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Short Communication

Repellency Effects of Essential Oils of *Cymbopogon winterianus*, *Eucalyptus globulus*, *Citrus hystrix* and their major Constituents against Adult German Cockroach (*Blattella germanica Linnaeus* (Blattaria: Blattellidae)

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Abstract

The German cockroach, *Blattella germanica*, has been considered a major source of allergens and pathogens. The application of repellents has received attention to keep these insects away from their hiding places such as kitchen cupboards. In this study, the repellent potency of essential oils (EOs) and the major components of *Cymbopogon winterianus*, *Eucalyptus globulus* and *Citrus hystrix* against adult German cockroach were assessed. The chemical compositions of EOs were investigated using the Gas chromatography-Mass spectrometry. The results demonstrated that all investigated EOs and their active monoterpenes including 1,8-cineol, citronellal, citronellol, geraniol, limonene and linalyl acetate, exhibited repellency effects against the insect. As a result, pure monoterpenes can also be considered as alternative repellents, as the standardization of the products is easier to perform. For further studies, active monoterpenes or combinations of EOs will be developed as products and their efficacy will be compared with commonly used insect repellents.

Keywords: Blattella germanica, Citrus hystrix, Cymbopogon winterianus, Eucalyptus globulus, German cockroach, Repellent

1. Introduction

The German cockroach, *Blattella germanica* (L.), is the most common indoor species found in houses, hospitals, and food processing facilities. It is considered as a potential vector of medically important pathogenic microorganisms (Menasria *et al*., 2014; Nasirian, 2017), and an important source of potent allergens causing asthma and other allergic diseases (Arlian, 2002). Their allergens are associated with fecal material, saliva, secretions, exoskeletons, and dead bodies (Arruda and Chapman, 2001). The allergens are found throughout the house with the highest levels in the kitchen (Arruda *et al*., 2001). As a result, pest management products that are safe to human health and environment-friendly are urgently needed.

Several insecticides, including organophosphates, carbamates, and pyrethroids have been used for the control of the German cockroach. However, the repeated use of these synthetic chemicals has resulted in the development of resistance which can affect human health and lead to environmental concerns. The development of alternatives to replace synthetic pesticides is therefore essential (Chang *et al*., 2010; Yeom *et al*., 2018). The application of repellents to keep the insects away from certain hiding places such as kitchen cupboards, food and beverage containers has received attention recently (Oz *et al*., 2013; Thavara *et al*., 2007). Several essential oils (EOs) and their pure constituents have been reported for their repellency activities and toxicities against many insects including the German cockroach (Jannatan *et al*., 2017; Lee *et al*., 2017; Yeom *et al*., 2015). Moreover, EOs and their constituents frequently show high volatility and can easily be decomposed after exposure to heat, humidity, light, and oxygen. Thus, there is little concern about their residue problems (Isman, 2006; Turek and Stintzing, 2013). However, the application of EOs to control cockroach is restricted by the difficulty of standardization of the active compounds (Rguez *et al*., 2018). This is because the chemical compositions of EOs depend on the harvest season, handling, and extraction processes, which may considerably affect their activities (Do *et al*., 2015). In order to obtain consistent activity, the active markers of EOs should be identified and quality controlled.

In general, the evaluations of toxicity of EOs and their isolated constituents on insects usually focus on acute fumigant and contact toxicity. Thus, the death of the insects has been used as the endpoint of the toxicity studies. The results could be useful for the development of insecticidal products (Yeom *et al*., 2015; Yeom *et al*. 2018). However, the information relating to their repellent potency is limited. The active substances of any repellent

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products should be able to deter, locally or from a distance, and prevent arthropods from getting in contact with the treated areas (Nerio *et al*., 2010).

In this study, the repellent activity of EOs of citronella (*Cymbopogon winterianus*), eucalyptus (*Eucalyptus globulus*) and kaffir lime (*Citrus hystrix*) is investigated against the adult German cockroach. The major constituents are identified and the repellent activity against the cockroach is assessed.

2. Materials and Methods

2.1. Chemicals

Standards of citronellal (≥95%), citronellol (≥95%), geraniol (≥98%), 1,8-cineol (≥99%) limonene (≥95%), and linalyl acetate (≥97%) were purchased from Sigma Aldrich (St. Louis, MO). EOs of citronella, eucalyptus and kaffir lime were obtained from Hong Huat Co., Ltd, Bangkok, Thailand. GC‐MS grade Hexane was supplied by Honeywell, Ulsan, Korea.

2.2. Insects

The German cockroaches were reared at the National Institute of Health (NIH) of Thailand without exposure to any insecticide. The cockroaches were provided with water and dried mouse food ad libitum. The insects were maintained at 30 ± 2 °C and 60 ± 5% RH under a 12:12 hour light:dark cycle.

2.3. GC-MS Analysis

The analytical methods of EOs of citronella, eucalyptus, and kaffir lime were modified from the studies of Silva *et al* (2016), Sebei *et al* (2015) and Warsito *et al* (2017), respectively. The GC-MS analysis was carried out using an Agilent Technology 6890 gas chromatograph coupled with a 5973 mass spectrometer. The carrier gas was ultra-high purity helium (99.999%), at a flow rate of 1.0 mL/min. The separation of the citronella oil was achieved on an INNOWAX capillary column (0.25 mm ID x 30 m, 0.25 µm film thickness). The sample injection was performed in pulse split mode (1:20). The oven temperature was programmed at 50 °C for three minutes, then 2 °C/min to 230 °C. For the eucalyptus and kaffir lime oils; the separation was performed on a HP-5MS capillary column (0.25 mm ID x 30 m, 0.25 µm film thickness). The sample injection was carried out in the split mode (1/50). Initial oven temperature was set at 50 °C for one minute, then increased by 4 °C/min to 220 °C. The temperature of the injection port, transfer line, and ion source were set at 230, 275 and 200 °C, respectively. The ion energy of electron impact ionization was 70 eV. The MS was operated in the scan mode with an acquisition range of 40 - 400 m/z. Volatile constituents were identified by comparing their mass spectra and retention index with those of the WILEY 7 and Adams 2001 libraries, and of the instrument's internal library creased from the previous studies. Each essential oil was diluted with hexane to obtain a final concentration of 1 µg/mL. The solution (2 µL) was injected into the GC-MS system.

2.4. Repellent Activity

This study was approved by the Institutional Animal Care and Use Committee of Mahidol University, Thailand (COA. No. MU-IACUC 2018/002). The experimental procedures were performed according to the study of Thavara *et al* (2007) which was the standard operation protocol of the NIH of Thailand. This method was employed for the registration of cockroach repellent products in Thailand.

The internal side walls of stainless-steel boxes (50 x 50 x 10 cm each) were greased with vaseline to prevent the escape of the insects. A filter paper (Whatman No.1, 50 x 50 cm) was divided in two halves which were placed at the bottom of the box. One of the halves was treated with an investigating compound corresponding to 10 ml/m^2, and the other was untreated (control). Ten adult male and female German cockroaches (aged 6-8 weeks) were shortly anesthetized (less than one minute) with CO_2 gas, and were placed at the center of each box. Water-soaked cotton wool and mouse food were provided in each area. The box was placed at the center of the Peet Grady Chamber (180 x 180 x 180 cm) which was kept in a dark and isolated environment to prevent disturbances from the surroundings. The experimental conditions were maintained at 30 ± 2 °C, 60 ± 5% RH, and under a twenty-four-hour dark cycle. The numbers of cockroaches in the treated and control areas were carefully observed at forty-eight hours after treatment. Each treatment was carried out in triplicate. The repellent rate was calculated according to the following equation:

Repellent rate (%) = U/(T+U) x 100

where U and T are the numbers of cockroaches in the untreated (control) and treated area, respectively. The oil and pure substance were considered to possess the repellent activity when the repellent rate was more than 80% (Thavara *et al*., 2007).

The repellent activity of EOs was investigated according to the above mentioned experimental procedure. Then, the pure major components of EOs were selected to investigate their repellent efficacy in order to identify the major constituents which contribute to the repellent activity.

2.5. Statistical Analysis

The mean ± SE values were reported. The data were subjected to one-way analysis of variance (ANOVA) followed by least significant difference (LSD) analysis using SPSS version 16 (SPSS Inc., Chicago, USA). Differences were considered significant at a value of $P<0.05$.

3. Results and Discussion

3.1. Chemical Constituents of Essential Oils

The GC-MS chromatograms and chemical constituents of EOs of citronella, eucalyptus, and kaffir lime oils are shown in Figure 1 and Table 1, respectively. The main constituents of the citronella oil were citronellal (39.43%), geraniol (17.66%) and citronellol (11.50%). For the eucalyptus oil, the major constituent was 1,8-cineol (80.20%) and the minor one was limonene (8.22%). The major compositions of the kaffir lime oil were linalyl acetate (38.49%) and limonene (32.92%) (Table1). The identified major compounds of EOs of citronella, eucalyptus and kaffir lime are in accordance with the previous studies of Silva *et al* (2016), Sebei *et al* (2015), Dosoky and Setzer (2018), respectively.

It is worth noting that there is a great variation in the chemical composition of EOs due to differences in genetic backgrounds, ripening stage, season, extraction method, etc. (Sebei *et al*., 2015; Warsito *et al*., 2017). This variation can also affect the biological activities of EOs (Rguez *et al*., 2018). Thus, it is necessary to standardize the EOs for practical use.

Table 1. Chemical constituents of *C. winterianus*, *E. globulus* and *C. hystrix* oils.

No.	Composition	Retention time (min)	%Relative area
Citronella oil			
1	Limonene	6.52	3.26
2	Citronellal	14.37	39.43
3	Isopulegon	16.90	3.21
4	β-Elemene	17.38	2.06
5	Citronellyl acetate	19.62	2.30
6	α-Amorphene	20.66	1.38
7	δ-Cadinene	22.05	2.84
8	Citronellol	22.52	11.50
9	Geraniol	24.69	17.66
10	α-Cubebene	29.58	1.83
11	Elemol	30.37	3.65
Eucalyptus oil			
1	α-Pinene	6.35	0.87
2	β-Pinene	7.56	0.18
3	β-Myrcene	7.97	0.34
4	l-Phellandrene	8.37	0.49
5	α-Terpinene	8.76	0.16
6	m-Cymene	9.03	4.20
7	Limonene	9.15	8.22
8	cis-Ocimene	9.50	0.23
9	1,8-Cineole	9.67	80.20
10	trans-Ocimene	9.77	0.13
11	γ-Terpinene	10.11	4.59
12	α-Terpinolene	11.07	0.27
Kaffir lime oil			
1	α-Pinene	6.34	0.75
2	Sabinene	7.46	0.85
3	β-Pinene	7.55	5.72
4	β-Myrcene	7.95	0.54
5	m-Cymene	9.01	0.51
6	Limonene	9.17	32.92
7	γ-Terpinene	10.09	6.43
8	Linalool	11.77	11.67
9	Linalyl acetate	17.04	38.49

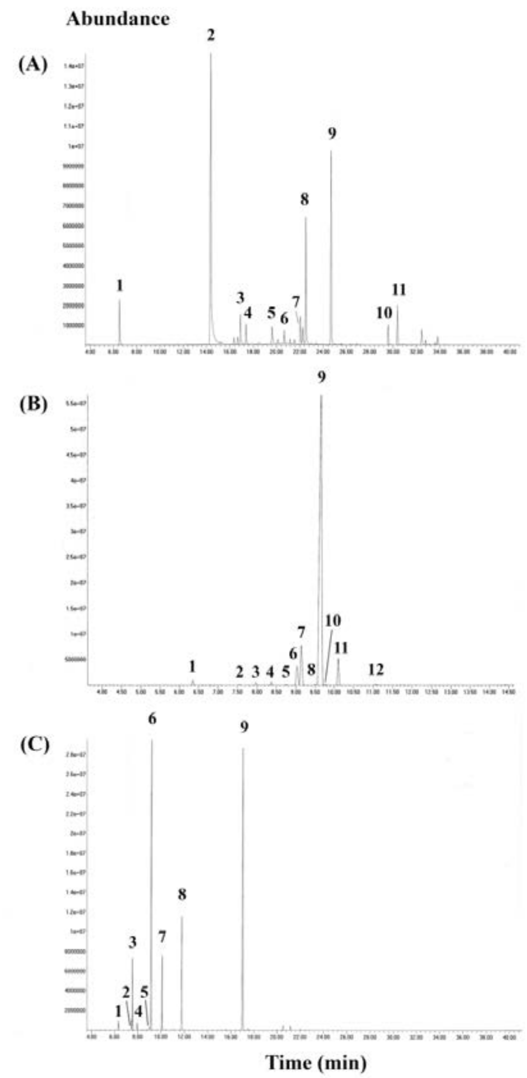

Figure 1. GC-MS Chromatogram of EOs of A. Citronella oil; (1) Limonene, (2) Citronellal, (3) Isopulegon, (4) β-Elemene, (5) Citronellyl acetate, (6) α-Amorphene, (7) δ-Cadinene, (8) Citronellol, (9) Geraniol, (10) α-Cubebene, and (11) Elemol, B. Eucalyptus oil; (1) α-Pinene, (2) β-Pinene, (3) β-Myrcene, (4) l-Phellandrene, (5) α-Terpinene, (6) m-Cymene, (7) Limonene, (8) cis-Ocimene, (9) 1,8-Cineole, (10) trans-Ocimene, (11) γ-Terpinene and (12) α-Terpinolene, C. Kaffir lime oil; (1) α-Pinene, (2) Sabinene, (3) β-Pinene, (4) β-Myrcene, (5) m-Cymene, (6) Limonene, (7) γ-Terpinene, (8) Linalool and (9) Linalyl acetate.

3.2. Repellent Potency of Oils and their Major Constituents

As shown in Table 2, the results demonstrated that the repellency rates of all investigated EOs and pure monoterpenes were more than 80% implying that these compounds possess a repellent activity against German cockroaches (Thavara *et al*., 2007). Although the activity of the tested EOs on the German cockroaches were not significantly different, (P>0.05), against one another, it was observed that the kaffir lime oil exhibited the highest repellency (93.33%), while the efficacy of citronella oil and eucalyptus oil were the same (85.00%).

For the activity of monoterpenes, it was found that the potency of citronellol > geraniol > citronellal > 1,8-cineol > linalyl acetate> limonene. Amongst these monoterpenes, the repellency of citronellol and geraniol were not significantly different from citronellal (*P*>0.05), but both were significantly higher than the repellency of 1,8-cineol, linalyl acetate and limonene (*P*<0.05). More interestingly, the results also demonstrated that the activities of citronellol and geraniol were significantly higher than those of the eucalyptus and citronella oils (*P*<0.05).

Table 2. Repellent rate of essential oils and pure major constituents.

Material	Repellent rate (%)[1]
Essential oil	
Citronella oil	85.00 ± 4.71 a[2]
Eucalyptus oil	85.00 ± 4.08 a
Kaffir lime oil	93.33 ± 1.36 abc
Monoterpenes	
1,8-Cineol	86.67 ± 1.36 a
Citronellal	91.67 ± 2.72 ab
Citronellol	96.67 ± 1.36 bc
Geraniol	95.00 ± 2.36 bc
Limonene	81.67 ± 1.36 d
Linalyl acetate	83.33 ± 1.36 d

[1] Data was expressed as mean ± SE; *n*=3

[2] Means within a column followed by the same lowercase letter are not significantly different using least significant difference multiple range test

From the previous study, the repellent activity of the kaffir lime oil against German cockroaches has been reported, but the chemical composition of the oil and the activities of its pure major constituents have not been investigated (Thavara *et al*., 2007). In addition, fumigant and contact toxicities of EOs of citronella and eucalyptus, and their active monoterpenes including 1,8-cineol, citronellal, citronellol, geraniol, limonene, and linalyl acetate, against the German cockroach have been reported (Alzogaray *et al*., 2011; Jannatan *et al*., 2017; Jang *et al*., 2005; Oz *et al*., 2013). Nevertheless, their repellent efficacy have not yet been investigated and compared. According to the toxicity studies, the investigated compounds were diluted with organic solvents and the experiments were performed in a close system with a volume less than 1.5 L. The death of the insects was considered as the endpoint of the studies (Yeom *et al*., 2015; Yeom *et al*. 2018). The results can be applied for the development of insecticidal products, but could not be applied to the repellent products.

In the view of the repellent products, the active compounds produce a vapor barrier that has an offensive smell or taste to insect, therefore, the products can be able to deter and prevent arthropods from getting in contact with the treated areas (Nerio *et al*., 2010). The repellent dose may be different from the insecticidal purpose. To obtain results correlating to the activity, our investigations were performed in the Peet Grady Chamber with the volume approximately of 5.83 m^3, and the endpoint of study was forty-eight hours after treatment. The results of this study demonstrate that plants EOs and their isolated compounds could be considered as potential repellents for the control of German cockroach. Nevertheless, it should be noted that natural products may cause irritation in some cases (Trumble, 2002). In addition, since all investigated monoterpenes demonstrated a repellent activity against the insect, these compounds could be utilized as active markers for establishing the quality specifications of the EOs and repellent products. Although the activity of EOs is generally attributed to some particular compounds, minor constituents may also contribute to the activity (Nerio *et al*., 2010) and act as synergists, enhancing the potency of major components (Regnault-Roger *et al*., 2012). Therefore, more researches on the functionality of the other compounds presented in the EOs need to be conducted.

4. Conclusions

This study identifies the active components of citronella, eucalyptus, and kaffir lime oils and demonstrates their repellent activities against adult German cockroaches. The EOs, pure active monoterpenes and/or their combinations could be considered as alternative repellents for cockroach control. For further studies, these EOs, pure monoterpenes and/or their combinations will be developed as repellent products and their repellency will be compared to the commonly used insect repellents such as N,N-diethyl-m-toluamide (DEET).

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Conflicts of Interest

The authors declare no conflict of interest.

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