International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2015; 2 (4): 29-35

Received: 15-10-2015
Accepted: 20-11-2015

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Synergistic effect of effective oils against *Aedes aegypti* female mosquito, vector of dengue and chikungunya

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Abstract
Dengue and chikungunya are deadly diseases transmitted by *Aedes aegypti*. Essential oil a natural product contains highly volatile chemical compounds and use as a personal protection against blood sucking mosquito is an effective and safe method. The objective of these studies was to evaluate the synergistic effect of 10 effective oils with the help of K & D module and Electroantennogram (GC-EAD). In the study of protection period, litsea + rosewood in the ratio of 1:1 (v/v) at 10% concentration showed 86% repellency for 4 hours against *Ae. aegypti* using K & D module. In the study of electrophysiological experiment, *p*-Menthane (4.13RT), Citronellol (4.25RT), *Z*-Citral (4.41RT), Citral (4.57RT) from the blend of ten selective essential oils showed strong antennal responses on the receptors of *Ae. aegypti*. In the present study, essential oil showed effective result are useful for developing safe, biodegradable, eco-friendly and effective insect repellent.

Keywords: *Ae. aegypti*, repellent, essential oils, K & D module, Electroantennogram.

1. Introduction
Several species of the mosquitoes are the prime vectors responsible for the spread of serious human diseases such as malaria, Japanese encephalitis, dengue, chikungunya, lymphatic filariasis, epidemic poly-arthritis as well as yellow fever. However, people of less privileged populations, throughout the tropical and subtropical regions of the world, are at greater risk and every year mosquito transmit disease to more than 700 million people [1]. Dengue and chikungunya are transmitted by *Aedes aegypti* and dengue a common and emerging mosquito-borne viral diseases that affect a wide range of people in the world and claimed more deaths than any other arboviral infection [2]. While dengue is a global concern, with a steady increase in the number of countries reporting the disease, currently close to 75% of the global population exposed to dengue are in the Asia-Pacific region. According to World Health Organization [3] over 50 – 100 million people with severe dengue require hospitalization each year, a large proportion of whom are children, about 2.5% of the affected children die.
The deadly diseases carried by insect and mosquito vectors and the annoyance caused by them encouraged the discovery of control measures including methods of personal protection. Mosquito control programs are essential to prevent spreading of mosquito borne diseases. Mosquito repellents are used as a personal protection which can provide practical and economical means of preventing mosquito-borne diseases [4]. Every year millions of people are affected by mosquito born diseases worldwide and the use of insect repellent to the skin is a common personal protection [5]. According to Charlwood [6], in 485 BC, Herodotus (Greek Historian) wrote that he observed Egyptian fishermen using castor-oil on their skin and they used the same oil in lamps to keep mosquitoes away at night. Just around the 4th Century, ancient Romans repelled mosquitoes by rubbing a combination of vinegar**,** manna and plant oil on their bodies. However, they (Romans) also burned herbs including black cumin, bay, galbanum and oregano to repel insects [7]. The burning of various plants or plant oils and other materials to produce smoke as a repellent is the oldest method recorded for repelling nuisance mosquitoes by humans and the burning of various plants, fish, bones, and dung to repel mosquitoes has been described in *Geoponika* (10th century) and the ancient Sanskrit *Yoga Ratnakara* (17th century) [8, 7].
Chemicals play an important role in influencing vital activities (search for food, opposite sex, an oviposition site or medium) of insects. It can be said that host seeking mosquitoes are attracted to human body odours, which have different substrates such as urine, feces, carbon

dioxide and lactic acid [9]. Host-seeking mosquitoes detect host-derived chemicals with the help of two major sensory organs. The maxillary pulps detect the level of CO_2 and olfactory receptors of antennae detect host-related odours [10]. At the time of Second Ward War only four repellents were known such as Citronella oil, Dimethyl phthalate, Indalone and Rutgers [11]. A large number of potential mosquito repellent compounds were screened by US Army and in 1953 *N, N*-diethyl-3-methylbenzamide also known as N, N-diethyl-m-toluamide (DEET), the "gold standard" repellent of modern days was discovered [12]. The mode of action of DEET has been a topic of controversy for some time. DEET may cause adverse toxic effects, especially in young children, pregnant and lactating women [13]. The synthetic repellents have disadvantages including strong odours, skin irritation, possible health effects, resistance development and environmental hazards. Due to these adverse effects, attempts are made to find safe and eco-friendly repellents derived from plant materials. In the last few years with the increase of public concern on the safety of many chemical products that were used previously as insecticides and repellents, several institutes and researchers were directed to the development of natural active ingredients especially from plant sources because they are believed to be safe to human use and are easily biodegradable [14, 15].

Essential oils are defined as natural volatile substances found in a variety of odoriferous plants. They are in the form of concentrated hydrophobic liquid containing volatile aroma compounds obtained from different parts of plants [16]. Essential oils are generally extracted by distillation method such as hydro distillation, steam distillation and solvent extraction are the commonly used techniques. In fact the essential oils are secondary plant metabolite and their major active constituents are monoterpenoids, sesquiterpenoids, benzenoids, phenylpropanoids etc. The composition of essential oils is highly diversified across different plant species and is directly affected by climatic and soil conditions. The properties and proportion of monoterpenoides depend on temperature, circadian rhythm and plant stages [17]. However, they are produced in aromatic species of higher plants belonging mostly to a few families, including the *Myrtaceae*, *Lamiaceae*, and *Asteraceae*. The accumulation and synthesis of essential oils are associated with complex secretory structures such as glandular trichomes (*Lamiaceae*), secretive (*Myrtaceae*, *Rutaceae*) and resin ducts (*Asteraceae*, *Apiaceae*) [18]. Depending on different species essential oils are stored in various parts of plants organs such as flowers, leaves, wood, roots, fruits, rhizomes and seeds. They are used in perfumes, cosmetics, soaps, for flavoring foods, drinks, and for adding scents to incense, household cleaning products and various essential oils have been used in medical formulations. The essential oils have been used by entomologist as mosquito larvicides and for repelling insect pests including haematophagous insects. Most commonly used essential oils as repellent are citronella, geranium, cedar, peppermint, rosemary, soybean and eucalyptus [19].

Vectors play an important role in transmission of deadly diseases. So insect repellents may be used to prevent and control the outbreak of insect borne diseases. Essential oils are composed of volatile components having minor and major constituents with pleasant fragrance to human beings. But this aroma may not be pleasant (attractive) to mosquitoes and has repelling action. In comparison of synthetic repellents, botanical repellents generally perform significantly less well, they have shorter protection time and hence they need frequent re-application. These drawbacks can be resolved by further investigations on repellency of essential oils. Thus there is a need to develop eco-friendly, safe, cost effective repellant for vector control. The proposed work is expected to provide us the information about repellency of effective essential oils against mosquito.

A better understanding of blend effect of effective essential oils repellent efficacy and finding effective repellent constituent present in essential oil is important for guiding research to develop newer, safe repellent for controlling transmission of mosquito borne diseases and also for new strategy or formulation against vector born insects.

2. Materials and Methods

2.1 Test Insect

The laboratory colony of *Ae. aegypti* mosquitoes were maintained in our insectary at 27±2 °C and 75±5% Relative humidity and utilized for all the experiments. Five to seven days old adult mosquitoes (50-75 pairs) were released for oviposition in a wooden rearing cage (750×600×600 mm) which has a sleeve opening on one side. Adults were given 10% sugar solution and the female mosquitoes were fed on rabbits for a blood meal initially for 2 days and then at every alternate day.

2.2 Essential Oils and Synthetic repellents

Ten essential oils namely litsea (*Litsea cubeba*), geranium (*Pelargonium graveolens*), rosewood (*Aniba rosaeodora*), cinnamon (*Cinnamomum zeylanicum*), citronella (*Cymbopogon winterianus*), lemongrass (*Cymbopogon citrates*), lemon scented (*Eucalyptus citriodora*), camphor (*Cinnamomum camphora*), galbanum (*Ferula galbaniflua*) and dill (*Anethum graveolens*) were obtained from the Fragrance and Flavour Development Center (FFDC), Kannauj, Uttar Pradesh, India. The synthetic repellent *N, N*- diethyl-m-toluamide (DEET) 98.5% pure was purchased from Sigma Aldrich chemicals and *N, N*-diethyl phenyl acetamide (DEPA) 99% pure was synthesized by chemists from Synthetic Chemistry Division of DRDE Gwalior.

3. Test Procedure and Methods

3.1 K & D module Bioassay

Tests were conducted in a laboratory using K & D module [20] by using Plexiglas. It has 5 cells for mosquito transfer in each cell with Stoppard at the bottom with 3cm × 4cm rectangular open hole with sliding door. The bottom shape of K & D module is slightly concave conformed to the curvature of a human thigh. Unfed *Ae. aegypti* females were drawn from the colony for repellent test. Tests were conducted during 10:00 hr to 16:00 hr in the light room and the laboratory temperature 27±2 ^{0}C and 70±5% RH with light was maintained for experiment. For repellent testing volunteer wearing short pants seated with horizontally extending legs and 3cm × 4cm outline were made using water soluble marker on a test area leaving alternate call blank to avoid contamination. Ten females (5-6 days old, blood meal unfed) were randomly selected and placed into three alternate cells in the K & D module. Duration of protection time for synergistic effects of effective essential oils at 10% concentrations was also tested against *Ae. aegypti*. The combinations were made for 10% concentration of essential oils by mixing equal concentrations of oils in 1:1 (v/v) ratio. Repellency activity was studied on treated area and observations on the number of bites were recorded at 0, 1, 2, 4 and 6 hr post treatment. Twenty five µl test solution in

Isopropanol was applied on marked area and after air drying a bioassay using K & D module was conducted. DEET and DEPA served as positive control for comparison and Isopropanol served as negative control. Observations conducted three times (n-3) on each repellent on 1 human volunteer (Age 28- 30) and the number of mosquitoes biting in each cell within a 5 minute exposure was recorded. A different set of mosquito population was used for replication.

3.2 Data Analysis

The percentage of repellency was calculated by comparing the number of bites for control against the number of bites for repellent treated volunteers. The percent repellency was calculated as follows = (C-T/C) (100), where C = number of mosquito bites in control and T = number of mosquito bites in treated [21].

3.3 Gas Chromatograph - Electroantennogram Detection (GC-EAD)

The sensitivity of *Ae. aegypti* to the components of essential oils was evaluated using gas chromatography-electroantennogram detection (GC-EAD). In a Gas Chromatograph (Agilent 7820A) is fitted with 30m × 0.32mm ID × 25µm DB 5MS column. The volatile chemicals which are very sensory system of mosquitoes can be easily identified using GC-EAD. The Gas Chromatograph was kept initially at 50 ˚C for 2 min then increased at the rate of 10 ˚C/min to 200˚C and held for 3 min. The GC effluents were split by a Y splitter (fused silica, Sigma). One end of the tube goes into FID and other end of tube was delivered to the antennal preparation through a heated transfer line kept at 230 ˚C. Continuous humidified air flow at the rate of 500ml/min for was delivered onto the antenna through which the GC effluent mixed with continuous air flow. Once the antenna stabilized the mixtures of 10 effective essential oils prepared at 1000ppm in HPLC grade Methanol at the rate of 1µl was injected into the GC and the response of the antenna was recorded in the GC-EAD program (Syntech, The Netherlands: Version 2.6).

4. Results

4.1 Synergistic effect of essential oils

In the present study, the ten selective essential oils namely litsea, geranium, rosewood, cinnamon, citronella, lemongrass, lemon scented, camphor, galbanum and dill in the ratio of 1:1 (v/v) at 10% concentration against *Ae. aegypti* using K & D module (Table 1) were examined. All combination showed complete protection soon after application. Litsea + rosewood, litsea + geranium, litsea + lemon scented, litsea + lemongrass showed 73% to 86% repellency for 4 hours and provided 50% to 56% 6 hours after application. However, rosewood + geranium, rosewood + lemon scented, rosewood + lemongrass, rosewood + citronella, rosewood + dill, rosewood + galbanum, rosewood + camphor showed 73% to 93% repellency for 2 hours and after application of 4 hours they showed 50% to 56% repellency against Ae. aegypti. Likewise, geranium + lemon scented, geranium + lemongrass, geranium + galbanum, geranium + camphor showed 73% to 86% repellency for 2 hours and provided 36% to 56% repellency for 4 hours after application. And the combination of dill + lemon scented, dill + lemongrass, dill + galbanum showed 83% to 93% repellency for 2 hours and showed 23% to 56% repellency for 4 hours after application. The blend of lemon scented + lemongrass exhibited 100% repellency for 2 hours and provided 26% to 50% repellency for 6 hours after application. However, lemongrass + cinnamon, lemongrass + citronella, lemongrass + rosewood, lemongrass + geranium, lemongrass + dill, lemongrass + galbanum, lemongrass + camphor showed 73% to 83% repellency for 2 hours and showed 16% to 26% repellency for 6 hours after application. Moreover, galbanum + lemon scented, galbanum + lemongrass, galbanum + cinnamon, galbanum + citronella, galbanum + dill, galbanum + camphor, citronella + lemon scented, citronella + cinnamon showed 73% to 86% repellency for 2 hours and provided 36% to 56% repellency for 4 hours after application. Cinnamon + lemon scented, showed 73 to 86% repellency for 2 hours and showed 53 to 56% repellency for 4 hours after application. While the blend of camphor + litsea, camphor + rosewood, camphor + lemon scented, camphor + lemongrass, camphor + cinnamon showed only 56% to 66% repellency for 4 hours and provided 16% to 30% repellency for 6 hours after application. The standard formulated synthetic repellent DEET and DEPA at 20% concentration provided much longer 100% protection up to 6 hours against *Ae. aegypti* mosquitoes.

Table 1: Mean percentage protection ± SE and protection period of 1:1 combination of 10% concentration of essential oils against *Ae. aegypti*.

S. No	Treatment 1:1	Mean Percentage Repellency ± SE				
		0hr	1hr	2hr	4hr	6hr
1	LS + RS	100 ± 0	100 ± 0	93.3 ± 0.34	86.6 ± 0.34	56.6 ± 0.34
2	LS + GRN	100 ± 0	100 ± 0	90 ± 0	86.6 ± 0.34	50 ± 0.59
3	LS + LMSN	100 ± 0	100 ± 0	83.3 ± 0.34	73.3 ± 0.34	50 ± 0.59
4	LS + LMNG	100 ± 0	100 ± 0	96.6 ± 0.33	86.6 ± 0.34	50 ± 0.59
5	LS + CNM	100 ± 0	86.6 ± 0.34	73.3 ± 0.34	53.3 ± 0.34	16.3 ± 0.34
6	LS + CITRO	100 ± 0	96.6 ± 0.33	86.6 ± 0.34	53.3 ± 0.34	23.3 ± 0.34
7	LS + DL	100 ± 0	96.6 ± 0.33	83.3 ± 0.34	56.6 ± 0.34	23.3 ± 0.34
8	LS + GLB	100 ± 0	100 ± 0	86.6 ± 0.34	56.6 ± 0.34	20 ± 0.59
9	LS + CMP	100 ± 0	86.6 ± 0.34	73.3 ± 0.34	53.3 ± 0.34	16.3 ± 0.34
10	RS + GRN	100 ± 0	100 ± 0	86.6 ± 0.34	56.6 ± 0.34	23.3 ± 0.34
11	RS + LMSN	100 ± 0	100 ± 0	73.3 ± 0.34	63.3 ± 0.34	26.6 ± 0.34
12	RS + LMSN	100 ± 0	100 ± 0	73.3 ± 0.34	46.6 ± 0.34	20 ± 0.59
13	RS + CMP	100 ± 0	100 ± 0	73.3 ± 0.34	66. 6 ± 0.34	30 ± 0.59
14	RS + DIL	100 ± 0	100 ± 0	76.6 ± 0.34	56.6 ± 0.34	26.6 ± 0.34
15	RS + CITRO	100 ± 0	83.3 ± 0.34	73.3 ± 0.34	43.3 ± 0.34	13.3 ± 1.36
16	RS + GLB	100 ± 0	83.3 ± 0.34	73.3 ± 0.34	43.3 ± 0.34	6.6 ± 0.34
17	RS + CNM	100 ± 0	83.3 ± 0.34	66.6 ± 0.34	33.3 ± 0.34	6.3 ± 0.34
18	GRN + CMP	100 ± 0	100 ± 0	73.3 ± 0.34	36.6 ± 0.34	16.6 ± 0.33

19	GRN + LMSN	100 ± 0	100 ± 0	73.3 ± 0.34	53.3 ± 0.34	26.6 ± 0.34
20	GRN + CITRO	100 ± 0	73.3 ± 0.34	66.6 ± 0.34	36.6 ± 0.34	6.3 ± 0.34
21	GRN + GLB	100 ± 0	83.3 ± 0.34	73.3 ± 0.34	43.3 ± 0.34	6.6 ± 0.34
22	GRN + LMNG	100 ± 0	83.3 ± 0.34	73.3 ± 0.34	56.6 ± 0.34	26.6 ± 0.34
23	GRN + DL	100 ± 0	76.6 ± 0.34	63.3 ± 0.34	36.6 ± 0.34	6.3 ± 0.34
24	GRN + CITRO	100 ± 0	73.3 ± 0.34	63.3 ± 0.34	36.6 ± 0.34	3.3 ± 0.33
25	GRN + CNM	100 ± 0	83.3 ± 0.34	73.3 ± 0.34	43.3 ± 0.34	6.3 ± 0.34
26	DL + LMSN	100 ± 0	100 ± 0	76.6 ± 0.34	56.6 ± 0.34	23.3 ± 0.34
27	DL + LMNG	100 ± 0	100 ± 0	83.3 ± 0.34	46.6 ± 0.34	26.6 ± 0.34
28	DL + CNM	100 ± 0	73.3 ± 0.34	63.3 ± 0.34	43.3 ± 0.34	16.6 ± 0.34
29	DL + GLB	100 ± 0	96.6 ± 0.33	83.3 ± 0.34	56.6 ± 0.34	23.3 ± 0.34
30	DL + CMP	100 ± 0	83.3 ± 0.34	53.3 ± 0.34	33.3 ± 0.34	6.3 ± 0.34
31	DL + CITRO	100 ± 0	70 ± 0.59	66.6 ± 0.34	36.6 ± 0.34	6.6 ± 0.34
32	CMP + LMSN	100 ± 0	100 ± 0	73.3 ± 0.34	63.3 ± 0.34	26.6 ± 0.34
33	CMP + LMNG	100 ± 0	100 ± 0	76.6 ± 0.34	56.6 ± 0.34	23.3 ± 0.34
34	CMP + GLB	100 ± 0	100 ± 0	73.3 ± 0.34	36.6 ± 0.34	16.6 ± 0.33
35	CMP + CNM	100 ± 0	100 ± 0	73.3 ± 0.34	56.6 ± 0.34	16.6 ± 0.34
36	CMP + CITRO	100 ± 0	83.3 ± 0.34	66.6 ± 0.34	33.3 ± 0.34	6.3 ± 0.34
37	LMSN + LMNG	100 ± 0	100 ± 0	100 ± 0	73.3 ± 0.34	30 ± 0.59
38	LMSN + CNM	100 ± 0	100 ± 0	83.3 ± 0.68	46.6 ± 0.34	20 ± 0.59
39	LMSN + GLB	100 ± 0	100 ± 0	86.6 ± 0.34	56.6 ± 0.34	16.6 ± 0.34
40	LMNG + CNM	100 ± 0	100 ± 0	73.3 ± 0.34	56.6 ± 0.34	16.6 ± 0.33
41	LMNG + GLB	100 ± 0	100 ± 0	83.3 ± 0.68	46.6 ± 0.34	23.3 ± 0.34
42	GLB + CNM	100 ± 0	100 ± 0	86.6 ± 0.34	56.6 ± 0.34	23.3 ± 0.34
43	CITRO + LMSN	100 ± 0	100 ± 0	73.3 ± 0.34	53.3 ± 0.34	26.6 ± 0.34
44	CITRO + LMNG	100 ± 0	100 ± 0	83.3 ± 0.68	46.6 ± 0.34	26.6 ± 0.34
45	CITRO + CNM	100 ± 0	100 ± 0	86.6 ± 0.34	56.6 ± 0.34	20 ± 0.59
46	CITRO + GLB	100 ± 0	100 ± 0	73.3 ± 0.34	56.6 ± 0.34	16.6 ± 0.34
47	DEPA	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
48	DEET	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
49	CONTROL	0	0	0	0	0

Keywords
CITRO: Citronella
CMP: Camphor
CNM: Cinnamon
DL: Dill
GLB: Galbanum
GRN: Geranium
LMNG: Lemongrass
LMSN: Lemon scented
LS: Litsea
RS: Rosewood

4.2 Electroantennogram response of the antenna of *Ae. aegypti* to blend of 10 effective oils

In the study of electrophysiological experiment the antenna of female *Ae. aegypti* responded to effective components of selected essential oils studied by Gas Chromatography - Electroantennogram Detection (GC-EAD) result are shown in Figure 1 and Table 2. The blend of ten selective essential oils is used for antennal responses. From the mixture of effective essential oils injected into the GC the components were clearly separated at the mentioned programs. There were 20 active fractions or components were founds in mixture of essential oils to which the antenna of *Ae. aegypti* responded. The chemical eluting at Linalool (2.19RT), Linalool oxide (2.35RT), Citronellal (2.67RT), Menthone (3.53RT), *p*-Menthane (4.13RT), *β*-Citronellol (4.25RT), *Z*-Citral (4.41RT), Cis-Geraniol (4.49RT), Citral (4.57RT), Cinnamaldehyde (4.90RT), Champagne (5.18RT), Geraniol (5.31RT), Citronellal (5.40RT), Geranyl acetate (5.90RT), *β*-Caryophyllene (6.18RT), Methoxycinnamaldehyde (6.58RT), Caryophyllene (7.15RT), Dillapole (8.16RT), Trans-Caryophyllene (9.38RT) and *β*-Citronellol (10.39RT) are very active. It's indicating that the effective essential oils that were showing very good repellent activity in the behavioural experiment contain active chemicals that are acting on the antennal receptors of *Ae. aegypti*.

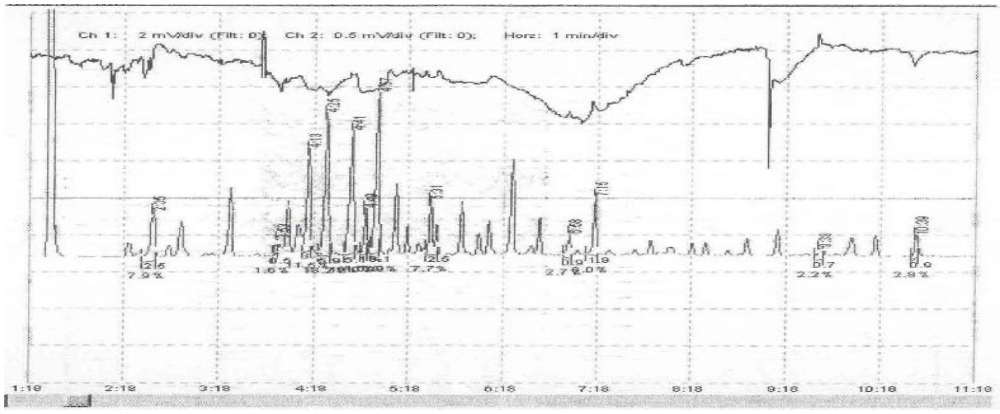

Fig 2: Gas Chromatograph Coupled - Electroantennogram Detection (GC-EAD): Antennal amplitude responses

Table 2: Blend of ten essential oils composition elicited antennal responses in GC-EAD analyses, using antenna of female *Ae. aegypti* as electroantennographic detector.

Peak	Blend Composition	Retention time
1	Linalool	2.19
2	Linalool oxide	2.35
3	Citronellal	2.67
4	Menthone	3.53
5	*p*-Menthane	4.13
6	*β*-Citronellol	4.25
7	*Z*-Citral	4.41
8	Cis-Geraniol	4.49
9	Citral	4.57
10	Cinnamaldehyde	4.9
11	Champagne	5.18
12	Geraniol	5.31
13	Citronellal	5.4
14	Geranyl acetate	5.9
15	*β*-caryophyllene	6.18
16	Methoxycinnamaldehyde	6.58
17	Caryophyllene	7.15
18	Dillapole	8.16
19	Trans-Caryophyllene	9.38
20	*β*-Citronellol	10.39

5. Discussion

The study of blood sucking mosquito repellency effect and behavioural responses of essential oils is one of the difficult studies, more complex and shows large differences between their results of researchers. Plant chemicals are highly volatile and different chemical constituent of essential oils having different repellent properties but in the contact repellent testing and other bioassay methods which particular chemical is responsible for repellent were unidentified [22]. Essential oils are composed of volatile components having minor constituents contain pleasant fragrance which are responsible for mosquito repellent and inhibit the orientation of blood sucking insects in three dimension environmental spaces [23, 24].

In the present study, the combination of litsea with dill, geranium, camphor, rosewood, galbanum, cinnamon, lemon scented, lemongrass and citronella showed effective repellent efficacy against *Ae. aegypti* mosquitoes. Likewise, blend of lemon scented and lemon grass also showed effective repellent against *Ae. aegypti*. Essential oil of litsea had high repellent efficacy against diurnally active mosquito *Ae. aegypti* and the result were supported by Tawatsin *et al*. [22], Amer and Mehlhorn [25] and Vongsombath *et al*. [26]. The repellent properties of some essential oils against many arthropods are based on their aromatic constituents [16]. The essential oils indicate that broad spectrum having large number of active ingredient or components which are responsible for repellency and mode of different activity of test mosquito depends on the blend effect of chemical components present in essential oils having different volatile properties [27]. The citronella oil also showed repellency against three different mosquitoes such as *Ae. aegypti*, *Cx. quinquefasciatus* and *An. dirus* [28, 25]. Dill, camphor, galbanum, cinnamon also showed repellency and the results was supported by Amer and Mehlhorn [25] against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. Likewise, the repellent efficacy of rosewood and geranium against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* was supported by Amer and Mehlhorn [25]. Essential oils such as lemongrass and lemon scented also showed effective repellency and the result were supported by Amer and Mehlhorn [25], Moore *et al*. [29] against various species of mosquitoes such as *Ae. aegypti*, *Cx. quinquefasciatus, An. gambiae, An. darling,* Mansonia spp. and *An. arabiensis*.

In the study of GC-EAD, in a blend of 10 effective oils, 20 active fractions showed in antennal responses. The behavioural experiment contains active quantitative constituents that are acting on the antennal receptors of *Ae. aegypti*. The mode of action of insect repellent appears to be based on interaction and inhibition of chemosensory neurons but it is difficult to point out which particular minor constituents of essential oil are responsible for repellency. The gas chromatography-electroantennogram detection (GC-EAD) technique was employed for identification of particular constituent of essential oil that is responsible for antennal response of *Ae. aegypti* female mosquito. In the present study, *p*-Menthane and Citronellol the components of geranium oil [30, 23], *Z*-Citral it is a mixture of geranial and neral [31] a dominant component of litsea oil and Citral a major component of lemongrass [32] from the blend of ten selective essential oils showed strong antennal responses on the receptors of *Ae. aegypti*. The other component of litsea oil such as Cis-geraniol also called Nerol and Geranyl acetate elicited responses in the antenna of *Ae. aegypti* female mosquito However, the major and minor component of rosewood oil is Linalool and Linalool oxide[33] and geranium oil is Menthone, *β*-citronellol and Trans-caryophyllene [30, 23] and the major component of lemon scented oil is citronella and *β*-Citronellol [34] elicited a spike response of the antenna of *Ae. aegypti* female mosquito. Likewise, linalool and geraniol are the components of lemongrass showed antennal response. The component of dill oil is Dillapole [35], camphor oil *p*-Menthane and Champene [36], cinnamon oil is Cinnamaldehyde, *β*-caryophyllene, Methoxycinnamaldehyde and Caryophyllene [23, 37], galbanum oil is Champene [36] and citronella oil is Citronellal and *β*-Citronellol [38, 23] showed spike response in the antenna of *Ae. aegypti.*

The result showed that, the repellent effect of selective essential oils is less then synthetic chemical. The blend of litsea oil with other effective oils and lemon scented with lemongrass gave effective and long lasting repellent effect over other combinations of effective oils against day biter mosquitoes such as *Ae. aegypti*. The K & D module bioassay is a kind of human skin bioassay in which the influence of test compounds (essential oils) might be influenced by human odour component and skin emanations. The EAG amplitude response and the repellent activity of essential oils studied with the help of various bioassay methods for comparison provide information of the effective repellent property.

The present study provides information for developing safe, biodegradable, eco-friendly and effective insect repellent formulation for topical application by using a non insecticidal chemical which can be used by individual and communities in specific situations to minimize the transmission of vector borne diseases. Further, it is based on this point of view that this study was conducted in the laboratory to evaluate the repellency of the essential oils.

6. Conclusion

Present study on synergistic effect of effective oils, only litsea oil showed effective repellency with the blend of other oils against *Ae. aegypti* female mosquitoes. Essential oils showed higher repellent activity against blood sucking mosquitoes are useful for development a newer anti-mosquito repellent from natural plant product.

7. Acknowledgements
The authors are grateful to Prof (Dr) M P Kaushik, Former Director, Defence Research and Development Establishment (DRDE), Gwalior and all the colleagues in the Department of Vector Management Division for their help in maintenance of the *Ae. aegypti* mosquito colony and their support while carrying out the above study on essential oils.

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