JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 11127–11133 11127
DOI:10.1021/jf9024486

Repellency of Essential Oils of *Cryptomeria japonica* (Pinaceae) against Adults of the Mosquitoes *Aedes aegypti* and *Aedes albopictus* (Diptera:Culicidae)

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The purpose of this study was to investigate the repellent activities of essential oils from *Cryptomeria japonica* (sugi) against adults of mosquitoes *Aedes aegypti* and *Aedes albopictus*. Comparison of essential oils from four different plant parts of *C. japonica* revealed that essential oil from its leaf exhibited the best repellent activity against mosquitoes. To understand the relationship between volatile organic compounds and repellent activity, the solid-phase microextraction (SPME) method was employed to analyze volatile organic compounds of leaf essential oil. The SPME fiber was coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS). The major volatile organic compounds in the cage were 3-carene, α-terpinene, limonene, γ-terpinene, and terpinolene at 0 min. Results demonstrated that (−)-terpinen-4-ol was the major volatile organic compound adsorbed by SPME fiber during repellent assays. Furthermore, the repellent activities of six compounds against adults of the mosquitoes were evaluated, and the results revealed that (−)-terpinen-4-ol exhibited the best repellent activity against *A. aegypti* and *A. albopictus*.

KEYWORDS: *Aedes aegypti*; *Aedes albopictus*; *Cryptomeria japonica*; essential oil; mosquito; repellency; SPME

INTRODUCTION

There are more than one million known animals in the world, of which 80% are arthropods, and insects account for 90% of all arthropods. Some insects can decrease the productivity of field workers because of the many distractions caused by the biting insects (*1*). Some species even spread dreadful diseases and have great effects on the health of human beings. Insect vectors, especially mosquitoes, are responsible for the spread of serious human diseases such as malaria, Japanese encephalitis, yellow fever, and filariasis as well as dengue (*2*). Dengue is prevalent in more than 100 countries and threatens the health of approximately 2.5 billion people. Around 80 million people are infected annually at an attack rate of 4% worldwide (*3*). The distribution and abundance of these diseases are strongly influenced by the presence of humans and the level of poverty (*4*). For dengue, there is no vaccine to prevent the infection, nor are there drugs to combat these diseases in infected persons, so vector control is the most commonly chosen solution available for reducing morbidity. *Aedes aegypti* and *Aedes albopictus*, the yellow fever mosquito, are also well-known vectors of dengue in Taiwan.

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Personal protection is one of the approaches to preventing mosquito bites. Apart from mosquito nets, the repellent plays an important role in protection against arthropods, because they can be used anywhere and anytime. When properly used, they are reported to reduce disease transmission (*1*). Current control involves mainly the use of synthetic repellents, which have a potential toxic effect on public health and the environment. Repeated use of synthetic repellents has disrupted natural biological systems and often resulted in the development of resistance (*2*, *5*). The phytochemicals derived from plant sources possess a complex of chemicals with unique biological activity (*6*). Over 2000 plant species contain chemicals with pest control properties (*7*); some of these plants have repellent activities against mosquitoes (*8*, *9*). Plants could be an alternative source of mosquito repellents because they constitute a potential source of bioactive chemicals and are typically free from harmful effects (*10*). Because of this, much interest has been focused on plant extracts or essential oils as potential mosquito repellent agents (*11*).

Natural repellents have been used in different communities for a long time and as the basis for most of the commercial repellents (*12*). Several phytochemicals extracted from various botanical sources have been reported to have detrimental effects on mosquitoes (*13*). Ansari et al. (*14*) evaluated the repellent

Published on Web 11/10/2009

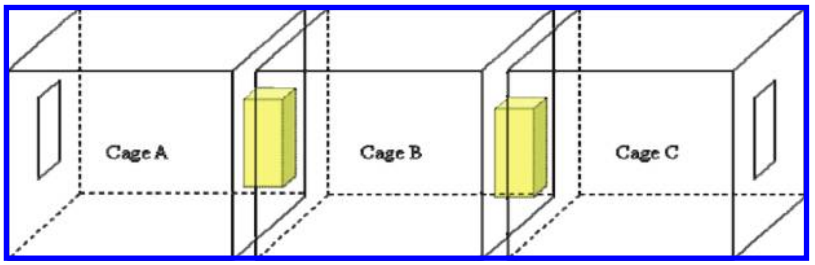

Figure 1. Apparatus for mosquito repellent assay: cage A (left), filter paper impregnated with ethanol; cage B (middle), mosquitoes only; cage C (right), filter paper impregnated with essential oil or compound. The top cover of the cage can be flipped open, and there are two extra doors on both sides. The connection between two cages was via the tunnel (yellow). Mosquitoes and filter papers were introduced from the door and the cover, respectively. The numbers of mosquitoes moving into each cage were recorded at 10 min intervals for 60 min.

activities of *Mentha piperita* (Lamiaceae) toward three mosquito species for 12 h. The results showed that the essential oil of *M. piperita* was highly effective as a repellent and gave 100, 92.3, and 84.5% protection against *Anopheles annularis*, *Anopheles culicifacies*, and *Culex quinquefasciatus*, respectively. Venkatachalam and Jebanesan (*15*) found that *Ferronia elephantum* (Rutaceae) leaf extract provided protection against *A. aegypti*. Gillij et al. (*16*) reported that some essential oils of aromatic plants grown in Argentina showed repellent activity against *A. aegypti*. At concentrations of 12.5%, *Baccharis spartioides* and *Aloysia citriodora* showed the longest repellent time (90 min).

Cryptomeria japonica, one of the important plantation tree species, is a widely distributed conifer in Taiwan (*17*−*19*). The woods of *C. japonica*, used as raw materials for the production of furniture and other wooden structures, are of different colors, including red, yellow, and black. They are of different chemical compositions and have different biological activities. In previous investigations, leaf essential oil and sapwood methanolic extract from red heartwood-type *C. japonica* were found to have excellent mosquito larvicidal activities (*20*, *21*). However, there are no references yet on the repellent activities of *C. japonica* against adults of mosquito. The purpose of this study is to evaluate the repellent activities of essential oil from *C. japonica* against adults of *A. aegypti* and *A. albopictus*.

MATERIALS AND METHODS

Mosquitoes. Cultures of *A. aegypti* and *A. albopictus* from the Kaohsiung strain were maintained in the laboratory without exposure to any insecticide. They were maintained at 27 °C and 60−70% relative humidity, under a 12:12 h light/dark cycle. Adult mosquitoes were fed a 10% sucrose solution and reared in the Department of Parasitology, Chang-Gung University.

Constituents of Essential Oil. The following compounds of essential oil constituents were purchased from Acros (Geel, Belgium): α-terpinene, 3-carene, γ-terpinene, *p*-cymene, limonene, (−)-terpinen-4-ol, (−)-α-terpineol, bornyl acetate, α-terpinyl acetate, sabinene, linalool, α-pinene, and β-pinene. β-Myrcene, terpinolene, and camphene were purchased from Sigma (St. Louis, MO), TCI (Japan), and ICN (Switzerland), respectively. β-Elemol, γ-eudesmol, and *ent*-kaur-16-ene were isolated from *C. japonica* leaf essential oil.

Essential Oil Distillation. Essential oils of different plant parts (leaf, twig, wood, and bark) from a 43-year-old black heartwood-type Japanese cedar (*C. japonica* D. Don) were extracted by hydrodistillation for 6 h. Materials were collected in February 2005 from the Experimental Forest of National Taiwan University located in Nantou County in central Taiwan. Voucher specimens (CJL005, CJT005, CJW005, and CJB005) have been deposited at the Laboratory of Wood Chemistry (School of Forestry and Resource Conservation, National Taiwan University).

Repellent Assay. Essential oils from leaf, twig, wood, and bark of *C. japonica* and six pure constituents [3-carene, α-terpinene, limonene, γ-terpinene, terpinolene, and (−)-terpinen-4-ol] were tested for their repellency against *A. aegypti* and *A. albopictus*. They were dissolved in ethanol to the desired concentrations. The modified method of Kim et al. (*22*) was employed to evaluate the repellency of the test materials against mosquitoes. A repellent apparatus test was carried out using a series of three connected cages (25 × 25 × 25 cm^3) (**Figure 1**). Two cages, cages A and C, positioned at either side of the central cage B, were screened with a wire (15 × 15 cm^2) to allow inflow of air. Each bioassay was conducted between 12:00 noon and 6:00 p.m. at 27 (±1) °C and 60−70% relative humidity. Briefly, essential oil (30 mg) in ethanol (300 μL) was applied to filter papers (7 cm in diameter), giving the equivalent of 1.92 μg/cm^3. The filter papers used in the control experiment were dispensed with ethanol (300 μL) only. Solvent was allowed to evaporate at room temperature for 2 min before the test. A repellent apparatus was switched on for 10 min after the treated paper was placed in cage C, which was connected to the right of the central cage. Fifty adult mosquitoes (7−12 days old) were released into the central cage B. Cage A had an ethanol-treated filter paper (control). The numbers of mosquitoes occupying each of the cages A and C were recorded at 10 min intervals for 60 min. DEET (purity = 98%), the commercial repellent purchased from Acros, served as a positive control for comparison. After completion of each bioassay, the cages were washed with 70% ethanol and allowed to dry properly. All bioassays were replicated five times. The mean percentage of distribution (%D) and percentage of repellency (%R) were calculated to evaluate the repellent activity of *C. japonica*. The formulas were

$$\%D = (T/50) \times 100$$

$$\%R = [1 - (T_a/(T_a + T_b))] \times 100$$

where T is the number of mosquitoes distributed in each cage, T_a is the number of mosquitoes distributed in treated cage C, and T_b is the number of mosquitoes distributed in control cage A.

Selection of SPME. Preparatory experiments were carried out to determine some of the operational parameters to be adopted throughout this research, such as the type of solid-phase microextraction (SPME) fiber to be employed, optimum adsorption time, and desorption time. These experiments were performed in the repellent apparatus. Leaf essential oil (30 mg) dissolved in ethanol (300 μL) was applied to filter papers (7 cm in diameter). The treated paper was placed in cage C, and the solvent was allowed to evaporate for 10 min. The SPME fiber was inserted into the cage containing essential oils or compounds. Four fibers with different coating materials, including 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 75 μm carboxen/polydimethylsiloxane (CAR/PDMS), and 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), were used. All fibers were purchased from Supelco (Bellefonte, PA) and conditioned in advance according to the manufacturer's instructions. A three-component fiber (CAR/PDMS/DVB) was found to be the most effective for this application. The optimum adsorption time and desorption time were both 5 min.

Collection of Volatiles from Essential Oil. The SPME technique was employed to collect volatiles released from the essential oils. An aliquot (300 μL) of the test solution was dispensed over a filter paper (7 cm in diameter). Control filter papers were dispensed with 300 μL of

ethanol only. The treated paper was placed in cage C, and the solvent was allowed to evaporate for 10 min. Volatiles emitted from leaf oil in the cage were collected using SPME at different times (0, 20, 40, and 60 min) during the repellent assay. The SPME fiber was inserted into the cage containing essential oils or compounds, followed by adsorption for 5 min. After sampling, the SPME fiber was immediately inserted into the GC injector for desorption. The relative quantities of the volatile compounds were obtained by integrating the peak area of the spectrograms.

Gas Chromatography−Flame Ionization Detector (GC-FID). The oil constituents were analyzed using a gas chromatograph (Trace-GC-Ultra, Thermo, Austin, TX) with a FID detector. A DB-5MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness) was used for separating the constituents. The split ratio was 1:10. Helium was used as the carrier gas, and the flow rate was maintained at 1 mL/min. The oil sample was dissolved in ethyl acetate at a concentration of 1 ppm, and 1 μL was injected into the GC for analysis.

Two temperature programs were employed to analyze the constituents of essential oil and volatiles extracted by SPME, respectively. One temperature program involved first setting the injector temperature to be 270 °C. The temperature was programmed from 60 to 280 °C at 5 °C/min and held at the final temperature (280 °C) for 5 min. The oil sample was dissolved in ethyl acetate at a concentration of 1 ppm, and 1 μL was injected into the GC. The other temperature program involved inserting the SPME fiber into the GC injector for desorption at 250 °C for 5 min. The GC oven temperature program included an initial holding at 50 °C for 2 min, increasing the temperature to 130 at 5 °C/min and then to the final temperature of 250 at 20 °C/min, and eventually holding at 250 °C for 5 min.

Gas Chromatography−Mass Spectroscopy (GC-MS). Constituents of plant oils were determined using a Trace GC chromatography, coupled with a PoLaris Q mass instrument (Thermo) and equipped with a 30 m × 0.25 mm DB-5MS capillary column with a film thickness of 0.25 μm. Helium was used as the carrier gas and maintained at 1 mL/min. The effluent of the capillary column was introduced directly into the ion source of the mass spectrometer. The ion trap mass spectrometer was operated in the electron-impact mode, with an ionization energy of 70 eV. The sector mass analyzer was set to scan from 50 to 650 amu. Components of essential oils were identified by comparison of mass spectrum from the Wiley library and confirmed by Kovats retention index and the authentic compounds [including α- and β-pinene, α- and γ-terpinene, camphene, sabinene, β-myrcene, 3-carene, *p*-cymene, limonene, terpinolene, linalool, (−)-terpinen-4-ol, α-terpineol, bornyl acetate, α-terpinyl acetate, β-elemol, γ-eudesmol, and *ent*-kaur-16-ene].

Statistical Analysis. Results are all shown as mean ± SD (n = 5). The significance of difference among individual mean was determined by Scheffe test in SAS (Statistical Analysis System) software. Results with $p < 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Repellency of Essential Oils from Different Parts against Mosquitoes. The repellency of essential oils of *C. japonica* leaf, twig, wood, and bark against *A. aegypti* and *A. albopictus* adults was evaluated by the repellent apparatus at 10 min intervals during a 60 min period. Results of the repellent assay showed that leaf essential oil offered the best repellency (**Figure 2**). After 20 min of treatment, leaf essential oil showed 81.0 and 71.3% repellency against *A. aegypti* and *A. albopictus*, respectively, whereas DEET showed only 55.0 and 63.0% repellency against *A. aegypti* and *A. albopictus*, respectively. After a 60 min exposure, leaf essential oil still showed strong repellent activity, giving 72.0 and 73.3% repellency against *A. aegypti* and *A. albopictus*, respectively, whereas the repellency of DEET against *A. aegypti* and *A. albopictus* reached 62.0 and 60.0%, respectively. Comparisons of these data revealed that the leaf essential oil of *C. japonica* exhibited more prominent repellent activity than DEET.

Furthermore, we analyzed the percentage of mosquito distribution in the three different cages to understand the effect of leaf essential oil on mosquitoes (**Table 1**). After a 20 min exposure, the

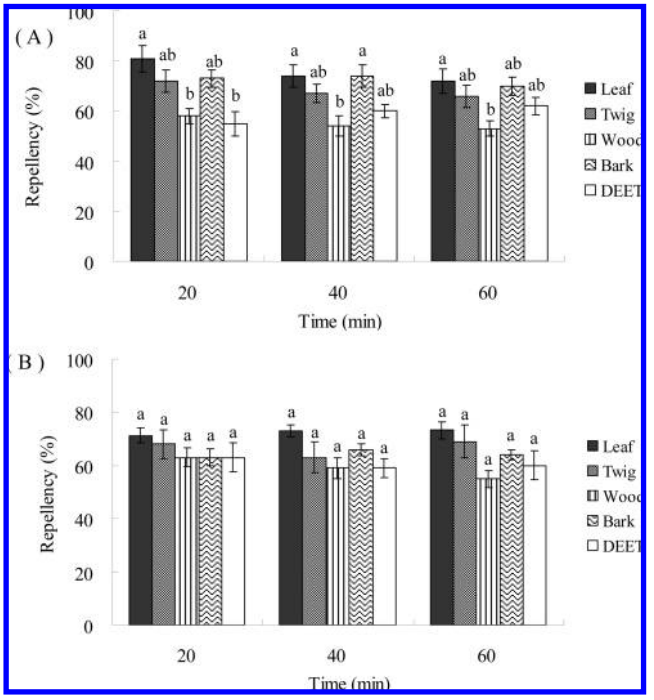

Figure 2. Repellency of four plant parts of essential oils from *Cryptomeria japonica* against *Aedes aegypti* (**A**) and *Aedes albopictus* (**B**) at 1.92 μg/cm^3 concentration. Bars marked by different letters are significantly different at the level of $p < 0.05$ according to the Scheffe test.

percentage of *A. aegypti* distribution was significantly lower in cage C, which contains a leaf essential oil-treated filter paper (D = 7.1%), than in cage A, which contains an ethanol-treated filter paper (D = 28.3%). When the exposure time reached 60 min, the percentage of *A. aegypti* distribution was 15.9% in cage C and 40.8% in cage A. The same trend was also observed for repellent activities against *A. albopictus* (**Table 2**). Thus, leaf essential oil also showed a strong repellent activity against *A. albopictus*. The percentages of mosquito distribution in cages C and A were 17.2 and 36.0%, respectively, after 20 min of exposure. When the exposure time reached 60 min, the percentage of *A. albopictus* distribution was still lower in cage C (D = 19.6%) than in cage A (D = 48.8%). Nevertheless, the numbers of mosquitoes in leaf essential oil-treated cages increased gradually as time elapsed.

Volatile Compounds of Leaf Essential Oil from *C. japonica*. Composition of essential oil from *C. japonica* leaf extracted by hydrodistillation was determined by gas chromatography. In addition, the SPME technique was employed to collect volatiles released into the cage containing the leaf essential oil-treated filter paper. The quantity of each major constituent was determined under identical GC-FID conditions. The constituents of leaf essential oil identified by both hydrodistillation and SPME are shown in **Table 3**. A total of 15 compounds and 4 types of terpenoids were identified in leaf essential oil of *C. japonica* by GC-MS. The major terpenoid of leaf essential oil was monoterpene hydrocarbons (46.87%). Other terpenoids of leaf essential oil were oxygenated diterpenes (21.74%), oxygenated sesquiterpenes (18.45%), and oxygenated monoterpenes (6.69%). In addition, the major constituents of leaf essential oil identified were *ent*-kaur-16-ene (21.74%), β-elemol (13.93%), 3-carene (13.05%), sabinene (10.29%), α-pinene (6.53%), and (−)-terpinen-4-ol (5.99%) in quantitative order.

Among four commercially available fibers examined, a three-component fiber (CAR/PDMS/DVB) was found to be the

Table 1. Percentage Distribution of *Aedes aegypti* in the Different Cages after Leaf Essential Oil Treatment at 1.92 μg/cm^3 Concentration (Cage A)[a]

	time					
cage	10 min	20 min	30 min	40 min	50 min	60 min
A	17.3 ± 1.3 b	28.3 ± 2.1 b	31.4 ± 1.0 b	35.4 ± 2.0 b	38.2 ± 2.6 a	40.8 ± 3.3 a
B	78.7 ± 2.2 a	64.6 ± 3.4 a	58.1 ± 2.3 a	53.8 ± 2.1 a	46.8 ± 2.0 a	43.3 ± 1.5 a
C	4.0 ± 2.1 c	7.1 ± 2.3 c	12.6 ± 3.6 c	12.9 ± 3.4 c	15.1 ± 2.6 b	15.9 ± 2.8 b

[a] Results are mean ± SE (n = 5). Different letters are significantly different at the level of $p < 0.05$ according to the Scheffe test.

Table 2. Percentage Distribution of *Aedes albopictus* in the Different Cages after Leaf Essential Oil Treatment at 1.92 μg/cm^3 Concentration (Cage A)[a]

	time					
cage	10 min	20 min	30 min	40 min	50 min	60 min
A	33.0 ± 3.5 b	36.0 ± 3.3 a	38.0 ± 4.0 a	43.6 ± 2.5 a	48.0 ± 1.8 a	48.8 ± 2.1 a
B	56.8 ± 5.8 a	46.8 ± 5.0 a	43.6 ± 4.9 a	37.2 ± 3.8 a	33.2 ± 2.1 b	31.6 ± 1.3 b
C	13.2 ± 2.9 c	17.2 ± 2.6 b	18.4 ± 1.9 b	19.2 ± 3.0 b	18.8 ± 3.7 c	19.6 ± 3.3 c

[a] Results are mean ± SE (n = 5). Different letters are significantly different at the level of $p < 0.05$ according to the Scheffe test.

Table 3. Constituents and Relative Content of Volatile Compounds of Leaf Essential Oil from *Cryptomeria japonica* Using DVB/CAR/PDMS Fiber

				relative contents (%)		
no.	constituent	KI[a]	rKI[b]	HD[c]	SPME[d]	identification
1	α-thujene	926	930		2.52	MS, KI
2	α-pinene	934	939	6.53	1.70	MS, KI, ST
3	camphene	951	954	0.63	0.50	MS, KI, ST
4	sabinene	973	975	10.29	5.83	MS, KI, ST
5	β-pinene	978	979		0.20	MS, KI, ST
6	β-myrcene	988	990	1.71	4.62	MS, KI, ST
7	3-carene	1007	1011	13.05	25.46	MS, KI, ST
8	α-terpinene	1016	1017	2.46	8.90	MS, KI, ST
9	*p*-cymene	1024	1025	0.52	4.98	MS, KI, ST
10	limonene	1029	1029	3.67	15.07	MS, KI, ST
11	γ-terpinene	1059	1060	4.25	14.86	MS, KI, ST
12	terpinolene	1084	1089	1.85	7.55	MS, KI, ST
13	linalool	1096	1097		0.17	MS, KI, ST
14	(−)-terpinen-4-ol	1180	1177	5.99	5.47	MS, KI, ST
15	α-terpineol	1193	1189		0.08	MS, KI, ST
16	bornyl acetate	1282	1289	0.7	0.59	MS, KI, ST
17	α-terpinyl acetate	1351	1349		0.05	MS, KI, ST
18	β-elemol	1553	1550	13.93		MS, KI, ST
19	*r*-eudesmol	1636	1632	4.52		MS, KI, ST
20	*ent*-kaur-16-ene	2050		21.74		MS, KI, ST
	monterpene hydrocarbons			46.87	92.19	
	oxygenated monoterpenes			6.69	6.36	
	oxygenated sesquiterpenes			18.45		
	oxygenated diterpenes			21.74		
	identified components			93.75	98.55	

[a] KI, Kovats index was determined on a DB-5MS column using C_9-C_{24} as external references. [b] rKI, Kovats index on DB-5MS column in reference to *n*-alkanes (*24*). [c] HD, hydrodistillation. [d] SPME, solid-phase microextraction.

most effective for this application and was thus employed to collect volatiles emitted from leaf essential oil of *C. japonica*. Rubiolo et al. (*23*) used SPME fibers coupled with GC-MS to examine two chemotypes of giant fennel (*Ferula communis*) with different biological activities. Several fibers including CAR/PDMS, PDMS, DVB/CAR/PDMS, and PDMS/DVB were tested. The results showed that DVB/CAR/PDMS was found to be the most effective in this application, and SPME in combination with GC-MS has also been found to be an efficient method for rapid and unequivocal discrimination of the two chemotypes. These results revealed that DVB/CAR/PDMS can be used for analyzing volatile compounds of essential oils.

SPME results showed that volatiles emitted from leaf essential oil of *C. japonica* included monoterpene hydrocarbons (92.19%) and oxygenated monoterpenes (6.36%) (**Table 3**). In contrast to the constituents identified by hydrodistillation, the volatiles of leaf essential oil extracted by SPME contained no sesquiterpenes or diterpenes. In addition, results from the analyses of the constituents and the relative abundance of leaf essential oil obtained by GC-MS revealed that there were over 10 constituents detected in the leaf essential oil. The major volatile constituents detected by SPME from leaf essential oil were 3-carene (25.46%), limonene (15.07%), γ-terpinene (14.86%), α-terpinene (8.90%), terpinolene (7.55%), sabinene (5.83%), and (−)-terpinen-4-ol (5.47%) in quantitative order.

Leaf essential oil exhibited the best repellent activity among essential oils from four different plant parts of *C. japonica*. To understand volatiles released from leaf essential oil, DVB/CAR/PDMS was employed to extract volatiles during repellent assay. Results of GC-MS chromatograms are shown in **Figure 3**. The strongest intensity was observed at the initial time during the repellent assay. However, the intensity decreased gradually as time elapsed. Most volatile compounds in the cage were monoterpenoids (98.55%) including α-thujene (t_R = 7.07 min), α-pinene (t_R = 7.28 min), sabinene (t_R = 8.39 min), β-myrcene (t_R = 8.86 min), 3-carene (t_R = 9.44 min), α-terpinene (t_R = 9.68 min), *p*-cymene (t_R = 9.91 min), limonene (t_R = 10.05 min), γ-terpinene (t_R = 10.91 min), terpinolene (t_R = 11.72 min), and (−)-terpinen-4-ol (t_R = 14.55 min) at 0 min (**Table 3**; **Figure 3**). Only (−)-terpinen-4-ol showed a strong peak in the GC-MS chromatogram at 20, 40, and 60 min, whereas the other volatile compounds showed no significant peaks.

Wang et al. (*25*) reported that the leaf essential oil of *C. japonica* had significant repellent activity against silverfish (*Lepisma saccharina*). The chemical composition of essential oil and the emissions from a test chamber were analyzed by GC-MS. The main constituents reported were 3-carene (21.03%), *p*-cymene (10.95%), limonene (9.49%), β-myrcene (9.39%), γ-terpinene (9.10%), α-terpinene (8.57%), and 4-terpineol (7.97%). Comparisons of these data revealed that the major volatile compound of leaf essential oil from *C. japonica* in this study was 3-carene, but its relative content was 25.46%. In addition, relative contents of other volatile compounds, such as γ-terpinene (14.86%), limonene (15.07%), and α-terpinene (8.90%), were similar with those previously reported by Wang et al. (*25*). However, relative contents of volatile compounds of *p*-cymene and β-myrcene were less, suggesting that materials from different sources led to differences in relative contents.

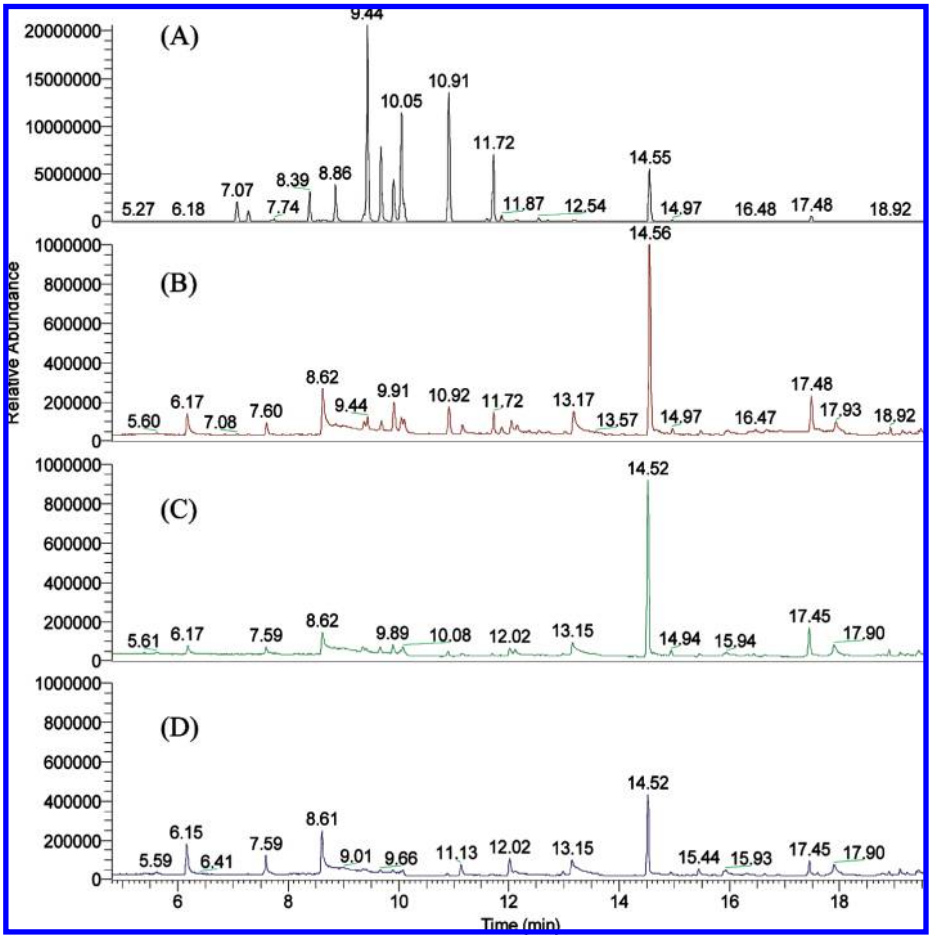

Figure 3. SPME-GC-MS chromatograms of volatile compounds from leaf essential oil at different times: (**A**) 0 min; (**B**) 20 min; (**C**) 40 min; (**D**) 60 min.

Different parts of plants contain a complex of chemicals with unique biological activity, which is thought to be caused by toxins and secondary metabolites, including phenolics, terpenoids, and alkaloids. They exist in plants and contribute to bioefficacy such as insecticidal, repellency, antifeeding, and ovicidal activities. The repellent constituents are mainly monoterpenoids (*26*). This may account for the repellent activity of leaf essential oil of *C. japonica* against *A. aegypti* and *A. albopictus* because volatile compounds from leaf essential oil are mainly monoterpenoids. In addition, some compounds, which also exist in *C. japonica*, have been reported for their repellent activities. Omolo et al. (*27*) evaluated six plant species growing in the Kenyan coast for repellency on the forearms of human volunteers against *An. gambiae*. The results showed that some of these constituents from the different oils, such as α-pinene, limonene, γ-terpinene, and α-terpinene, showed high individual repellencies. Traboulsi et al. (*28*) reported that α-pinene, β-pinene, limonene, and γ-terpinene from six aromatic plants had repellent activities against *Culex pipiens* and protected the human body from being bitten. Their results suggested that leaf essential oil of *C. japonica* had repellent activity due to these compounds.

Repellency of Major Compounds from Leaf Essential Oil. The major volatile compounds of leaf essential oil in the cages were found to be 3-carene, α-terpinene, limonene, γ-terpinene, and terpinolene. However, these volatile compounds released quickly as time elapsed, and the major volatile compound of leaf essential oil in the cages became (−)-terpinen-4-ol. Then, we evaluated six volatiles from leaf essential oil against *A. aegypti* and *A. albopictus*. Repellency of volatile compounds from leaf essential oil against mosquitoes is shown in **Figure 4**. As can be seen, after 20 min of treatment, (−)-terpinen-4-ol exhibited great repellent activity, 96 and 90% repellency against *A. aegypti* and *A. albopictus*, respectively, at 1.92 $\mu g/cm^3$ concentration. After 60 min of exposure, (−)-terpinen-4-ol still showed strong repellent activity, 92.0 and 82.2% repellency against *A. aegypti* and *A. albopictus*, respectively. Compared with essential oil, 3-carene, limonene, and (−)-terpinen-4-ol had more prominent repellent activity. In conclusion, (−)-terpinen-4-ol exhibited the best repellent activity. The (−)-terpinen-4-ol present in *C. japonica* leaf essential oil may also account for its repellent activity against mosquitoes.

Ma and Zhang (*29*) reported that terpinen-4-ol, the main insecticidal composition in the essential oil of *Sabina vulgaris*, was very toxic to third- and fourth-insta larvae of the armyworm (*Mythimna separata*). Kordali et al. (*30*) evaluated the essential oils of the aerial parts of three *Artemisia* species (*A. absinthium*, *A. santonicum*, and *A. spicigera*) for their toxicity against granary weevil, *Sitophilus granarius*. The results showed that terpinen-4-ol, the predominant component of the oils, was more toxic among the tested pure compounds. Five monoterpenoids [terpinen-4-ol, 1,8-cineole, linalool, *R*-(+)-limonene, and geraniol] in vapor form against different stages of *Tribolium confusum* had been reported (*31*). Analysis of the toxicity data showed that terpinen-4-ol exhibited the highest activity against the immature stages and adults of *T. confusum*. Park et al. (*32*) showed that terpinen-4-ol exhibited strong toxic action against larvae of *Lycoriella ingenue*. It can be concluded that terpinen-4-ol is a potential control agent against insects.

In conclusion, for the practical use of *C. japonica* leaf oil and terpinen-4-ol as novel mosquito repellents, further research on

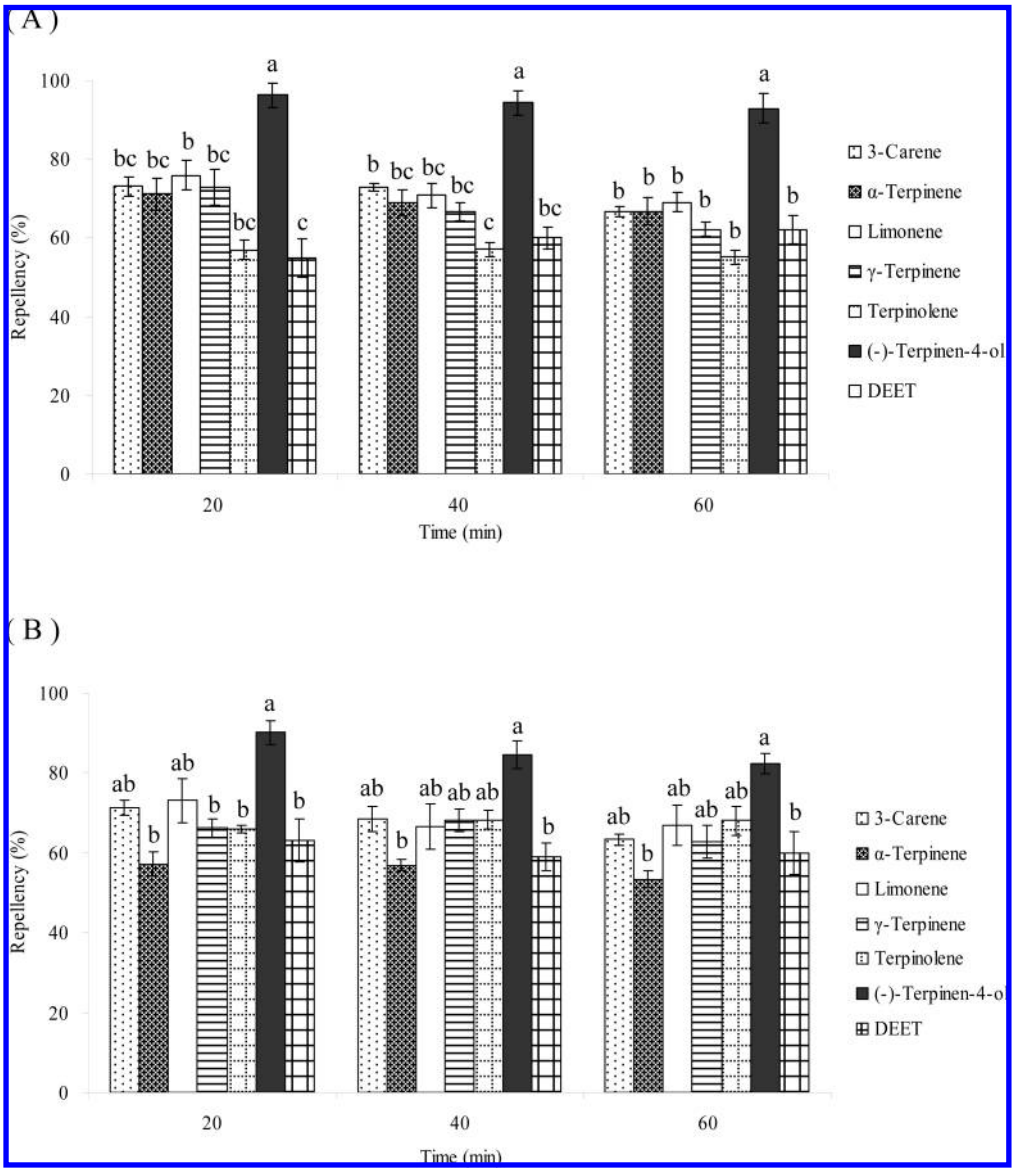

Figure 4. Repellency of major compounds from leaf oil (1.92 μg/cm^3) against *Aedes aegypti* (**A**) and *Aedes albopictus* (**B**). Bars marked by different letters are significantly different at the level of $p < 0.05$ according to the Scheffe test.

safety issues as well as formulations for improving repellent efficacy and stability and for reducing the cost is necessary.

ACKNOWLEDGMENT

We thank Experimental Forest of National Taiwan University for supplying *C. japonica* materials.

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Received for review July 16, 2009. Revised manuscript received October 16, 2009. Accepted October 21, 2009. Financial support from the Taiwan Forestry Bureau of Council of Agriculture of Executive Yuan is gratefully acknowledged.