

Four Monoterpene Compounds: Repellency Evaluation Against *Aedes aegypti*

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ABSTRACT Repellency activity has attracted scientist in accordance of their usefulness as a control against vector mosquito. It can provide protection against the insects bite especially insects of public health importance. Repellency was defined as the stimulation by a chemical of oriented movements away from the source, or the prevention of the insect from approaching the source. Of such interest, four monoterpene compounds, i.e., citronellal, citronellol, geraniol and limonene were tested individually for their repellency activity against adult female vector mosquito of dengue and dengue haemorrhagic fever, *Aedes aegypti*. Repellency activity was evaluated using repellency kit with human as volunteers. Results indicated that citronellol gave the strongest activity with median effective concentration value (EC_{50}) of $0.00011 \text{ mg cm}^{-2}$. It was followed by geraniol ($0.00018 \text{ mg cm}^{-2}$), citronellal ($0.00025 \text{ mg cm}^{-2}$) and limonene ($0.00268 \text{ mg cm}^{-2}$). Standard repellent deet was also evaluated for the repellency activity and gave EC_{50} value of $0.0005 \text{ mg cm}^{-2}$. This paper then provides information on the repellency activity of the four commercial monoterpenes, which could be found abundantly in the essential oil of plant kingdom.

ABSTRAK Aktiviti repelensi telah menarik perhatian penyelidik disebabkan oleh kegunaannya di dalam mengawal vektor nyamuk. Ia boleh memberi perlindungan daripada gigitan serangga terutamanya serangga yang berkepentingan kesihatan. Repelensi didefinisikan sebagai suatu stimulasi kimia dengan orientasi penjarahan daripada sumber atau pencegahan serangga daripada menghampiri sumber. Di atas minat ini, maka empat kompoun monoterpene iaitu citronellal, citronellol, geraniol dan limonene telah diuji aktiviti repelensinya secara berasingan terhadap vektor nyamuk denggi dan denggi berdarah betina dewasa *Aedes aegypti*. Aktiviti repelensi dinilai menggunakan kit repelensi dengan beberapa orang individu sebagai sukarela. Keputusan menunjukkan citronellol memberikan aktiviti terbaik dengan nilai kepekatan berkesan median (EC_{50}) sebanyak $0.00011 \text{ mg cm}^{-2}$. Ini diikuti oleh geraniol ($0.00018 \text{ mg cm}^{-2}$), citronellal ($0.00025 \text{ mg cm}^{-2}$) dan limonene ($0.00268 \text{ mg cm}^{-2}$). Repelen piawai, deet juga telah dinilai untuk aktiviti ini dan memberikan nilai EC_{50} sebanyak $0.0005 \text{ mg cm}^{-2}$. Kajian ini memberikan maklumat berkenaan aktiviti repelensi empat monoterpene yang boleh ditemui dengan banyaknya di dalam minyak pati alam tumbuhan.

(Repellency activity, *Aedes aegypti*, citronellal, citronellol, geraniol, limonene, Deet)

INTRODUCTION

Repellents are commonly used to prevent insect bites during outdoor activities and help in interrupting disease transmission. This repellency activity has attracted considerable attention of researchers especially in controlling vector mosquitoes of public health importance. Moreover, demand from public for convenient, safe and effective repellent gave wide

opportunity in exploring products for repellency activity. In view of this, the studies of biologically active plant materials with repellency properties have gained noticeable interest among researchers [1-3]. Among biologically active plant materials for repellency properties, essential oils always to be one of the main choices, mainly due to the nature of specific chemical constituents in the oils such as the monoterpenes. Monoterpenes are naturally

occurring compounds produced widespread in plants and often producing odour. They are of commercial interest as they are commonly used in food, food flavourings, beverages, perfumes and cosmetic and in many cleaning products. One of the most popular insecticidal monoterpene was pyrethroids from *Chrysanthemum* species. It is a desirable pesticide with low environmental persistence and low mammalian toxicity [4].

Monoterpenes compound selected in this study, i.e., citronellal, citronellol, geraniol and limonene were important compounds that can be found abundantly in a few plant species such as in *Thymus sipylues* [5], *Eucalyptus citriodora* [6] and *Xanthoxylum acanthopodium* [7]. Other than the property studied, the monoterpenes also showed termiticidal activity [8], antitumour activities [9] and can be acted as agent for cancer chemoprevention [10]. The aim of the study was to determine the repellency properties of four monoterpenes standard compounds after tested individually against female mosquito *Aedes aegypti*. Standard repellent, deet was also tested against the mosquito to compare their repellency properties.

MATERIALS AND METHOD

Standard compounds

The standard compounds were purchased from different company, i.e., citronellal (ICN Pharmaceuticals, Inc.), citronellal (Sigma), geraniol (Fluka) and limonene (Fluka). The compounds then were further diluted with ethanol (95% v/v) to the appropriate concentration needed.

Repellency Assessment

Mosquito repellency 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 [11] with slight modification. The test procedure was similar to that described by previous researchers [12, 13]. The flexor regions of the forearms of 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 ethanol (0.0006 – 0.0379 mg cm²) and 0.025 ml of the diluent was applied randomly to the marked areas of first, second, fourth and fifth circles. Ethanol 95%

was applied at the middle, which was the third circle as the control test. DEET (diethyl-m-toluamide) was used as a standard repellent. 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. Fifteen female mosquitoes, three to five days old were introduced into each cage and the number of biting was recorded at the end of 120 sec. The test procedure was replicated three times for each oil sample and statistically reliable estimates of their median effective concentrations (EC₅₀) were obtained by probit analysis program [14]. Percentage repellency was determined by the formula described [15]:

$$\text{Repellency} = 100 - \left[\frac{T}{C} \times 100 \right]$$

Where T is the total number of bites on treatment area and C is the total number of bites on control area.

RESULTS AND DISCUSSION

Table 1 shows the % repellency of the standard compounds and standard repellent, deet toward *Aedes aegypti* at various concentrations. The compounds showed significant degree of repellency, with comparable values than the standard repellent, deet. Complete protection was achieved with citronellol, citronellal and geraniol at 0.04 mg cm⁻², 0.02 mg cm⁻² and 0.04 mg cm⁻² concentration, respectively. Limonene at 0.04 mg cm⁻² concentration only provided 85.7% repellency against mosquito bites. Deet gave complete protection at 0.0025 mg cm⁻² concentration. Table 2 shows the repellency effect of the standard compounds and standard repellent, deet against the *Aedes aegypti* mosquito with median effective concentration (EC₅₀) ranging from 0.00011 to 0.00268 mg cm⁻². Amongst these, citronellol with EC₅₀ of 0.00011 mg cm⁻² was the most effective, comparable to the value of the standard repellent, deet (0.00050 mg cm⁻²). The repellency activity values were followed by geraniol (0.00018 mg cm⁻²), citronellal (0.00025 mg cm⁻²) and limonene (0.00268 mg cm⁻²).

From this result we suggested that these compound might responsible for the repellency activity of the essential oils in the plants as in our previous study [16], which showed that

Cymbopogon nardus essential oil that rich with monoterpene compounds gave the most effective repellency activity with values of 0.0009 mg cm⁻². These compounds also may contribute in the knockdown effect of the *C. nardus* as showed in our previous study in evaluation of smoke from mosquito coils [17]. The effect of a repellent heavily depends on its uniform distribution over the surface being treated, and this can be best achieved with repellents recommended type such as in aerosol form [18]. In another study, scientists suggested that vanillin formulated in the essential oils could improve the repellency efficacy [3]. Although deet has been recommended as one of the personal protection over four decades, they also showed serious adverse effect especially in infants and young

children [19]. We suggested that plant that rich with the monoterpenes should be the alternative in the development of the insect repellent.

CONCLUSION

The significant results obtained in these study suggest that essential oils from plant species, which have these four monoterpenes abundantly, may also have the repellency properties and the potential to be developed into commercial repellents in various forms. However, the residual lifespan as well as their performance under field conditions and the full extent of the repellency activities need to be determined.

Table 1. Percentage repellency of standard compounds and deet toward *Aedes aegypti*

Concentration mg cm ⁻²	citronellal (%)	citronellol (%)	geraniol (%)	limonene (%)	Deet (%)
0.0800	100	100	100	85.7	100
0.0400	100	100	100	78.6	100
0.0200	95.8	100	95.8	64.3	100
0.0100	87.5	91.7	95.8	64.3	100
0.0050	83.3	87.5	95.8	63.6	100
0.0025	83.3	87.5	87.5	54.5	100
0.0012	75.0	87.5	82.1	54.5	83.3
0.0008	-	75.0	-	-	-
0.0006	-	-	71.4	27.3	50
0.0003	-	-	71.4	-	33.3
Control	0	0	0	0	0

Table 2. Median effective concentrations (EC₅₀) of standard compounds and deet against *Aedes aegypti*

Compound	EC ₅₀ (mg cm ⁻²)	95% CI	Slope ± SE
citronellal	0.00025	0.00006 – 0.00055	0.89 ± 0.15
citronellol	0.00011	0.00002 – 0.00026	0.86 ± 0.16
geraniol	0.00018	0.00006 – 0.00033	1.06 ± 0.17
limonene	0.00268	0.00165 – 0.00392	0.69 ± 0.08
Deet	0.00050	0.00027 – 0.00091	2.75 ± 0.54

Note: EC₅₀=Median effective concentration; 95% CI= 95% Confidence Interval; SE=Standard Error

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