DEVELOPMENT OF ENVIRONMENT-FRIENDLY INSECT REPELLENTS FROM THE LEAF OILS OF SELECTED MALAYSIAN PLANTS

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The leaf oils of Litsea elliptica, **Cinnamomum mollissimum,** ***Cymbopogon nardus*** *and Pogostemom cablin were evaluated for their repellency effect against adult female mosquito of Aedes aegypti. The leaf oil of Cy.* ***nardus*** *was the most effective with median effective dosage (ED_{50} of 0. 0009 mg cm^{-2} comparable to the value obtained with dimethyl phthalate (0. 000 7 mg cm^{-2}), the standard repellent used in this study. The essential oils from other species studied also showed significant repellency effect with ED_{50} ranging from 0.0023 to 0.0065 mg cm^{-2}. These essential oils were then employed as active ingredients in the preparation of insect repellents used in our field trials. Results ftom the field trials indicate that an aqueous cream containing 15% of the leaf oils* ***(L elliptica, C.mollissimum, Cy. nardus*** *in the ratio of 1: 1: 1) provided 96 6% protection against mosquito bites for the duration of the test.*

INTRODUCTION

Essential oils from some plants such as citronella and pennyroyal have been employed as insect repellents since ancient times. Insect repellent formu1ations consisting of oil of citronella, spirits of camphor, oil of tar, oil of pennyroyal and castor oil have been shown to provide long-lasting protection against insects (Freeborn, 1928; Dover, 1930; Skinner and Johnson, 1980).

Penfold and Morrison (1952) reported the effectiveness of forty essential oils against mosquitoes, march flies and sand flies; and of these the most effective were those of *Dacrydium franklini, Backhousia myrtifolia, Mela-leuca bracteata* and *Zieria smithii.* Pine oil was found to be effective as repellent against the striped ambrosia beetle *Trypodendron lineatum* (Dubbel, 1992). Volatile oils from *Eucalyptus* and *Caryophyllum* species have also been reported to possess satisfactory repellency effects (Mayer, 1952). The monoterpenes, α-pinene, limonene, terpinolene, citronellol, citronellal and camphor which are common constituents of some oils have been reported to possess high repellent properties against various insects (Perttunen, 1957; Moore, 1974). Isolated compounds (benzaldehyde, cinna-maldehyde and eugenol) from the essential oil of *Pogostemon parviflorus* have been reported as repellents against *Sitophilus oryzae* and *Bruchus chinensis* (Saxena and Koul, 1982). Eisner (1964) found that vapours of nepetalactone, a cyclopentanoid monoterpene isolated from catnip plant *Nepeta catana* can be used as repellent against seventeen species of insects. This study reports on repellency properties of the essential oils of four Malaysian plants, viz. *Litsea elliptica, Cinnamomum mollisimum, Cymbo-pogan nardus* and *Pogostemon cablin* against the biting of *Aedes aegypti* and the use of these oils as active ingredients in the preparation of insect repellents.

MATERIALS AND METHODS

Preparation of Plant Materials

The plant materials were collected from Pasoh *(Litsea elliptica* and *Cinnamomum mollissimum),* Kuala Pilah *(Pogostemon cablin)* and Kepong *(Cymbopogon nardus)* in Peninsular Malaysia. The plants were identified and voucher specimens were deposited at the Herbarium of the Forest Research Institute Malaysia. Dimethyl phthalate was purchased from Aldrich Chemicals, Milwaukee, USA. The essential oils of the samples were obtained by subjecting their leaves to water distillation for 8 hours.

Assessment of Repellency

Mosquito repellent activity was assessed by using the test cage described in the American Society for Testing and Materials (ASTM) standard E951-83 "Laboratory testing of non-commercial mosquito repellent formulations on the skin" (Anonymous, 1983). The test procedure was similar to that described by Buescher *et al.* (1982) and Gupta *et al.* (1989). The flexor region of the forearms of four volunteers were outlined with five circular 29 mm diameter test areas. A volume of 0.025 ml of serial dilutions of the essential oils in absolute ethanol (0.0006 - 0.0379 mg cm^2*)* and 0.025 ml of the diluent were applied randomly to the marked areas. The test cages were positioned securely on the arms of each volunteer with Velcro tapes to ensure that only the test areas were exposed for mosquito bites. 15 mosquitoes were introduced into each cage and the number.of bitings was recorded at the end of 90 sec. The test procedure was replicated four times for each oil sample and statistically reliable estimates of their median effective dosage (ED_{50}) were obtained by probit-log concentration analysis (Finney, 1971). Dimethyl plithalate, applied in a similar manner was used as the standard repellent. Percentage repellency was determined by the formula described by Weaving and Sylvester (1967).

$$\text{Percent Repellency} = 100 - \frac{\text{number of bites on treated arm} \times 100}{\text{number of bites on control arm}}$$

Preparation of Insect repellent products

Insect repellent cream An aqueous cream (oil in water type) was prepared by emulsifying the leaf oils in water with an emulsifying wax. The formula for the insect repellent cream is as follows;

Leaf oil of *Cinnamomum mollissimum*	*5.0ml*
Leaf oil of *Litsea elliptica*	*5.0ml*
Leaf oil of *Cymbopogon nardus*	*5.0ml*
Cetostearyl alcohol	4.0g
Sodium lauryl sulfate	0.4g
Triethanolamine	5.0ml
Stearic acid	10.0g
Methyl p-hydroxybenzoate	0.2g
Purified water to	100ml

Insect repellent candle The candle was made up of a mixture of hard paraffin and methyl stearate as the hydrocarbon bases. The formula for the insect repellent candle is as follows;

Leaf oil of *Litsea elliptica*	*5ml*
Leaf oil of *Cinnamomum mollissimum*	5ml
Leaf oil of *Cymbopogon nardus*	10ml
Leaf oil of *Pogostemon cablin*	*2ml*
Hard paraffin	60g
Methyl stearate	18g

FIELD TRIALS OF INSECT REPELLENT CREAM

The aqueous cream was applied at 0830 hours each day for three days to each arm below the elbow of four volunteers. Individuals treated with the repellent cream were required to expose their treated skin constantly for 3 hours period. The secondary forest at the Forest Research Institute Malaysia was selected as the test site because of high mosquito populations and rarity of mosquito-borne disease. The volunteers were allowed to roam freely in the forested test site and the number of mosquito bites on the treated skin was counted throughout the hour. This procedure was repeated on volunteers who were not treated with the cream. The number of bites on the untreated arms of volunteers was recorded. The percent protection provided by the repellent cream can be expressed by the formula (Lillie *et al.,* 1988);

$$\text{Percent Protection} = \frac{\text{Bites on control} - \text{bites on treated}}{\text{Bites on check}} \text{ X } 100$$

RESULTS AND DISCUSSION

Table 1 and 2 show the mean number of mosquito bites per 90 seconds period and the % repellency of the leaf oils towards *Aedes aegypti* at various concentrations. The leaf oils showed significant degree of repellency, however, they had lower values than the standard repellent, dimethyl phthalate. The oils of *Cymbopogon nardus* and *Pogostemon cablin* at 0.0047 mg cm^2 concentration provided 72.2 and 71.4% protection against mosquito bites respectively. Complete protection was achieved with *Litsea elliptica* and *Cinnamomum mollissimum* at 0.0379 mg cm^2 . Table 3 shows the repellency effect of the essential oils against the mosquito with ED_{50} ranging from 0.0007 to 0.0065 mg cm^{-2} . Amongst these the oil of *Cymbopogan nardus* with a ED_{50} of 0-0009 mg cm^{-2} was the most effective, comparable to the value of the standard repellent (0.0007 mg cm^{-2}). The essential oils from the other species also showed significant repellency effect ranging from 0.0023 to 0.0065 mg cm^{-2} .

Table 1. Mean Number of Aedes aegypti Bites Received per Test

Concentration mg cm^{-2}	**Dp**	**Le**	**Cm**	**Cn**	**PC**
0.0379	-	0	0	-	-
0.0189	-	1.00	0.50	-	-
0.0095	-	1.75	0.75	-	0.75
0.0047	0	2.50	2.25	1.25	1.00
0.0024	1.00	-	-	1.25	2.00
0.0012	2.00	-	-	2.25	2.25
0.0006	3.50	-	-	2.50	-
Control	6.00	4.25	3.25	4.50	3.50

C.I. - Confidence interval; **Dp** - Dimethyl phthalate; **Le** - L*itsea elliptica;*
Cm - C*innamomum mollissinium;* **Cn** - C*ymbopogon nardus;* **PC** - *Pogostemon cablin.*

Table 2. Percent Repellency of Plant Samples Toward Aedes aegypti

Concentration mg cm^{-2}	**Dp**	**Le**	**CM**	**Cn**	**PC**
0.0379	-	100	100	-	-
0.0189	-	76.47	84.62	-	-
0.0095	-	58.82	76.92	-	78.57
0.0047	100	41.18	30.77	72.22	71.43
0.0024	83.33	-	-	72.22	42.86
0.0012	66.67	-	-	50.00	35.71
0.0006	41.67	-	-	44.44	-
Control	0	0	0	0	0

C.I. - Confidence interval; **Dp** - Dimethyl phthalate; **Le** - L*itsea elliptica;*
Cm - C*innamomum mollissinium;* **Cn** - C*ymbopogon nardus;* **PC** - *Pogostemon cablin.*

Table 3. Median Effective Dosages of Plant Samples against Aedes aegypti

	ED_{50}	***95% CI***
Dp	0.0007	0.0005 - 0.0008
Le	0.0060	0.0045 - 0.0075
Cm	0.0065	0.0055 - 0.0075
Cn	0.0009	0.0005 - 0.0013
PC	0.0023	0.0017 - 0.0029

C.I. - Confidence interval; **Dp** - Dimethyl phthalate; **Le** - L*itsea elliptica;*
Cm - C*innamomum mollissinium;* **Cn** - C*ymbopogon nardus;* **PC** - *Pogostemon cablin.*

Table 4. Mean Number of Mosquito Bites Received per Person per Hour on Treated and Untreated Skin

Treatment	**Mosquito bites per hour**	**Percent protection**
Repellent cream on skin	1	96.6
No treatment	30	0

The aqueous cream containing 15% of the leaf oils *(Lelliptica, C. mollissimum, Cy. nardus* in the ratio 1: 1: 1) provided high level of protection against mosquito bites. Subjects treated with the cream received only 1 mosquito bite per hour (Table 4). The mean number of mosquito bites received per person for untreated volunteers was 30. This indicated that the cream provided 96.6% protection against mosquito bites for the duration of the test. Further tests need to be carried out to determine the duration of protection provided by the cream.

The repellent effect of the essential oils indicated that they contained active principles responsible for the repellency activity. The chemical compositions of the essential oils have been determined previously (Wijesekera, 1973; Moorkherjee et al., 198 1; Dayar Arbain, 1990; Ibrahim & Goh, 1990; Ibrahim & Goh, 1992). The high levels of citronellal and citronellol in *Cymbopogan nardus,* benzyl benzoate in *Cinnamomum molfisimum,* 10-undecen-2-one in *Litsea elliptica* and patchouli alcohol in *Pogostemon cablin* could be responsible for the high repellency activity of the essential oils. The major components of the oils when in combination with other compounds of diverse structure in the oils could exhibit different mode of action against the test organism, contributing towards their repellency activity.

The significant repellency effect exhibited by the leaf oils towards *Aedes aegypti* and the excellent protection from mosquito bites provided by the repellent cream suggested that the essential oils have the potential to be developed into commercial chemical repellents. Although the leaf oils were less effective than the standard repellent, dimethyl phthalate, the herbal repellent cream and candle developed from them are environment-friendly, with pleasant natural aroma and less

harmful than synthetic repellents which have been reported to cause many undesirable side effects to human (Reynolds, 1989).

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