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Repellent effect, knockdown study and electrophysiological responses of essential oils against *Aedes aegypti*

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Abstract
Aedes aegypti mosquito is a major vector of dengue and chikungunya in tropical and sub-tropical region. Personal protection by using repellents is one of the effective ways to prevent epidemic dengue from mosquito biting. In the protection period essential oil of litsea at 20% was found effective till 2 hrs while DEET and DEPA provided complete protection ranging from 5 to 6 hrs against *A. aegypti* mosquito at same forngs litsea oil exhibited 10%, 76%, 100% knockdown effect at 0.1%, 1% and 5% concentrations and Malathion showed 50% 86%, 100% knockdown activity at above concentrations. Analysis by GC-EAD the dominant component of litsea oil i.e. *Z*-citral elicited strong antennal responses in the antenna of *A. aegypti* mosquito. Based on the study it may be concluded that essential oils are effective and safe to human being as a personal protection against mosquitoes.

Keywords: Essential oils, repellent, knockdown, GC-EAD, *Aedes aegypti*.

1. Introduction
Aedes aegypti is a vector of dengue, dengue hemorrhagic fever (DHF) and chikungunya. More than 100 countries every year 80 to 100 million people infected due to dengue virus but no effective vaccines or anti viral therapy are present for controlling dengue and chikungunya virus a deadly disease [1, 2]. N, N-diethyal-3-methylbenzamide (DEET) is an effective mosquito repellent but most of the researches observed some adverse effects such as skin irritation, unpleasant smell, skin eruption and harmful for young children, lactating women and also for environment [3, 4]. Insecticides application is a major application for controlling mosquito population but regular use of single synthetic insecticides can result in resistance amongst the target insects [5, 6]. Natural plant products use as a personal protection as a repellent and botanical insecticide is alternative source for controlling deadly diseases spread by mosquitoes.
Use of plant based natural chemicals is a safe alternative method to synthetic chemicals for repelling mosquitoes. And plant products generally recognized as an important natural source for insecticides [7]. Many researchers studied on bio-efficacy of essential oils against different mosquito species with the help of laboratory and field bioassay methods [8, 9, 10-2-11, 12]. However, Bacot and Talbot [13] used first time case bioassay experiment for evaluation of mosquito repellent using *A. aegypti* mosquitoes.
In the present study, we evaluated the duration of protection and knockdown activity of 23 essential oils against *A. aegypti*. From effective essential oils we also determined the electrophysiological responses using Gas Chromatography Coupled - Electroantennogram Detection (GC-EAD) for identification of particular constituents which are responsible for antennal response acting on the antenna of *A. aegypti* female mosquito.

2. Material and Methods
2.1 Test Insect
The laboratory colony of *A. aegypti* mosquitoes was maintained in insectary at 27±2 °C and 75±5% RH and utilized for all the experiments. 5 to 7 days old adult mosquitoes (50–75 pairs) were released for oviposition in a wooden rearing cage (750×600×600 mm) having a sleeve opening on one side. Adults were given 10% sugar solution and the female mosquitoes were fed on rabbits for blood meal initially for 2 days and then at alternative days.

2.2 Essential Oils

Twenty three essential oils as mentioned in Table 1 were obtained from the Fragrance and Flavour Development Center (FFDC), Kannuj, 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. Malathion was purchased from Sigma Aldrich chemicals. Experiment in 2013.

Table 1: List of essential oils obtained from different plant sources used for the repellent study against Aedes aegypti mosquitoes (Source of oils: Fragrance and Flavour Development Center, Kannuj, U.P)

S. No	Name of Essential Oils	Name of the Plant	Name of Plant Family
1	Amyris	*Amyris balsamifera*	Rutaceae
2	Basil	*Ocimum basilicum*	Lamiaceae
3	Black pepper	*Piper nigrum*	Piperaceae
4	Camphor	*Cinnamomum camphora*	Lamiaceae
5	Catnip	*Nepeta cataria*	Lamiaceae
6	Chamomile	*Anthemis nobilis*	Asteraceae
7	Cinnamon	*Cinnamomum zeylanicum*	Lauraceae
8	Citronella	*Cymbopogon winterianus*	Poaceae
9	Dill	*Anethum graveolens*	Apiaceae
10	Frankincense	*Boswellia carteri*	Burseraceae
11	Galbanum	*Ferula galbaniflua*	Apiaceae
12	Geranium	*Pelargonium graveolens*	Geraniaceae
13	Jasmine	*Jasminum grandiflorum*	Oleaceae
14	Juniper	*Juniperus communis*	Cupressaceae
15	Lavender	*Lavandula angustifolia*	Lamiaceae
16	Lemon scented	*Eucalyptus citriodora*	Myrtaceae
17	Lemongrass	*Cymbopogon citrates*	Poaceae
18	Litsea	*litsea cubeba*	Lauraceae
19	Peppermint	*Mentha piperita*	Lamiaceae
20	Rosemary	*Rosmarinus officinalis*	Lamiaceae
21	Rosewood	*Aniba rosaeodora*	Lauraceae
22	Tagetes	*Tagetes minuta*	Asteraceae
23	Thyme	*Thymus serpyllum*	Labiatae

2.3 Cage Bioassay

The protection time was tested by the method described by Rao *et al.* [14]. The protection time of 23 essential oils was performed on human volunteer in March 2013. DEET and DEPA served as a positive control and Isopropanol served as a negative control. The hand was washed with tap water and dried with towel and then the essential oil was applied. 20% concentration of repellent were applied on the external surface of the fist of human hand over an area of about 150 cm^2 at the rate of 1 mg/cm^2. The treated surface was exposed to 200 non blood fed female mosquitoes (5 - 7 days old) in 75 × 60 × 60 cm. Tests were conducted during 10:00 hr to 16:00 hr in the light room at 27±2 ^{0}C and 70±5% RH. Each evaluator counted five minute biting counts and the experiments were performed in triplicate (n = 3). Tests were repeated at interval of 30 min and the experiment was completed when 5 mosquitoes had bitten. The number of insects landing or biting was recorded for two volunteers.

2.4 Cone Bioassay

Study of essential oils toxicity was carried out against *A. aegypti* female mosquito using WHO bioassay method (WHO) [15] with slight modifications in June 2013. 0.1%, 1.0% and 5% stock solutions were prepared by dissolving a known weight in acetone and 2 ml of each concentration were impregnated on filter papers (Area: 122.65). Malathion served as a positive control for comparison and acetone served as negative control. Impregnated papers were left to dry at room temperature for 1hr prior to testing. Batch of 10 *A. aegypti* female mosquitoes (5-6 days old) were randomly selected from the pool of 200 adults placed into WHO bioassay kit for knockdown observation and results were recorded every 10 minutes for one hour. After one hour exposure the knockdown mosquitoes were placed in holding cups with 10% sugar solution for observation of recovery and mortality at 24 hour at 27 ± 2 ^{0}C and 70 ±5% RH. And the test was replicated 3 times for each test concentration.

2.5 Data Analysis

Abbott's formula will be applied for Mortality [16].

$$\text{Mortality \%} = \frac{\text{Test mortality\% - Control mortality\%}}{\text{100 - Control mortality\%}}$$

The values of effective dose KT_{50} were obtained through Probit Analysis and by use of POLO PLUS-PC 2.0 software and the values were drawn from three replicates. Effectiveness of the test oils was determined by comparing the 95% confidence intervals of the KT_{50} values.

2.6 Gas Chromatograph Coupled-Electroantennogram Detection (GC-EAD)

The sensitivity of *A. aegypti* to the components of essential oils was evaluated using Coupled - Electroantennogram Detection (GC-EAD) in September 2013. In Gas Chromatograph (Agilent 7820A) is fitted with 30 m x 0.32 mm ID x 25 µm 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 where split by 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 500 ml/min for was delivered onto the antenna through which the GC effluent mixed with continuous air flow. Once the

antenna stabilized the 10 effective essential oils prepared at 1000 ppm in HPLC grade Methanol at the rate of 1 µl was injected into the GC and the response of antenna was recorded in the GC-EAD program (Syntech, The Netherlands: Version 2.6).

3. Results

3.1 Cage bioassay

In the present study, twenty three essential oils were screened for repellent effect against *Aedes aegypti* under laboratory conditions using cage bioassay method showed in Table 2. Essential oil of litsea was found effective till 2 hours while other oils namely rosewood, lemon scented, geranium, lemongrass and dill were effective till 1.5 hours. Three oils namely cinnamon, galbanum and citronella were effective till 1 hour. While other oils such as catnip, camphor, thyme, rosemary, jasmine, basil, frankincense, lavender, amyris, peppermint, tagetes and chamomile provided complete protection up to 30 minutes. In juniper and black pepper no protection was found. The technical DEET and DEPA provided complete protection ranging from 5 to 6 hours against *A. aegypti* mosquito.

3.2 Cone Bioassay

In the present study, the knockdown effect occurred at 0.1%, 1% and 5% for all 23 tested essential oils for one hrs of exposure litsea showed effective knockdown activity against *A. aegypti* mosquito showed in Table 2 and initial mortality with KT_{50} after 24 hrs showed in Table 3. In 60 minute litsea oil exhibited 10 ± 0 %, 76 ± 0.34 %, 100 ± 0 % knockdown effect at 0.1%, 1% and 5% on *A. aegypti* and showed least effective knockdown dose value KT_{50}: 1.427% (95% CI= 0.752 - 3.085) followed by rosewood with 10 ± 0 %, 66 ± 1.80%, 100 ± 0% and KT_{50} with 2.029% (95% CI= 1.236 - 3.689), geranium with 10 ± 0 %, 60 ± 0%, 100 ± 0% and KT_{50} with 2.489% (95% CI= 1.51 - 4.826). The Malathion showed 100% knockdown activity over all tested oils at 0.1%, 1% and 5% and the effective knockdown dose value ranging from 0.278% (95% CI= 0.162 - 0.442).

3.3 Electrophysiological experiment

In the study of electrophysiological experiment, the antenna of female *A. aegypti* responded to effective components of selective 10 essential oils studied by (GC-EAD) result are showed in Table 5. Antenna of female *A. aegypti* responded to 25 components in 10 essential oils namely litsea, geranium, rosewood, cinnamon, citronella, lemongrass, lemon scented, camphor, galbanum and dill from 6 different plant families. The predominant component of litsea oil is *Z*-citral a mixture of geranial and neral elicits a spick response in the antenna of *A. aegypti* female mosquito and the other components were identified as *cis*-geraniol also called nerol and geranyl acetate. However, the antennal stimulatory component of rosewood oil is linalool and linalool oxide elicit a spick response and the active component of geranium oil is *β*-citronellol, menthone, *p*-menthane and Trans-caryophyllene. Moreover, the EAD active major component of lemon scented oil is citronellal, *β*-citronellol, geraniol. Lemongrass oil is citral, linalool and camphene elicited a strong response of the antenna of *A. aegypti* female mosquito. However, an electroantennography active component of cinnamon oils includes cinnamaldehyde, *β*-caryophyllene, methoxycinnamaldehyde and camphor oil includes *p*-menthane, cinnamaldehyde. Camphene and dillapole was the only component of gabanum and dill oil that elicited antennal response respectively. Two components of citronella oil, citronellal and *β*-citronellol were EAD active.

Table 2: Repellency effects of 23 essential oils against *A. aegypti* mosquitoes using cage bioassay in comparison with synthetic insect repellents Diethyl m tolumide (DEET) and Diethyl phenyl acetamide (DEPA).

S. No	Name of Compound	Protection Period (hours)
1	Litsea	2
2	Geranium	1.5
3	Rosewood	1.5
4	Lemon grass	1.5
5	Lemon scented	1.5
6	Dill	1.5
7	Cinnamon	1
8	Galbanum	1
9	Citronella	1
10	Camphor	0.5
11	Catnip	0.5
12	Thyme	0.5
13	Rosemary	0.5
14	Jasmine	0.5
15	Basil	0.5
16	Frankincense	0.5
17	Lavender	0.5
18	Amyris	0.5
19	Peppermint	0.5
20	Tagetes	0.5
21	Chamomile	0.5
22	Black pepper	0
23	Juniper	0
24	DEPA	5.5
25	DEET	6

Table 3: The knockdown effect for the 1 hrs exposure of twenty three essential oils on *A. aegypti* at 0.1%, 1% and 5% concentrations in comparison with Malathion.

Compound	% Conc.	10 min	20 min	30 min	40 min	50 min	60 min
		KT_{50} ± SE	KT_{50} ± SE	KT_{50} ± SE	KT_{50} ± SE	KT_{50} ± SE	KT_{50} ± SE
Amyris	5%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	1%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	0.10%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Basil	5%	76.67 ± 0.34	90 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	1%	13.33 ± 0.34	30 ± 0.59	40 ± 1.02	46.67 ± 0.68	70 ± 0.59	86.67 ± 0.34
	0.10%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Black pepper	5%	0 ± 0	76 ± 0.34	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	1%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	0.10%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Camphor	5%	0 ± 0	0 ± 0	3 ± 0	6 ± 0	6 ± 0	6 ± 0
	1%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

	0.10%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Chamomile	5%	0 ± 0	0 ± 0	20 ± 0	23.33 ± 0.34	33.33 ± 0.34	33.33 ± 0.34
	1%	0 ± 0	0 ± 0	10 ± 0	10 ± 0	13.33 ± 0.34	13.33 ± 0.34
	0.10%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Catnip	5%	100 ± 0	0 ± 0	1000 ± 0	100 ± 0	100 ± 0	100 ± 0
	1%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	6 ± 0.34	46 ± 0.34
	0.10%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Cinnamon	5%	0 ± 0	0 ± 0	30 ± 0	33.33 ± 0.34	40 ± 0	43.33 ± 0.34
	1%	0 ± 0	0 ± 0	10 ± 0	13.33 ± 0.34	16.67 ± 0.34	20 ± 0
	0.10%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Citronella	5%	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	1%	0 ± 0	36 ± 0.34	50 ± 0	53 ± 0.9	76 ± 0.34	100 ± 0
	0.10%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Dill	5%	36 ± 2.07	83 ± 1.70	90 ± 0	100 ± 0	100 ± 0	100 ± 0
	1%	3.0 ± 0.34	10 ± 0	16 ± 0	0 ± 0	43 ± 1.22	70 ± 1.56
	0.10%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Frankincense	5%	13.33 ± 0.34	16.67 ± 67	23.33 ± 0	30 ± 0	33.33 ± 0.34	43.33 ± 0.34
	1%	6.67 ± 0.34	10 ± 0	13.33 ± 0	20 ± 0	23.33 ± 00.34	30 ± 0
	0.10%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Galbanum	5%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	1%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	0.10%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Geranium	5%	20 ± 1.02	60 ± 0	86 ± 0.34	93 ± 0	100 ± 0	100 ± 0
	1%	6 ± 0.35	16 ± 0.34	26 ± 1.22	46 ± 1.8	53 ± 1.89	60 ± 0
	0.10%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	10 ± 0	10 ± 0
Jasmine	5%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	1%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	0.10%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Juniper	5%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	1%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	0.10%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Lavender	5%	10 ± 0.68	23 ± 0.14	50 ± 2.94	60 ± 2.35	73 ± 2.23	83 ± 1.70
	1%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	0.10%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Lemon scented	5%	20 ± 0	50 ± 1.18	76 ± 1.89	83 ± 1.22	90 ± 1.02	90 ± 1.02
	1%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	3 ± 0.34
	0.10%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	3 ± 0.34
Lemongrass	5%	16 ± 0.34	76 ± 0.34	0 ± 0	100 ± 0	100 ± 0	100 ± 0
	1%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	3 ± 0.34	3 ± 0.34
	0.10%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Litsea	5%	26.67 ± 0	80 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	1%	0 ± 0	26 ± 1.22	43.33 ± 0.34	56.67 ± 0.34	60 ± 2.35	76 ± 0.34
	0.10%	0 ± 0	0 ± 0	0 ± 0	10 ± 0	10 ± 0	10 ± 0
Peppermint	5%	56 ± 0.34	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	1%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	0.10%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Rosemary	5%	23.33 ± 0.34	26.67 ± 0.34	40 ± 0	43.33 ± 0	53.33 ± 0.34	50 ± 0
	1%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	0.10%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Rosewood	5%	22.33 ± 0.34	73.33 ± 0.34	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	1%	0 ± 0	23.33 ± 0.34	36.67 ± 0.68	50 ± 0	56.67 ± 0.34	66 ± 1.80
	0.10%	0 ± 0	0 ± 0	0 ± 0	10 ± 0	10 ± 0	10 ± 0
Tagetes	5%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	1%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	0.10%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Thyme	5%	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	1%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	33 ± 0.34	33 ± 0.34
	0.10%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	3 ± 0.34	3 ± 0.34
Malathion	5%	53.33 ± 1.36	80 ± 0.59	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	1%	33.33 ± 0.34	76.67 ± 0.34	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	0.10%	0 ± 0	6.67 ±0.34	16.67 ± 0.34	46.670 ± 0.34	76.67 ± 0.34	100 ± 0

Table 4: Mortality and knockdown effect of twenty three essential oils on *A. aegypti* adults exposed continuously for 1 hour at different concentrations in comparison with Malathion

compound	Mortality and KT_{50} after 24 hrs						
	Mortality% ± SE			KT_{50}	95%	Slope ± SEM	X2(df=2)
	0.10%	1%	5%		Lower Limit to Upper limit		
Amyris	3.30±0.34	16.70 ± 0.90	43.30 ± 0.68	7.824	3.409 - 73.955	1.009 ± 0.289	0.104
Basil	3.30±0.34	23.30 ± 0.38	43.3 ± 0.34	6.911	2.993 - 63.061	0.951 ± 0.272	0.137
Black pepper	3.30 ± 0.34	16.70 ± 0.68	40 ± 0	9.635	3.816 - 165.550	0.945 ± 0.286	0.032
Camphor	3.30 ± 0.34	23.30 ± 1.36	56.67 ± 0.90	3.758	2.047 - 11.003	1.197 ± 0.285	0.045
Catnip	3.30 ± 0.34	23.30 ± 0.34	43.30 ± 0.68	8.255	3.625 - 77.989	1.046 ± 0.300	0.497
Chamomile	3.30 ± 0.34	16.67 ± 0.68	36.67 ± 0.34	12.273	4.314 - 509.783	0.882 ± 0.284	0.001
Cinnamon	3.30 ± 0.34	23.30 ± 0.34	60 ± 0	3.345	1.886 - 8.518	1.262 ± 0.290	0.131
Citronella	3.30 ± 0.34	20 ± 0	63.30 ± 0.38	3.192	1.864 - 7.311	1.362 ± 0.304	0.707
Dill	3.30 ± 0.34	16.67 ± 0.34	56.70 ± 0.34	4.232	2.333 - 12.576	1.266 ± 0.304	0.793
Frankincense	3.30 ± 0.34	23.30 ± 0.34	43.30 ± 0.34	6.911	2.993 - 63.061	0.951 ± 0.272	0.137
Galbanum	3.30 ± 0.34	30 ± 0	56.67 ± 0.34	3.307	1.786 -9.403	1.146 ± 0.271	0.158
Geranium	3.30 ± 0.34	23.30 ± 0.34	70 ± 0	2.489	1.51 - 4.826	1.468 ± 0.306	0.700
Jasmine	3.30 ± 0.34	16.67 ± 0.34	36.70 ± 0.34	12.273	4.314 - 509.783	0.882 ± 0.284	0.001
Juniper	3.30 ± 0.34	10 ± 0	36.67 ± 0.34	13.52	4.854 - 531.772	0.955 ± 0.307	0.720
Lavender	3.30 ± 0.34	10 ± 0	40 ± 1.67	10.633	4.297 - 177.509	1.023 ± 0.310	0.976
Lemon scented	3.30 ± 0.34	23.30 ± 0.34	66.67 ± 0.34	2.726	1.620 - 5.683	1.396 ± 0.300	0.451
Lemongrass	3.30 ± 0.34	2.33 ± 0.9	43.30 ± 0.68	6.911	2.993 - 63.061	1.362 ± 0.304	0.707
Litsea	36.67 ± 0	36.67 ± 0.34	100 ± 0	1.427	0.752 - 3.085	1.059 ± 0.229	0.205
Peppermint	3.30 ± 0.34	16.67 ± 0.34	53.30 ± 0.34	4.814	2.542 - 17.180	1.200 ± 0.300	0.557
Rosemary	3.30 ± 0.34	23.30 ± 0.34	43.30 ± 0.34	6.911	2.993 - 63.061	0.951 ± 0.272	0.137
Rosewood	3.30 ± 0.34	73.30 ± 0.34	73.30 ± 0.34	2.029	1.236 - 3.689	1.482 ± 0.296	0.168
Tagetes	3.30 ± 0.34	10 ± 0	43.30 ± 0.34	13.52	4.854 - 531.772	0.955 ± 0.307	0.720
Thyme	3.30 ± 0.34	16.67 ± 0.34	36.67 ± 0.34	7.372	3.198 - 68.836	0.978 ± 0.280	0.001
Malathion	50 ± 0.59	86.67 ± 0.68	100 ± 0	0.278	0.162 - 0.442	1.749 ± 0.306	0.725

Table 5: List of components that elicited antennal responses in GC-EAD detection, using antenna of female *A. aegypti* as electroantennographic detector.

Essential oil	Antennal Stimulatory Constituent	Retention time
Dill	dillapole	8.16
Galbanum	champagne	5.18
Geranium	menthone	3.53
	p-menthane	4.13
	β-citronellol	4.25
	Trans-caryophyllene	9.38
Camphor	*p*-menthane	4.13
	champagne	5.18
Cinnamon	cinnamaldehyde	4.9
	β-caryophyllene	6.18
	methoxycinnamaldehyde	6.58
	caryophyllene	7.15
Litsea	Z-citral	4.41
	cis-geraniol	4.49
	geranyl acetate	5.9
Rosewood	linalool	2.19
	linalool oxide	2.35
Lemon scented	citronellal	2.67
	Citronellal	5.4
	β-citronellol	10.39
Citronella	citronellal	2.67
	β-citronellol	10.39
Lemongrass	linalool	2.19
	citral	4.57
	geraniol	5.31

4. Discussion

The screening of effective essential oil as a mosquito repellent is highly valuable for making safe, nontoxic as a personal protection for protecting from deadly diseases transmitted by blood sucking mosquitoes. Essential oils are highly volatile plant product with various compounds and the quality of essential oils depends on various factors such as plant varieties, methods of extraction [17]. In the present study, litsea oil showed effective results against *A. aegypti* and the result was supported by various researchers against different mosquitoes such as *A. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* [2-10]. Rosewood, lemongrass, lemon scented geranium also showed effective results against *A. aegypti*, and the result was supported by various researchers *A. aegypti*, *An. stephensi*, *Cx. quinquefasciatus*, *Cx. quinquefasciatus, An. gambiae, An. darling, Mansonia spp* and *An. arabiensis* [10-22]. The citronella oil also showed repellency against three different mosquitoes such as *A. aegypti*, *Cx. Quinquefasciatus* and *An. dirus* [10-17]. Dill, camphor, galbanum, cinnamon also showed effective results against *A. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* [10]. The other 13 oils namely tagetes, juniper, chamomile, frankincense, basil, peppermint, black pepper, amyris, jasmine, thyme, catnip, lavender and rosemary also showed small range of repellent efficacy against *A. aegypti* mosquitoes at various concentrations against different mosquitoes species such as *A. aegypti*, *Ae. albopictus*, *An. stephensi*, *An. dirus*, and *Cx. quinquefasciatus* [2-10].

According to researches, the repellent effect of essential oils against mosquitoes are quite different due to some factors such temperature, wind, humidity and the blend effect of plant volatile chemical called phytochemicals [2-18, 19]. Moreover, mosquito age, body size, density in cage also provide different results [20]. Plant based chemicals are highly volatile and different chemical constituent of essential oils have different repellent properties. Due to high volatile property of essential oil provide short period of protection against blood sucking mosquito as compared with synthetic repellent and most of the researches used different chemical compounds such as synthetic or natural product as a fixative as coconut oil, mustard oil, vanillin, salicylic acid for reducing the volatility of essential oil

[21,22-17]. In the present study, variation of protection period, knockdown and mortality showed effective results which are effective parameters for development of plant products for controlling mosquito born diseases.

Electroantennogram (GC-EAD) is an advanced technology for detection of quantitative minor phytochemicals and this technique is useful for fast screening of particular volatile component which are responsible for antennal responses [23]. In the study, the predominant component of litsea oil is *Z*- citral elicits a spick response in the antenna of *A. aegypti* female mosquito [24, 25]. However, the major component of rosewood oil is Linalool showed strong response in the antenna of *A. aegypti* female mosquito [26]. The major component of geranium oil is *β*-Citronellol elicit a spick response of the antenna of *A. aegypti* female mosquito [23-27]. Moreover, the major component of lemon scented oil is citronella and lemongrass oil is citral elicits a spick response of the antenna of *A. aegypti* female mosquito [28, 29]. Dillapiole [31], camphene [31], *β*-caryophyllene [32], camphene [23] and citronella [23] are major components showed strong response in the antenna of *A. aegypti* female mosquito from dill oil, camphor oil, cinnamon oil, galbanum oil and citronella oil respectively.

Plant based products are safe and eco-friendly as compared to synthetic repellents and the present study provides information for developing safe, biodegradable, eco-friendly and effective insect repellent.

5. Conclusion

The present study evaluates the essential oils of twenty three plant species for their repellency against *A. aegypti* a major vector of dengue and chikungunya in the laboratory condition. The result shows that essential oils provide repellent activity against *A. aegypti* female mosquito and its giving protection against the arthropod transmitted diseases. During experimentation, essential oils did not show any adverse affects such as skin irritation, rashes, discomfort and other allergic reactions to the volunteer. Our research is being continued for searching efficacy of effective essential oils and repellent activity against blood sucking mosquito. The further study will provide information for developing a new anti mosquito product from plant based material and also alternative repellent to synthetic repellents.

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