay methods, it is not yet established that which particular chemical is responsible for repellent (Twastin, 2006). A recent study revealed that the synergistic properties of 10 efficient oils were evaluated with the help of K & D module against *Aedes aegypti* mosquito and among these oils litsea oil showed effective repellency against *Aedes aegypti* mosquito with the combination of other studied oils; similarly we have also observed that litsea oil showed highest repellency against *Aedes albopictus*. Other researchers i.e., (Santiwitchaya, 2004., De-Boe et al. 2010., Vongsombath et al. 2012) too have carried similar type of studies. In the same manner, the repellent efficacy of rosewood, geranium and litsea against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* mosquitoes was evaluated by Amer and Mehlhorn (2006).

The K & D module bioassay is a type of human skin bioassay in which the influence of test compounds (essential oils) might be influenced by human odour component and skin emanations, so it is necessary that such type of studies should also correlate with other repellent studies i.e., EAG (Electroantennogram) and arm in cage method.

In support of present study we can say that the groundwork for considerate the repellent efficiency of efficient essential oils for manufacture effective, long-lasting, safe, nontoxic, biodegradable insect repellent and useful for controlling mosquito borne diseases (Figure 2).

In the column, Mean % repellency ± SD; ANOVA followed by Tukey's test of multiple comparison. The values in the column are not significantly different ($p>0.05$).

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