

Evaluation of botanicals as repellents against mosquitoes

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Repellent properties of three plant extracts—essential oil (steam distillate) of *Zanthoxylum limonella* (fruits), *Citrus aurantifolia* (leaf) and petroleum ether extract of *Z. limonella* (fruits) were evaluated as repellent against *Aedes* (S.) *albopictus* mosquitoes in mustard (Dhara) and coconut (Parachute) oil base under laboratory conditions. Three concentrations—10, 20 and 30% of the repellents were evaluated. Repellents in mustard oil afforded longer protection time against the bites of *Aedes* (S.) *albopictus* mosquitoes than those in coconut oil. At 30% concentration, 296–304 min protection time was achieved by the test repellents in mustard oil base while repellents in coconut oil exhibited 223.5–245 min protection time at the same concentration. Oil of *Z. limonella* gave the highest protection time against the bites of *Aedes* (S.) *albopictus* mosquitoes at all the concentrations than other herbal repellents tested both in mustard and coconut oil.

Key words *Aedes* (S.) *albopictus* – herbal repellents – laboratory trial – repellent chamber

Repellency is known to play an important role in preventing the vector borne diseases by reducing man-vector contact. Synthetic chemicals and insecticides used for control of vectors are causing irreversible damage to the eco-system, as some of them are non-degradable in nature. Some repellents of synthetic origin may cause skin irritation and affect the dermis¹. Majority of commercial repellents are prepared by using chemicals like allethrin, N-N-diehyl-m-toluamide (DEET), dimethyl phthalate (DMP) and N, N-diethyl mendelic acid amide (DEM). It has been reported that these chemical repellents are not safe for public use^{2,3}. Because of unpleasant smell, oily feeling to some users^{4,5} and potential toxicity^{6–8} some prefer to use natural insect repellent products. Repellents of plant origin do not pose hazards of toxicity to human and domestic animals and are easily biodegradable. Natural products are safe for human when compared to that of synthetic compounds^{9,10}.

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Therefore, it is the hour to launch extensive search to explore eco-friendly biological materials for control of insect pests.

The phytochemicals derived from plant resources can act as larvicides, insect growth regulators, repellents and ovipositional attractants, having deterrent activities observed by different researchers^{11–13}. The plant products have been used traditionally to repel or kill the mosquitoes in many parts of the world. Novak¹⁴ emphasised the urgent need for the investigation of phytochemicals as repellents for mosquito control. Certain natural products have been investigated for repellent activity against mosquitoes. *Zanthoxylum armatum*, DC. syn. *Z. alatum* Roxb. (Rutaceae); *Azadirachta indica* (Maliaceae) and *Curcuma aromatica* (Zingiberaceae) were among them and have been reported to possess repellent properties against mosquitoes¹. *Callistemon rigidus* (bottle brush), *A. indica* (neem) and *Z. armatum*

(timur) have been reported to have repellent activity against land leeches also¹⁵. Repellent action of neem oil in the form of mats⁹ and neem cream¹⁶ have been evaluated against mosquitoes. Benzene and methanol extracts of *Artemisia vulgaris* have been reported to have repellent activity against *Ae. aegypti*¹⁷. Quelling, the insect repellent produced in China, derived from the extract of the lemon grass and eucalyptus plants were evaluated against mosquitoes. Essential oil obtained from *Vitex negundo* was used as repellent against *Aedes aegypti*¹⁸. Repellent properties of *Lantana camara* (Verbanaceae) flowers against *Aedes* mosquitoes reported by Dua *et al*¹⁹.

In the present communication, an attempt has been made to evaluate the repellent efficacy of three plant materials against *Aedes* (S.) *albopictus* mosquito under laboratory conditions.

Material & Methods

The essential oils used as mosquito repellents were obtained by steam distillation of the fruits of *Zanthoxylum limonella* (Bajarmani), leaves of the *Citrus aurantifolia* (Lemon) and petroleum ether extract of the fruits of *Z. limonella*. Mustard oil 'Dhara' (M/s. National Dairy Development Board, P. B. No. 40, Anand) and Coconut oil 'Parachute brand' (M/s. MARICO Industries Ltd., Bombay) were obtained from the market and used as bases of the repellents.

The repellent trials were conducted in a repellent test chamber (30 x 30 x 62.5 cm) under laboratory conditions. Three concentrations—10, 20 and 30% of the repellents in mustard and coconut oil were evaluated on the hands of human subjects. Before application of the repellents, hands were washed and cleaned thoroughly with rectified spirit. Male and female *Aedes* (S.) *albopictus* mosquito progenies obtained from laboratory colony were maintained in honey solution in a cloth cage (50 x 50 x 50 cm) under controlled temperature (28 + 2°C) and relative humidity range (75–80%). About 50 to 60 hungry (3 days old) female *Ae.* (S.) *albopictus* were introduced into the repellent chamber through the hole on top. Aliquot of 0.3 ml of the test solution was

smear on dorsal side of one hand (wrist to finger tips) of each of the subject. After 30 min of application, the hand was placed inside the repellent chamber for 10 min through a hole up to wrist and plugged with cotton to prevent escape of mosquitoes in order to facilitate the female mosquitoes to bite on. The test was repeated at every 30 min interval. The interval between the application of repellent and the first two consecutive bites occurring within 30 min was considered as protection time against the bites afforded by each of the concentrations of the test repellents²⁰. The test was replicated 10 times for each concentration of the repellents. Control readings were obtained by placing hand inside the repellent chamber without applying any repellent before the experiment. Results obtained were statistically analysed as per Randomize Block Design test (RBD).

Results & Discussion

It was observed that the three herbal repellents exhibited better protection against the bites of *Aedes* (S.) *albopictus* mosquitoes in mustard oil than in coconut oil. Maximum protection time (296–304 min) was afforded by the herbal repellents at 30 per cent concentration in mustard oil (Table 1), while 223.5–245 min protection time was recorded with the herbal repellents in coconut oil at the same concentration (Table 2). Oil of *Z. limonella* exhibited the highest protection time against mosquito bites at all the concentrations (10, 20 and 30%) in both mustard and coconut oils.

On statistical analyses, steam distillate oil and petroleum ether extract of *Z. limonella* were found equally effective and afforded significantly the better protection time than the oil of *C. aurantifolia* at 10% concentration in mustard oil. But at 20% concentration, oil of *Z. limonella* gave significantly the highest protection against the bites of *Aedes* (S.) *albopictus* mosquito (> 0.01) while steam distillate oil of *C. aurantifolia* and petroleum ether extract of *Z. limonella* were found at par. However, at the highest concentration (30%), there was no significant difference in protection time among the three herbal repellents in mustard oil (Table 1). At 30% concentration, essential oil of *Z. limonella* yielded significantly the better protection time (> 0.05). At 20% concentration, oil

Table 1. Relative repellency of three herbal repellents against *Aedes (S.) albopictus* mosquito (in mustard oil base)

Conc. (%)	Repellent	Range (min)	Treat. mean (min)	Variance ratio	LSD	Tabulated F value	
						1% level	5% level
10	<i>Z. limonella</i> oil (Steam distillate)	175–193	184*	93.7	9.18	6.01	3.55
	<i>C. aurantifolia</i> oil (Steam distillate)	122–138	129.5				
	<i>Z. limonella</i> (Petroleum ether extract)	171–185	178*				
20	<i>Z. limonella</i> oil (Steam distillate)	260–290	275.5*	28.075	11.58	6.01	3.55
	<i>C. aurantifolia</i> oil (Steam distillate)	230–250	239.5				
	<i>Z. limonella</i> (Petroleum ether extract)	230–250	240.5				
30	<i>Z. limonella</i> oil (Steam distillate)	290–315	304.5*	3.5	6.01	6.01	3.55
	<i>C. aurantifolia</i> oil (Steam distillate)	285–315	302.5				
	<i>Z. limonella</i> (Petroleum ether extract)	280–300	296				
Control	Mustard oil	30–40	35				

*Least significant difference, >0.01.

Table 2. Relative repellency of three herbal repellents against *Aedes (S.) albopictus* mosquito (in coconut oil base)

Conc. (%)	Repellent	Range (min)	Treat. mean (min)	Variance ratio	LSD	Tabulated F value	
						1% level	5% level
10	<i>Z. limonella</i> oil (Steam distillate)	134–150	142	1.654	11.23	6.01	3.55
	<i>C. aurantifolia</i> oil (Steam distillate)	121–143	132				
	<i>Z. limonella</i> (Petroleum ether extract)	122–148	135.5				
20	<i>Z. limonella</i> oil (Steam distillate)	180–210	195*	12.293	11.23	6.01	3.55
	<i>C. aurantifolia</i> oil (Steam distillate)	159–181	170.5				
	<i>Z. limonella</i> (Petroleum ether extract)	178–214	191*				
30	<i>Z. limonella</i> oil (Steam distillate)	232–258	245*	4.47	15.93	6.01	3.55
	<i>C. aurantifolia</i> oil (Steam distillate)	214–242	228				
	<i>Z. limonella</i> (Petroleum ether extract)	209–237	223.5				
Control	Coconut oil	20–30	25				

*Least significant difference, >0.01.

and petroleum ether extracts of *Z. limonella* were found at par and gave significantly better protection time than the oil of *C. aurantifolia* (> 0.01) in coconut oil. There was no significant difference in protection time among the three herbal repellents at 10% concentration (Table 2).

On comparing with the earlier observations, all the three test repellents were found to give better protection time against the bites of *Aedes (S.) albopictus* mosquitoes than the seed extract of *Tephrosia purpuria* Linn. Pers²¹. Das *et al*¹, reported 7.4, 6.5 and 6.4 h protection against the bites of mosquitoes with 60% (0.57 mg/cm²) concentration of essential oil of *Z. armatum* (fruits), *Curcuma aromatica* (rhizomes) and oil of *A. indica* respectively in mustard oil base in field conditions. It is stated that petroleum ether extract of *Vicoa indica*, *Buddleja asiatica*, *Chenopodium ambrosoides*, *Clerodendrum inerme* and methanol extract of *Solanum erinthum* gave 3 h protection against mosquitoes at 9% concentration²². It is reported that at 1% of garlic extract gave 8 h protection against *Culex fatigans*²³.

Contrary to 'per cent protection' reported by earlier workers, total protection from mosquito bites with herbal repellents was considered in the present trial. Four hours protection time yielded by *Z. limonella* in mustard oil base (ranged between 3 and 5 h) and three hours in coconut oil (ranged between 2.3 and 4 h) respectively.

An insect repellent of plant origin ought to be well-defined and harmless to human and other non-target organisms. Therefore, use of these botanical derivatives 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 three plant extracts confirmed their broad-spectrum mosquito repellent properties. Further studies on identification of active compounds, toxicity and field trials are needed to recommend the active fraction of these plant extracts for development of eco-friendly chemicals and indigenous plant base oil for protection against the bites of haematophagous insects.

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