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Repellency of Medicinal Plant Extracts Against Dengue Vector Mosquitoes, *Aedes albopictus* and *Ae. aegypti* (Diptera: Culicidae)

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Abstract.- This study was carried out to evaluate the repellency potential of the essential oils extracted from the branches and leaves of eucalyptus (*Eucalyptus globules*), neem (*Azadirachta indica*), peppermint (*Mentha piperita*), niazbow (*Ocimum basilicum*) and rhizome of ginger (*Zingiber officinale*) against the adult females of *Aedes albopictus* and *Aedes aegypti*. The essential oils were extracted by using a Soxhlet apparatus with petroleum ether as a solvent. The repellency of essential oils was determined by a human - bait technique. The bites were recorded for 3 min after each 15 min from 1100 h to 1400 h. A control treatment was also run using canola oil. All other oils were mixed separately with canola oil to make 10% solution. The ANOVA and Duncan analysis revealed a highly significant difference in repellency duration and biting protection among the repellents tested under this experiment. All essential oils tested provided protection for > 33 min than the control. Neem oil gave the longest protection *viz.*, 246±15.78 and 256±14.87 min, against *Ae. aegypti* and *Ae. albopictus*, respectively, while ginger oil gave the shortest protection time against both *Aedes* species. All the studied factors (type of oil as a repellent, repellency duration, and biting protection) and their interactions were found to be highly significant statistically. The oils extracted from *M. piperita, A. indica, Z. officinale* and *O. basilicum* have strong repellent action against the adults of *Ae. aegypti* and *Ae. albopictus* and could be safely used to avoid these mosquitoes.

Keywords: Dengue mosquitoes, medicinal plant extracts, repellent activity.

INTRODUCTION

Mosquitoes are vectors of many diseases such as malaria, dengue fever, yellow fever, filariasis and chikungunya throughout the world, particularly in tropical and subtropical areas (Sritabutra *et al*., 2011). *Anopheles*, *Culex*, and *Aedes* are three medically important genera of mosquitoes that are responsible for the spread of these diseases (Samidurai *et al*., 2009). The *Aedes* genus is mainly responsible for the spread of dengue fever, dengue haemorragic fever (DHF), and yellow fever. *Aedes aegypti* (L) and *Ae. albopictus* Skuse are the major vectors for dengue fever (Service, 2004; Jahan *et al*., 2011). Due to this disease, more than 22,000 deaths are reported annually mainly in children and youth (below 20 years) (Riaz, 2011). There is neither a vaccine nor a treatment available for this viral disease. Thus, the only solution is to avoid and prevent mosquito bites. Mosquitoes can be avoided either by controlling them or avoiding their bites (Tjahjani, 2008). For control, different chemicals are used that are not only toxic to human health or other creatures but also cause resistance in mosquitoes against these chemicals (David *et al*., 2007). Different chemicals known as mosquito repellents are used to prevent mosquitoes from landing on or biting human skin. Generally, these are synthetic chemicals that are not completely safe for human beings, particularly for babies, small children, and developing faetus in pregnant women. These repellents may cause respiratory diseases or irritation, rashes, allergy, and hot sensation to human skin (Das *et al*., 2003). Due to these reasons, researchers are seeking alternative herbal products as repellents which are both safe for human health and non-hazardous for the environment. Many researchers reported repellency of essential oils from plants against adult mosquitoes (Govindarajan and Karuppannan, 2011) showing that essential oils from neem (*Azadirachta indica*), *Eucalyptus* spp.,

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Citrus spp., peppermint (*Menta piperita*), lemongrass (*Cymbopogon citrates*) and other plants show weak to strong repellency against mosquito species such as *Ae. aegypti*, *Ae. albopictus*, *Culex quinquefasciatus* and some *Anopheles* spp. (Choochote *et al*., 2005; Makhaik *et al*., 2005). Many researchers used these oils separately (Makhaik *et al*., 2005) while others used them in combinations to enhance their repellency (Sritabutra *et al*. 2011). Some of these oils provided 6-8 h protection against mosquito bites while others gave only 2-3 h protection (Makhaik *et al*., 2005; Sritabutra *et al*., 2011). Thus, the present study was carried out to test the repellent efficacy of medicinal plant extracts against *Ae. aegypti* and *Ae. albopictus* under laboratory conditions.

MATERIALS AND METHODS

In this study, the skin repellent activity of petroleum ether extracts of *E. globules*, *A. indica*, *M. piperita*, *O. basilicum* and *Z. officinale* in canola oil were evaluated against blood starved adult females of *Ae. aegypti* and *Ae. albopictus* under laboratory conditions. Canola oil was used as the control. All other oils were mixed separately with canola oil to make 10% solution. Each was given a code number E1-E6 that was allotted to all oils used in this study.

Collection and rearing of mosquitos

Larvae, pupae and adults of *Aedes (Ae. aegypti* and *Ae. albopictus*) mosquitoes were collected from different catchment areas such as tree holes, tires, water storage tanks, and fish water ponds. Larvae and pupae were collected by using standard dippers while adults with aspirators and stored in a plastic bottle tied up with muslin (Sritabutra *et al*., 2011). The collected specimens were carried to the Department of Zoology, Government College University, Faisalabad for sorting, rearing, and identification. The larvae and pupae were separated. The larvae were kept in rearing trays and pupae in beakers inside the cages in the laboratory at 26±1°C and 75±5% RH. The larvae were fed fish diet and adults 10% glucose solution (Kumar *et al*., 2011). Four days old 100 females of both species were kept in separate cages and starved for 8 h before starting the experiment.

Collection of plant material

For the oil extraction, branches and leaves of *Ocimum basilicum* and rhizomes of *Azadirachta indica*, *Eucalyptus globules*, *Mentha piperita*, *Zingiber officinale* different plants and their parts were collected from Government College University, Faisalabad (31°30'N, 73°05' E), washed with tap water to remove dust particles and dried at room temperature in shade and then for 48 h at 60°C in an electric oven. The dried material was ground in a grinder and the powder was stored in plastic bottles for oil extraction.

Extraction of oil

Essential oils were extracted from the selected plant material using a Soxhlet apparatus (Cheng *et al*., 2009b). Twenty five grams powder of each plant material with 250 ml of solvent (petroleum ether) was used to extract oil through Soxhlet apparatus for 8 to 24 h (Vogel, 1978). After extraction, vacuum evaporator was used to evaporate solvent to attain filtrate in dehydrated form. Acquired extract was stored in an airtight jar.

Repellency test

The repellency of essential oils was conducted using a human-bait technique. Prior to every test, the hand and forearm of a human volunteer were washed with odorless impartial cleanser, thoroughly cleaned, and dehydrated for 10 min before applying the extract. A part of (5 ×5 cm) on each forearm of five human volunteers was marked out by a fixed marker. Approximately 0.1 mL of the 10 % oil (respective oils were mixed in canola oil) was applied to the marked part of one forearm of every volunteer, while the other forearm was treated with canola oil as control. In the test, the forearm was protected by a paper cover except the zone marked open. The control and treated arms were placed concurrently into the testing cage. An effort of the mosquito to introduce its stylets was measured as a bite. The bites were recorded for 3 min after each 15 min from 11:00 h to 14:00 h.

Protection time was documented as the time elapsed among repellent use and the observation period instantly foregoing that in which a definite

bite was attained. If no bites were recorded at 180 min, tests were stopped, and protection time was documented as 180 min. If no mosquito bit the control arm, the sample was discarded and the test was reinstated with a new consignment of mosquitoes to confirm that lack of bites was due to repellency. The trials were conducted three times in distinct cages, and in every test, different volunteers were recycled to nullify some results of skin alterations on repellency. Twenty five female mosquitoes were used in each cage. The median protection time was used to compare the tested plant extracts. Significant differences were analyzed by one-way analysis of variance (ANOVA) and Duncan's new multiple range test (DMRT). Based on the formula of Kumar *et al*. (2011) and Sritabutra *et al*. (2011), the percentage protection was calculated:

$$\% \text{ protection} = \frac{\text{No. of bites received by control arm} - \text{number of bites received by treated arm}}{\text{No. of bites received by control arm}} \times 100$$

RESULTS

The ANOVA and Duncan analysis revealed a highly significant difference in repellency duration and biting protection among the repellents and control (canola) oil was lower than all other test oils. All essential oils tested provided protection for at least > than 33 min (Table I) and thus may be included in the standard list of repellents for *Aedes* species.

Neem oil repellent (E2) had the best repellency against both species of *Aedes* mosquitoes in which the protection times were (246±15.78) and (256±14.87) min, against *Ae. aegypti* and *Ae. albopictus* respectively. The protection time of the canola oil used as control was (14.34±0.00) and (19.24±0.00) min against *Ae. aegypti* and *Ae. albopictus* respectively. The least protection was provided by ginger oil *viz.,* 35.76±4.99 and 33.76±4.99 min against *Ae. aegypti* and *Ae. albopictus,* respectively. All other oil formulations were in between these two oils and statistically significant from each other (neem oil + mint oil + eucalyptus oil + niazbow oil + ginger oil + control oil) as shown in Table I. These results showed that for this level of bite protection, neem oil must be re-applied after every 4 h and mint oil every 1.5 h. Also, Neem oil (E2) had the best percent biting efficiency against both species (*Ae. aegypti* and *Ae. albopictus*) in which the biting percentage was 0.27 and 0.25%, respectively. All the tested botanical oils showed the biting percentage against these two *Aedes* mosquitoes ranged from 0.6 to 0.97% except for neem oil and control (Table I). The canola oil was used as control gave the least biting protection. The protection time (min) and biting percentage (%), in case of different oils for *Ae. aegypti* and *Ae. albopictus.* The graph shows the neem oil provide maximum protection time and least biting percentage among all five test oils and canola oil as a control measure. Maximum biting % was observed in the case of control treatment. All the oils were statistically at par with each other.

DISCUSSION

The essential oil extracts obtained from *M. piperita, E. globules, A. indica, Z. officinale,* and *O. basilicum* showed a varying degree of repellency against the two mosquito species, *Ae. aegypti* and *Ae. albopictus.* The neem oil provided the longest protection time against both species, while ginger oil provided the least protection time. Our results showed that *Ae. aegypti* was slightly more sensitive to some essential oils (eucalyptus and ginger oils) than was *Ae. albopictus* with neem, mint, and niazbow oils. These results were statistically in agreement with those of Tawatsin *et al*. (2006) who found that *Ae. albopictus* was more sensitive to all essential oils (repellency 4.5–8 h) than *Ae. aegypti* (repellency 0.3–2.8 h). Other researchers (Yang and Ma, 2005; Jaenson *et al*., 2006) also reported that there was more sensitivity of *Ae. albopictus* than *Ae. aegypti* when exposed to eucalyptus extract. In case of eucalyptus oil, about 1.5 h protection time was noted in this study in contrast to Yang and Ma (2005) who noted double protection time (3 h). In this study, maximum protection time of 4 h and minimum biting percentage of 27% were provided by neem oil while Amer and Mehlhorn (2006) noted a maximum protection time of 8 h and 100%

Table I.- Efficacy of herbal oil formulations (10% oil mixed in canola oil) from different plant sources as repellents and biting protector against *Ae. aegypti* and *Ae. albopictus* (mean ±SD).

Repellents	Protection time		Biting %	
	Ae. aegypti	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*
Eucalyptus	82.54±12.05^{c}	78.64±13.15^{bc}	0.63^{b}	0.68^{b}
Neem	246±15.78^{a}	256±14.87^{a}	0.27^{a}	0.25^{a}
Mint	97.87±13.76^{b}	97.87±13.76^{b}	0.94^{b}	0.92^{b}
Niazbow	64.34±10.45^{d}	69.34±10.35^{c}	0.97^{b}	0.80^{b}
Ginger	35.76±4.99^{e}	33.76±4.99^{d}	0.92^{b}	0.85^{b}
Canola	14.34±0.00^{f}	19.24±0.00^{e}	3.98^{c}	4.14^{c}

repellency against *Aedes* species with *Melaleuca leucadendron*. In this study with *M. piperita*, 100% protection was shown till 98 min after which 0.94% biting was noted for the rest of the time, while Kumar *et al*. (2011) noted 100% protection till 150 min after which only 1-2 bites were reported for next 30 min after the application of *M. piperita*. We noted about 70 min protection time against *Aedes* species from petroleum ether extract of *O. basilicum* but Padilha de Paula *et al*. (2003) noted a high bite protection of 8 h with liquid paraffin solution of *O. basilicum*.

We used canola oil to make 10% concentration of the repellent oils in this study, while Das *et al*. (2003) used 10, 20 and 30% concentration of the repellents in mustard and coconut oils and maximum protection was noted with mustard oil. Both canola and mustard crops belong to the same family Brassicea. Karunamoorthi *et al*. (2008) also reported 8 h bite protection against different mosquito species in the field using petroleum ether extracts of the plant. However, the protection time of this study was short in some oils and formulations could improve the protection time. Some early workers showed that repellency can be improved by adding 5-10% Vanillin (Tawatsin *et al*., 2001; Choochote *et al*., 2007). Usually, botanical oils do not provide long protection, while synthetic repellents can provide more than 6 h. These differential responses induced by same plant extracts against mosquitoes may be due to some extrinsic and intrinsic factors, such as plant species, parts of plant used, solvents used in extraction, geographical location where the plants are grown, and evaluation methods (Sukumar *et al*., 1991). However, results from this study can be used to develop and improve the production of essential oil extracts, for greater effectiveness as insect repellents.

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