Original Article

Behavioral Response of *Aedes aegypti* Mosquito towards Essential Oils Using Olfactometer

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Abstract

Background: *Aedes aegypti* mosquito is responsible for transmitting human diseases like dengue and chikungunya. Personal or space protection with insect repellents is a practical approach to reducing human mosquito contact, thereby minimizing disease transmission. Essential oils are natural volatile substances from plants used as protective measure against blood-sucking mosquitoes.

Methods: Twenty-three essential oils were evaluated for their repellent effect against *Ae. aegypti* female mosquito in laboratory conditions using Y-tube olfactometer.

Results: The essential oils exhibited varying degree of repellency. Litsea oil showed 50.31%, 60.2 %, and 77.26% effective mean repellency at 1 ppm, 10 ppm and 100 ppm respectively, while DEET exhibited 59.63%, 68.63%, 85.48% and DEPA showed 57.97%, 65.43%, and 80.62% repellency at respective above concentrations. Statistical analysis revealed that among the tested essential oils, litsea oil had effective repellency in comparison with DEET and DEPA against *Ae. aegypti* mosquito at all concentration. Essential oils, DEET and DEPA showed significant repellence against *Ae. aegypti* ($P < 0.05$) at all 3 concentration tested.

Conclusion: Litsea oil exhibited effective percentage repellency similar to DEET and DEPA. The essential oils are natural plant products that may be useful for developing safer and newer herbal based effective mosquito repellents.

Keywords: Essential oils, *Aedes aegypti*, Repellent, Y-maze olfactometer, Flight orientation

Introduction

Mosquito borne diseases such as malaria, Japanese encephalitis and dengue are a major concern in both developed and developing countries. Dengue and chikungunya are important diseases transmitted by *Aedes aegypti* mosquito which lives in urban habitats and breeds mostly in man-made and natural containers (Julian 2009, WHO 2012). Its peak host seeking activity is early in the morning and in the evening before dusk (WHO 2012). These diseases have many economical and sociological adverse effects on human, domestic animal populations (WHO 2013) and Dengue fever is one of the main causes of child mortality in Asia and Africa (Gupta et al. 2012). Since there is no effective vaccine available for the control of these deadly diseases (Norashiqin et al. 2008, Gu et al. 2009), mosquito control programs are essential to prevent spread of these diseases. Insect repellents are used as a personal protection that can provide a practical and economical means of preventing mosquito-borne diseases. The most common mosquito repellent DEET (N,N Diethyl-m-toluamide), a gold-standard of synthetic repellent, which is currently available in the market has shown repellency for 6 to 8 hours against mosquito (Yap 1986, Kweka et al. 2012) and other blood sucking insects (Browne et al. 1997). Even

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though DEET is mostly used as a mosquito repellent, but it has the side effects on human being such as skin irritation, affects the central nervous system, mucous membranes and mild toxic (Maibach and Johnson 1975, Reuveni and Yagupsky 1982, Phasomkusolsil et al. 2010, Miot et al. 2011) and strong solvent for plastic and other synthetic products (Kazembe et al. 2012). Every year more than 29 billion mosquito coils are sold for personal protection which contain pyrethrum daisy a plant product obtained from the flower of Asteraceae family basically used for repellent and insecticidal purpose against blood sucking insects (WHO 1998, Lawrance et al. 2004). Due to these adverse effects, attempts are made to find safe and ecofriendly repellents derived from plant extracts/ materials.

Several characteristics such as insecticidal, repellent and growth reducing properties are found in plant essential oils. The major families, which contain essential oils as insecticidal and repellent properties, include Lamiceae, Asteraceae, Myrtaceae and Lauraceae. Plant essential oil from Myrtaceae family has effective repellent activity against mosquitoes (Tapondjou et al. 2003, Maia et al. 2011). However, Labiatae, Apiaceae, Lamiaceae contain insect repellent, antifeedent activity and insecticidal properties (Regnault-Roger et al. 1994, Vasilakoglou et al. 2007). Plant family Poaceae, Rutaceae also showed effective repellent activity against mosquitoes (Regnault-Roger 1997, Maia et al. 2011). Essential oil from plant family such as Geraniaceae, Oleaceae, Piperaceae, Cupressaceae and Burseraceae also exhibited repellent activity against blood sucking mosquitoes (Amer and Mehlhorn 2006). According to Moore et al. (2006) plant families such as Lamiaceae, Myrtaceae and Poaceae are best known species as insect repellent. However, *p*- menthone-3,8- diol (PMD) from *Eucalyptus maculate citrodon* a lemon eucalyptus and citronella oil from *Cymbopogon nardus* are effective natural repellent preferred by various users (Curtis et al. 1987, Trigg et al. 1996, Trigg 1996, Trongtokit et al. 2005, Hsu et al. 2013) and no adverse effect reported since 1984 (US EPA 1999)

Essential oils are the alternative source against synthetic repellent because they are non-toxic for human and other organisms (Das et al. 2003, Tarek et al. 2012). Essential oils from several plants have shown effective repelling against mosquitoes (Barnard 1999). Natural plant based repellents have also demonstrated good efficacy against some mosquito species in tests examining as adulticidal activity (Yang et al. 2005, Manimaran et al. 2012), larvicidal activity (Ansari et al. 2000, Adebajo et al. 2012), repellent activity (Amer and Mehlhorn 2006, Gleiser et al. 2011) and adulticidal, repellent, larvicidal, oviposition deterrent activity (Prajapati et al. 2005). The effectiveness of any plant essential oil as repellent depends on several factors such as quality, type of repellent, mode of action, temperature, humidity, biting response of mosquito, volatility, methods of extractions (Tawatsin et al. 2001). Factors affecting the quality of essential oils include plant species, cultivating conditions, and maturation of harvested plants, plant storage, plant preparation and methods of extraction (Tawatsin et al. 2001, Norashiqin et al. 2010). Behavioral studies on essential oils are very important research to identify effective plant oils and their constituent responsible for exhibiting repellent effects against mosquitoes and there is a need to develop eco-friendly, safe, cost effective repellants for blood sucking insects.

In the present study, an attempt has been made to study repellency of 23 various essential oils obtained from Fragrance and Flavour Development Center, Kannuj, U P India, against *Ae. aegypti* mosquitoes in laboratory condition.

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Material and Methods

Test Insect

The test mosquitoes *Ae. aegypti* were reared in the laboratory conditions in wooden cages (750 X600 X600 mm) for feeding and egg laying on a filter paper strip in a plastic container/bowl containing 250 ml of water. Cotton with 10% sugar solution was provided for nourishment and the female mosquitoes were fed on rabbits for blood meal initially for 2 days and then at every alternative days. The eggs were collected and transferred to a bowl containing two liters of water, for rearing of hatched larvae up to adult stage. Brewer's yeast powder was provided as food for larvae and water was changed on alternate days. The pupae were collected and kept in small cages (550 X450 X 50 mm) covered with cotton cloth for emerging into the adult. *Aedes aegypti* (five to six days old) adults were drawn from the stock colony maintained at 27±2 ˚C and 70±5% RH for all the evaluations.

Essential Oils and Synthetic repellents

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.

Flight orientation

The repellent behavior response of *Ae. aegypti* exposed to 23 essential oils was studied using Y- maze olfactometer is shown in Fig. 1 described by Erler et al. (2006) slightly modified. It is made up of glass tube having internal diameter of 2.5 cm and 45 cm length from main arm containing one arm for testing repellent and the other arm for control. The olfactometer was kept on table, pressurized air was flow continuously into the olfactometer at the rate of 1.5 L/min. Filter paper strips (1cmx 5cm) were loaded with 100 µl of different concentration viz, 1 ppm, 10 ppm, 100 ppm of individual essential oils, DEET, and DEPA dissolved in isopropanol. DEET and DEPA were used as positive controls and the paper strips treated with solvent isopropanol alone was used as control. After the test oil was applied on treated paper, it was fixed in one arm and solvent treated paper in another arm of Y-maze olfactometer as control. Twenty *Ae. aegypti* females (5 to 6 days old) were used for each test. The mosquitoes were released into the main arm located at the other end of the olfactometer has a circular tube (1 inch) having circular (1cm diameter) access hole for introduction of mosquitoes and cotton was used the keep the access hole closed. Numbers of mosquitoes present in test and control arm of Y -maze were counted after three minutes of exposure and all mosquitoes were removed soon after completion of experiment. After completion of one experiment the treated and control were interchanged, for each assay at different concentrations six replication were taken for test mosquito. The experiment was carried out during day time from 1000 hrs to 1600 hrs at room temperature 27±2 ˚C, Relative Humidity 70±5% with light intensity of 120–125 lux (bioassay room was illuminated by three 36 W fluorescent lamps). Six replicates of tests were conducted for each treatment and for each replicate a new set of twenty female mosquitoes were used. After every treatment, Y-maze and set up connections were washed with acetone and dried soon to avoid any interference of other essential oils scents.

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Data Analysis

The percent repellency of essential oils was calculated as per Erler et al. (2006). Where PR= (C-T)/(C+T) X 100 where C= number of insects in control arm and T= number of insects in treated arm, insects that remained in the main arm were not taken into account. The data were subjected to statistical analysis for comparison analyzed by using statistical software (Sigma Stat V2.03). The values for repellency and mean were analyzed using one way ANOVA for determination of variance ratio and least significant difference (LSD).

Results

The results obtained from behavioral response of *Ae. aegypti* to essential oils using Y-maze olfactometer are present in Table 2. At 1 ppm concentration, litsea, geranium and rosewood exhibited significantly higher repellency (50.31%, 48.74% and 46.00%) in comparison with the other oils and similar repellency was observed for DEET (59.63%) and DEPA (57.97%) (F= 16.948, df= 149, P< 0.001). At higher concentration of 10 ppm, litsea (60.26%), rosewood (59.22%) and geranium (56.84%) also showed significant repellency over other oils but similar to that of DEET (68.63%) and DEPA (65.432%) (F= 18.238, df= 149, P< 0.001) followed by lemon scented (45.12%). A further increase in the concentration to 100ppm also showed similar trend, where litsea (77.26%), rosewood (66.90%) and geranium (66.11%) exhibited significantly higher repellence's of mosquitoes over other oils but lesser effects than DEET (85.48%) and DEPA (80.62%), (F= 12.677, df= 149, P< 0.001) followed by lemongrass (57.49%).

Based on the repellency of essential oils against *Ae. aegypti* mosquitoes, the order of effective percentage repellency can be arranged as follows, litsea > rosewood > geranium > lemongrass > lemon scented > camphor > citronella > galbanum > dill > cinnamon > basil > frankincense > lavender > black pepper > thyme > rosemary > jasmine > catnip > peppermint > chamomile > juniper > amyris > tagetes.

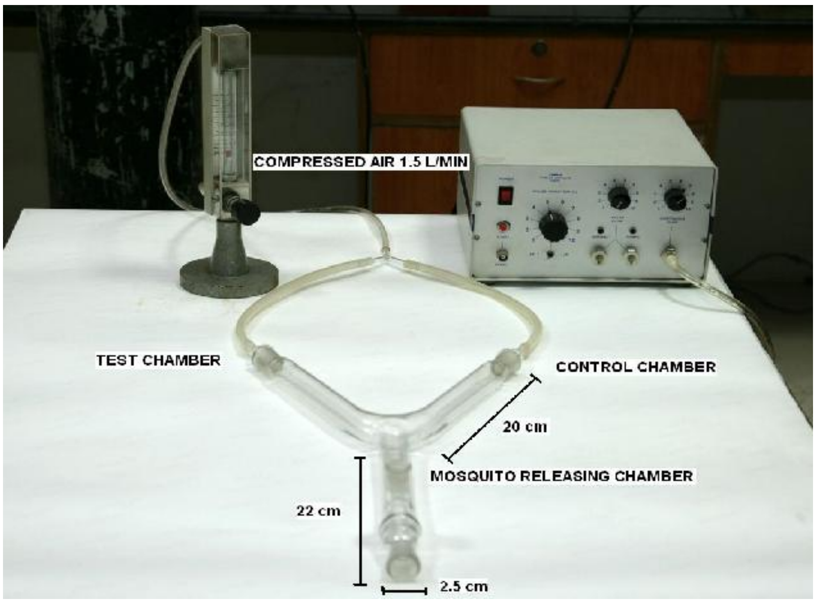

Fig. 1. 'Y' maze olfactometer showing behavioural bioassay assembly air flow meter and regulating unit

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

Name of Material	Plant Name	Family	Origin of plant	Distillation	Part Used
Amyris	*Amyris balsamifera*	Rutaceae	Haitai, Jamaica	Steam	wood
Basil	*Ocimum basilicum*	Lamiaceae	India	Steam	Fresh Plant
Black pepper	*Piper nigrum*	Piperaceae	India	Steam	Seed
Camphor	*Cinnamomum camphora*	Lamiaceae	China	steam	Leaves
Catnip	*Nepeta cataria*	Lamiaceae	France, Canada	steam	Leaves
Chamomile	*Anthemis nobilis*	Asteraceae	France, Italy	Steam	Leaves
Cinnamon	*Cinnamamomus zeylanicum*	Lauraceae	Sri Lanka	Steam	Bark
Citronella	*Cymbopogon winterianus*	Poaceae	Indonesia, Central Nepal	Steam	Leaves
Dill	*Anethum graveolens*	Apiaceae	Hungary	Steam	Seed
Frankincense	*Boswellia carteri*	Burseraceae	Somalia	Steam	Tree resin
Galbanum	*Ferula galbaniflua*	Apiaceae	Turkey	Steam	Tree resin
Geranium	*Pelargonium graveolens*	Geraniaceae	South Africa, Egypt	Steam	Leaves, Stalk
Jasmine	*Jasminum grandiflorum*	Oleaceae	India, South Asia	Hydro	Flower
Juniper	*Juniperus communis*	Cupressaceae	India	Steam	Fruit
Lavender	*Lavendula angustifolia*	Lamiaceae	France	Steam	Flower
Lemon grass	*Cymbopogon citrates*	Poaceae	Southeast Asia.	Hydro, Steam	Leaves
Lemon scented	*Eucalyptus citriodora*	Myrtaceae	Australia	Steam	Leaves
Litsea	*Litsea cubeba*	Lauraceae	China	Steam	Fruit
Peppermint	*Mentha piperita*	Lamiaceae	India	Steam	Leaves, Flower
Rosemary	*Rosmarinus officinalis*	Lamiaceae	Spian, Tunisia	Steam	Shrub
Rosewood	*Aniba rosaeodora*	Lauraceae	Brazil	Steam	Wood
Tagetes	*Tagetes minuta*	Asteraceae	South America	Steam	Flower
Thyme	*Thymus serpyllum*	Labiatae	Europe and North Africa	Steam	Leaves

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Table 2. Repellency of 23 essential oils against *Aedes aegypti* mosquitoes using "Y" maze olfactometer in comparison with synthetic insect repellents N, N-diethyl phenyl acetamide (DEPA) and N, N- diethyl-m-toluamide (DEET)

S. No	Compound	Mean% repellency ± SE		
		1 ppm	10 ppm	100 ppm
1	Litsea	50.31 ± 5.03^{a}	60.26 ± 2.91^{a}	77.26 ± 2.94^{ab}
2	Rosewood	46.00 ± 4.85^{ab}	59.22 ± 2.56^{a}	66.90 ± 4.23^{b}
3	Geranium	48.74 ± 4.55^{a}	56.84 ± 2.97^{ab}	66.11 ± 2.16b^{bc}
4	Lemongrass	21.45 ± 3.83^{cd}	43.60 ± 3.15^{c}	57.49 ± 1.83^{c}
5	Lemon scented	33.99 ± 2.49^{bc}	45.12 ± 2.91^{bc}	55.39 ± 3.8^{c}
6	Camphor	23.12 ± 3.35^{c}	41.5 ± 2.98^{c}	54.21 ± 1.73^{c}
7	Citronella	24.56 ± 3.18^{c}	34.22 ± 3.03^{c}	52.95 ± 4.75^{c}
8	Galbanum	13.54 ± 2.95^{d}	30.77 ± 3.37^{d}	51.38 ± 3.04^{c}
9	Dill	8.10 ± 2.56^{d}	33.29 ± 2.38^{cd}	51.04 ± 2.98^{c}
10	Cinnamon	13.75 ± 4.23^{d}	27.67 ± 3.62^{d}	50.82 ± 2.3^{c}
11	Basil	20.29 ± 3.66^{d}	39.88 ± 3.5^{c}	50.56 ± 5.14^{c}
12	Frankincense	23.70 ± 4.67^{c}	40.68 ± 3.73^{c}	50.46 ± 2.29^{c}
13	Lavender	19.62 ± 3.17^{d}	28.74 ± 2.42^{d}	49.92 ± 4.06^{c}
14	Black pepper	22.22 ± 4.14^{c}	33.90 ± 2.67^{c}	48.96 ± 4.29^{c}
15	Thyme	20.11 ± 3.11^{d}	36.14 ± 4.16^{c}	48.61 ± 2.6^{c}
16	Rosemary	18.78 ± 5.22^{d}	31.07 ± 3.29^{d}	46.01 ± 2.49^{c}
17	Jasmine	15.70 ± 3.35^{d}	24.55 ± 3.32d^{e}	46.08 ± 3.85^{c}
18	Catnip	20.34 ± 5.53^{d}	34.16 ± 5.21^{c}	44.30 ± 3.63^{c}
19	Peppermint	27.64 ± 6.02^{c}	34.72 ± 2.8^{c}	42.15 ± 3.55^{c}
20	Chamomile	11.65 ± 4.39^{d}	30.31 ± 2.7^{d}	40.73 ± 3.86^{c}
21	Juniper	11.04 ± 4.38^{d}	29.98 ± 4.46^{d}	39.92 ± 3.11^{cd}
22	Amyris	12.28 ± 3.28^{d}	20.85 ± 3.43^{e}	31.37 ± 3.75^{d}
23	Tagetes	13.94 ± 3.67^{d}	16.65 ± 3.16^{e}	30.52 ± 5.29^{d}
24	DEPA	57.97 ± 2.8^{a}	65.43 ± 2.84^{a}	80.62 ± 2.48^{a}
25	DEET	59.63 ± 2.28^{a}	68.63 ± 2.53^{a}	85.48 ± 2.3^{a}

Mean percentage repellency ±SE, Data followed by the different letters are significantly different ($P< 0.05$, by one-way ANOVA and Least Significance Difference).
The values showed by the same letter are statistically non-significant ($P> 0.05$)

Discussion

Essential oils are composition of volatile components having minor constituents contain pleasant fragrance which are responsible for mosquito repellency and inhibit the orientation of blood sucking insects (Campbell et al. 2010). Host-seeking insects, orient to a host by using chemical stimuli precede visual and thermal stimuli emanating from the host. Different mosquito species develop different host preferences, and it is generally assumed that host selection and discrimination is mainly based on olfactory cues (Takken 1991). Results obtained in the present study using olfactometer bioassay showed that volatile essential oils exhibited concentration dependent spatial repellency against *Ae. aegypti.*

Litsea, rosewood and geranium oil showed effective repellency against *Ae. aegypti* mosquito but the effectiveness of essential oils was not superior over synthetic insect repellents DEET and DEPA. The result of litsea oil repellent efficacy was supported by Vong-

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sombath et al. (2012) against *Armigeres*, *Culex* and *Aedes*, likewise, Amer and Mehlhorn (2006) against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. They have also supported the repellent efficacy of geranium and rosewood. In this study, DEET and DEPA were used as positive controls and the effective repellent activities of DEET against mosquitoes as compared with other essential oils are supported by Kazembe et al. (2012). The effective repellent activity of DEET and DEPA against *Ae. aegypti* and *An. stephensi* mosquito was reported by Debboun and Wagman (2004).

Hu et al. (2011) reported that *Z*- citral and limonene as the predominant component of litsea oil extracted from the plant *Litsea cubeba* an evergreen tree found in Japan, southern China, and some parts of Southeast Asia. However, Rosewood oils from the plant *Aniba rosaeodora* contains linalool and 1,8-Cineole as the main chemical constituent in rosewood oil (Guilherme et al. 2007). Moreover, Geranium oil from *Pelargonium graveolenes* reported to contains *β*-Citronellol as main component (Campbell et al. 2010). Essential oils contain more than 20 to 80 minor and major highly volatile chemical constituents of which the major components showed effective repellent against *Ae. aegypti* mosquito (Campbell et al. 2010). Plant oil which contains limonene, linalool, citronellol showed effective repellent activity against different mosquito species (Barnard 1999, Tawatsin et al. 2001, Kline et al. 2003, Kang et al. 2009, Hsu et al. 2013). Due to high volatile property, essential oil exhibited effective but short duration of protection against mosquitoes.

Synthetic repellent has low rate of vaporization and more effective than essential oils but they cause adverse effect on human health (Maibach and Johnson 1975, Choochote et al. 2007). Plant based repellents are safe, non-toxic and ecofriendly. Therefore most of the repellent manufacturers use different substances, chemicals or natural products as fixative compounds such as vanillin, salicylic acid, coconut oil, mustard oil with essential oils for reducing the rate of evaporation of volatile components and for improvement of long lasting repellency against mosquitoes (Tawatsin et al. 2001, Das et al. 2003, Kongkaew et al. 2011, Sritabutra et al. 2013). However, Amer and Mehlhorn (2006) found that several essential oils namely litsea, niaouli and catnip prepared in vanillin demonstrated good repellent efficacy against different mosquito species as compared to synthetic chemical DEET. Moreover, Adeniran and Fabiyi (2012) using formulated cream base lemongrass oil against *Ae. aegypti* mosquito. Whereas, several essential oils with synthetic chemicals as formulated cream, spray, lotion showed effective repellency against *Ae. albopictus*, *Cx. nigripalpus* and *Ochlerotatus triseriatus* were reported by Barnard and Xue (2004) and formulated neem cream exhibited effective repellency against *Aedes*, *Culex* and *Anopheles* mosquitoes (Dua et al. 1995). Hence, essential oils are alternative source as a mosquito repellent as compared to synthetic mosquito repellent.

Present olfactometer bioassay elicited sensory response of *Aedes* mosquito in the absence of any skin emanations. However, mosquito response to essential oils applied on skin may give a different result. Behavior of mosquito towards oils in the presence of skin emanations and other unidentified human odor components were found different (Bernier et al. 2005, Hao et al. 2012). Although many essential oils have been found to be potently repellent, because of the high volatility of major constituents of most oils, very few have found potential in personal protection. On the other hand, they have shown great potential in space protection. Using an olfactometer test is quick and effective way to evaluate the behavioural responses of mosquitoes towards volatile

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stimuli of essential oil. These effective essential oils can be used as plant based product for provided a protection against various mosquito- borne diseases. There is a need for promoting the use of herbal products because of their safety to individual and communities.

Conclusion

Based on the above studies on repellent study of essential oils, litsea, rosewood and geranium showed nearly effective repellency like synthetic repellent DEET and DEPA against *Ae. aegypti* female mosquitoes. Our research is continued in this line of work for searching effective essential oils with higher repellent activity against blood sucking mosquitoes to develop a newer anti mosquito repellent from herbal-based product as an alternative repellent to synthetic insect repellents.

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