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Utility of y-maze olfactometer to check behavioral response of *Aedes albopictus* mosquito: A vector of dengue and chikungunya using essential oils

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Abstract
Aedes albopictus mosquito is responsible for transmitting human diseases like dengue and chikungunya. Personal 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. In this present study, five essential oils were evaluated for their repellent effect against *Ae. albopictus* female mosquito in laboratory conditions using Y-tube olfactometer. The essential oils exhibited varying degree of repellency. Litsea oil showed -0.30±1.10, -0.43±7.0 and -0.57±0.9 effective mean repellency at 1 ppm, 10 ppm and 100 ppm respectively, while DEPA showed -0.14±0.75, -0.34±0.6 and -0.62±1.0 repellency at respective above concentrations. Statistical analysis revealed that among the tested essential oils, litsea oil had effective repellency in comparison with DEPA against *Ae. albopictus* mosquito at all concentration. Essential oils and DEPA showed significant repellence against *Ae. albopictus* (P< 0.05) at all 3 concentration tested. Litsea oil exhibited effective percentage repellency similar to DEPA. The essential oils are natural plant products that may be useful for developing safer and newer herbal based effective mosquito repellents.

Keywords: *Aedes albopictus,* Dengue, Y-maze olfactometer, essential oils

Introduction

Today vector-borne diseases are the principal cause for majority of the morbidity and mortality in the developing countries including India. These are communicable diseases reflected in the form of Malaria, Dengue, Chikungunya, Japanese Encephalitis (JE), Kala-azar and Lymphatic filariasis which are transmitted by mosquitoes and other vectors. According to the WHO World Malaria Report 2015, there were 214 million cases of malaria globally in 2015 [1]. Dengue fever represents high disease burden in endemic countries [2]. An estimated 3.6 billion people live in high risk areas worldwide. It is also presumed that over 230 million bear the load of infection and approximately 2 million suffer with dengue fever and its severe forms with 21,000 deaths as reported. India also saw a doubling up of cases of dengue from 2014 to 2015 and the worst hit city was Delhi in recent years. The number of cases of Chikungunya is also increasing day-by-day. Apart from that, Kala-azar and Lymphatic filariasis also poses a severe threat to the Indian society.

Behavioural attributes of the vectors is one of the important aspect in the determination of epidemiology of vector borne diseases. Nets, impregnated/protective clothing and repellents are mainly used for the purpose of personal protection. For decades, repellents have been in use in the reduction of man-vector contact and it is employed as a major tool in protection against biting insects. Since 1957, the most commonly used insect repellent formulations have contained N,N- Diethyl-m-toluamide (DEET), which is effective against a broad spectrum of insects [3] but there have also been concerns over the toxicity of DEET [4, 5]. Antwi *et al.* [6] found no significant toxicological risks from typical usage of DEET topical insect repellents. Other insect repellent N, N-Diethyl phenyl acetamide is known to have multi-insect repellent property against various haematophagous insects [7, 8].

Insect repellent as a weapon against these vectors has been in utilization for decades. But, these repellents which are currently available in the market have reasonable limitations. Since they are synthetic in nature they may poses the risk of side-effects. They have maximum repellency effect of 6 – 8 hours.

The rapid evolution of resistance against vector borne diseases especially malaria have compelled the researchers to go for long-lasting, stable, eco-friendly alternative tool against these vectors. In view of the above circumstances natural oils may prove to be healthy alternative against these synthetic repellents. Essential oils have immense potential in influencing the behavioural attributes of the vectors. They are highly concentrated aromatic substances found in the glandular cells of plants and flowers. Most plants produce a single essence, however, some plants produce multiple essences. Plant essential oils may have a number of properties like growth-retardant, growth-promoting, repellent and insecticidal. Presently the total world production of essential oil is about 1,10,000 – 1,20,000 m tones, out of which India produces 15%, holding, third place after China and Brazil in the world production, while in terms of value its share is much better with 21-22 % [2]. Essential oils from several plants have shown effective repelling against mosquitoes [9]. Natural plant based repellents have also demonstrated good efficacy against some mosquito species in tests examining as adulticidal activity [10]. One of the oldest insecticide known, pyrethrum is also the strongest insecticide allowed under National Organic Standards guidelines. Made from the dried flowers of a little white daisy now classified as *Tanacetum cinerariifolium*, pyrethrum insecticides are known for their fast knock-down of unwanted insects specially in case of mosquitoes. Insects typically become paralyzed as soon as they come into contact with pyrethrum, so it's often used in wasp sprays. Essential oils derived from different families *viz.* Asteraceae, Lamiceae, Lauraceae, Myrtacea have shown remarkable repellent properties against arthropod species. A number of natural repellents are also available in the market under different brand names i.e., MosQuick Natural Mosquito Repellent Oil, Natural Mosquito Repellent Citronella Incense Sticks (Agarbatti), Bodyguard Premium Natural Mosquito Repellent Patches etc.

A small effort in the present study has been made to show the repellency effect of six essential oils against the *Ae. albopictus* using Y-Maze olfactometer, since, an important determinant controlling the mosquito-host interaction is the ability for mosquitoes to accurately identify their hosts using several sensory modalities and the most prominent one is olfaction [11]. An olfactometer was utilized for determining the behavioral response of mosquito against essential oils of various types. This study has enlighten us about the prominent role of essential oils as an effective repellent against vectors.

Materials and Methods

Test Insect

Colony of *Ae. albopictus* 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 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. Non-blood-fed females (5–7 days old) were used in the olfactometer bioassay.

Essentials oils

Essential oils were purchased for this study from Surajbala Exports Pvt. Ltd, Gurgaon, New Delhi.

Table 1: List of essential oils

S. No	Essential Oils Name	Plant Name	Family
1	Litsea	*Litsea cubeba*	Lauraceae
2	Rosewood	*Aniba rosaeodora*	Lauraceae
3	Lemongrass	*Cymbopogon citrates*	Poaceae
4	Geranium	*Pelargonium graveolenes*	Geraniaceae
5	Lemon scented	*Eucalyptus citriodora*	Myrtaceae

Y-tube olfactometer

Flight orientation studies on non-blood fed *Ae. albopictus* females using Y-tube olfactometer was conducted according to Seenivasagan *et al.* [12] to different doses of essential oils. For each replicate, mosquitoes which readily oriented to human hand were aspirated out from the cage in a group of twenty and were used in the experiment. Before stimulation by the test odor, the mosquitoes were given 5 min to acclimatize in the release chamber. Between the replicates, a constant flow of fresh air flushed the olfactometer. The essential oils (200 µl of odor stimuli) were applied onto a piece of filter paper (10×2.5 cm), placed in an odor cartridge after evaporation of solvent. The airflow was maintained at 50 L/min during the experiment, which was split into two halves by a T-splitter which carried the odor of test chemicals downwind [13]. Seven replicates of orientation experiments were conducted for each dose of test chemicals in a dual choice bioassay, in which the positions of test stimuli were alternated between every replicate. The bioassay was conducted for 3 min; in the given time, all mosquitoes have fled from the release chamber and entered into upwind end of the olfactometer. The olfactometer was cleaned thoroughly after testing each of the chemical. The number of mosquitoes collected in respective chambers of control treated arm were counted manually to assess the odor preference and used for analysis.

Data Analysis

The following formulas were used to determine the preference index (PI), and percentage no response (NR) of the mosquitoes to the test oils as described previously (Afify *et al.* 2014; Seenivasagan *et al.* 2012). Preference index (PI) = Tn-Cn/Tn+Cn; %No response (NR) = Nr/OnX100.

Tn= Number of mosquitoes in test chamber

Cn= Number of mosquitoes in control chamber

On= Number of mosquitoes released in one replicate (i.e. 20)

Nr= Number of mosquitoes not responding to either odor

Flight orientation experiments were analyzed by analysis of variance (ANOVA) followed by Tukey test of multiple comparison.

Results

Behavioural avoidance and repellency response of *Ae. albopictus* to three different concentrations of five essential oils and DEPA was documented. Figure 1 is behavioural analysis curves that compare the patterns of PI and the NR of mosquitoes remaining in the test chamber Y-maze olfactometer under contact and noncontact trials against litsea oil, rosewood oil, lemongrass oil, geranium oil, lemon grass oil, lemon scanted and DEPA at different concentrations over a 3 min test period. The results obtained from behavioural response of *Ae. albopictus* to essential oils using Y-maze olfactometer are present in Table 1. Overall, higher escape responses were seen from the contact chambers than those from paired noncontact chambers, regardless of test compounds and concentrations. Among five oils, essential oil from litsea and rosewood produced the strongest irritant and repellent escape responses at the lowest concentration (1%). At 1% concentration, litsea, rosewood and lemon grass oils exhibited significantly higher preference index (-0.30±1.10, -0.24±0.63 and -0.18±0.81) in comparison with the other oils and the lower repellency was observed for DEPA (-0.14±0.75) ($p < 0.05$). For non-response, at 1% concentration, litsea, rosewood and lemon grass oils exhibited significantly higher (3.0±0.89, 2.33±1.0 and 2.11±0.75) and similar NR was observed for DEPA (2.83±0.75) in comparison with other oils. A further increase in the concentration to 10 %, litsea (-0.43±7.0) and rosewood oils (-0.33±0.9) also showed significant repellence over other oils but similar to that of DEPA (-0.34±0.6) (P< 0.05) followed by geranium oil (-0.22±1.0). At higher concentration of 100%, also showed similar trend, where litsea (-0.57±0.9) and rosewood (-0.49±0.75) exhibited significantly higher repellence's of mosquitoes over other oils but lesser effects than DEPA (-0.62±1.0) (P< 0.05) followed by lemon grass oils (-0.37±0.1). There was no significant difference in preference index among the five essential oils at 1, 10 and 100% concentration (Table 1). The order of effective percentage repellence can be arranged as follows, litsea > rosewood > lemongrass> geranium> lemon scanted.

Table 2: Details profile of essential oils

Essential oils	Concentration (% w/v)						
	1		10		100		P value
	Pi	Nr	Pi	Nr	Pi	Nr	
Litsea	-0.30±1.10	3.0±0.89	-0.43±7.0	2.33±0.81	-0.57±0.9	2.83±0.75	< 0.05
Rosewood	-0.24±0.63	2.33±1.0	-0.33±0.9	2.16±0.75	-0.49±0.75	2.33±1.0	< 0.05
Lemon grass	-0.18±0.81	2.11±0.75	-0.17±1.2	2.16±0.75	-0.37±0.1	2.0±0.63	< 0.05
Geranium	-0.14±0.54	1.6±0.51	-0.22±1.0	1.5±0.54	-0.34±1.2	1.66±0.51	< 0.05
Lemon scanted	-0.10±0.75	1.66±0.51	-0.18±1.0	2.0±0.63	-0.35±0.54	1.5±0.54	< 0.05
Depa	-0.14±0.75	2.83±0.75	-0.34±0.6	2.16±0.75	-0.62±1.0	2.16±0.75	< 0.05

PI= Preference index; NR=Non response; *p* value < 0.05 considered significant

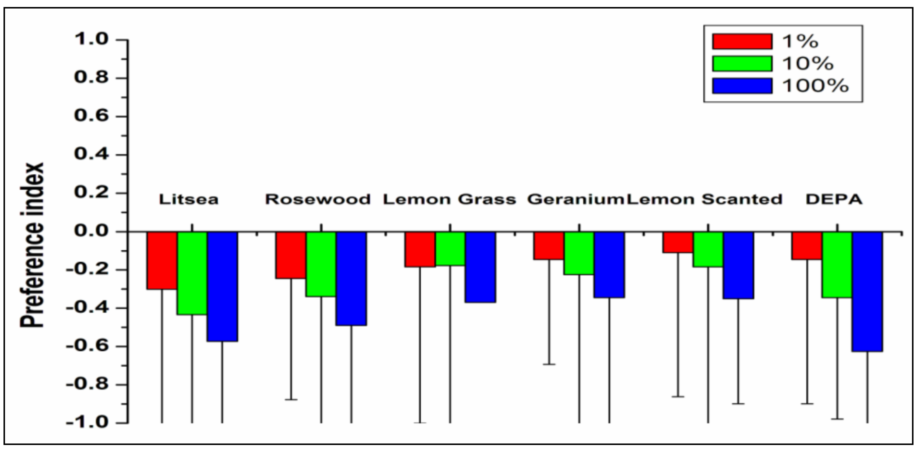

Fig 1: Behavioral response of *Aedes albopictus* mosquito towards essential oils using olfactometer

Discussion

Plants have been used for centuries in the form of crude fumigants where plants were burnt to drive away nuisance mosquitoes and later as oil formulations applied to the skin or clothes. Many plant extracts and oils repel mosquitoes, with their effect lasting from several minutes to several hours. Essential oils are volatile naturally occurring, complex compounds classified by a strong odour and are formed by plants as secondary metabolites. Essential oils contain more than 20 to 80 minor and major highly volatile chemical constituents of which are responsible for mosquito repellency and inhibit the orientation of blood sucking insects [15]. Essential oils are the alternative source against synthetic repellent because they are non-toxic to human and other organisms [16, 17].

Insect repellent is a substance applied to skin, clothing or other surfaces which discourages insects from landing or climbing on that surface. Hamisi M. Malebo *et al.*, showed the most common insect repellents available contain N, N–diethyl-3-methyl-benzamide or also called DEET and DEPA have shown strong protection against mosquitoes. Long term application of chemical substances for controlling, repelling and killing of hazardous insects make serious anxieties for environment and human health. Therefore uses of environment friendly and biodegradable natural insecticides of plant origin have received renewed attention as agents for vector control [18].

Das *et al.* [19] evaluated the repellent properties of *Zanthoxylum armatum*, *Zanthoxylum alatum*, *Curcuma aromatica*, and *A. indica* against mosquitoes in two bases of mustard oil (Brassica sp.) and coconut oil (Cocos sp.). They found that all the herbal oils and dimethyl phthalate (DMP) exhibited a better protection against the bites of mosquitoes in mustard oil base than in coconut. The effectiveness of attractants and repellents has not been systematically evaluated under well controlled conditions including insect densities, temperatures, air-flow rates, different geographic subpopulations and time durations.

In previous studies, a newly constructed olfactometer system, was used for evaluation of mosquito attractants and repellents, was demonstrated [24]. Biological and environmental parameters were optimized for effective analysis of attractants and repellents against *Ae. albopictus* female adults. The influence of mosquito age and density on repellent tests has been reported previously [25]. However, the method used was a bioassay in a Y-maze olfactometer, but not spatial repellency bioassay in a wind tunnel bioassay system, and the test species was *Ae. albopictus.*

Sharma *et al.* reported that the repellent effect of neem oil and showed protection against *Cx. quinquefasciatus,* whereas in the present study, essential oils of litsea and rosewood showed better repellency against *Ae. albopictus.* Most of the plant based active ingredients tend to be highly volatile, so although they are effective repellents for a short period after application, they rapidly evaporate leaving the user unprotected [26]. More research is needed to develop new repellents from substance of natural origin that can offer effective mosquito management to reduce the indiscriminate use of harmful insecticides. The results of the present study of behavioural response of dengue and chikungunya vector showed the potential of *litsea oil and rosewood oils* using Y-maze olfactometer.

Using an olfactometer test is quick and effective way to evaluate the behavioural responses of mosquitoes towards volatile stimuli of essential oil. These effective essential oils can be used as plant based products for providing 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.

An insect repellent of plant origin of essential oil ought to be well-defined and harmless to human and other non-target organisms. Therefore, use of these essential oils in mosquito control, instead of synthetic insecticides, could reduce the cost and environment effects. The results of the preliminary screening of laboratory evaluation of repellent activity of five essential oils confirmed their mosquito repellent properties. Currently, there is no effective vaccine against dengue, chikungunya, and due to insecticide resistance, many of our chemical control strategies are no longer effective for controlling the vector populations. Despite this fact, vector control remains a critical means of fighting this mosquito-borne disease. One alternate method of chemical control is the use of repellents for personal protection. Prevention of mosquito bites by natural insect repellents has become more popular, and there are many commercially available repellents on the market. In north-east region, many botanical plants hold the potential as spatial repellents for use against disease-carrying mosquito species, evaluations of these plant species are required, however, to determine the effectiveness and proper dosing of the essential oils derived from botanical plants to develop effective repellents for protection against vector-borne diseases.

Conclusion

Based on the above studies of essential oils, litsea, rosewood, lemongrass, geranium and lemon scanted showed effective repellency against *Ae. albopictus* female mosquitoes. Our research will continue to identify effective essential oils with higher repellent activity against blood sucking mosquitoes to develop a newer herbal-based mosquito repellent.

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