Insecticidal activities of leaf essential oils from *Cinnamomum osmophloeum* against three mosquito species

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Abstract

The larvicidal activities of leaf essential oils and their constituents from six chemotypes of indigenous cinnamon (*Cinnamomum osmophloeum* Kaneh.) trees were evaluated against three mosquito species. Results of larvicidal tests demonstrated that the leaf essential oils of cinnamaldehyde type and cinnamaldehyde/cinnamyl acetate type had an excellent inhibitory effect against *Aedes albopictus* larvae, and their LC₅₀ values in 24 h were 40.8 µg/ml (LC₉₀ = 81.7 µg/ml) and 46.5 µg/ml (LC₉₀ = 83.3 µg/ml), respectively. Results of the 24-h mosquito larvicidal assays also showed that the effective constituents in leaf essential oils were *trans*-cinnamaldehyde and benzaldehyde and that the LC₅₀ values of these constituents against *A. albopictus* larvae were below 50 µg/ml. In addition, cinnamaldehyde type leaf essential oil and *trans*-cinnamaldehyde have also exhibited great larvicidal performance against *Culex quinquefasciatus* and *Armigeres subalbatus* larvae. Comparisons of mosquito larvicidal activity of *trans*-cinnamaldehyde congeners revealed that α -methyl cinnamaldehyde, benzaldehyde, and *trans*-cinnamaldehyde exhibited strong mosquito larvicidal activity.

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1. Introduction

The search for new strategies or natural products to control destructive insects and vectors of diseases is desirable due to the prevalent occurrence of vector resistance to synthetic insecticides and the problem of toxic non-biodegradable residues contaminating the environment and undesirable effects on nontarget organisms (Jantan et al., 2005). It has been shown that the use of plant essential oils as mosquito control agents can be effective, and it has been shown to minimize the impact that most pesticidal com-

pounds impose on the environment (Fatope et al., 1993). Mosquitoes play a predominant role in the transmission of some important diseases such as dengue fever, Japanese encephalitis, malaria, and viral diseases which are today among the greatest health problems in the world (Cheng et al., 2004; Hemingway, 2004; Kuo et al., 2007). In recent years, dengue fever cases have increased significantly in Taiwan (Lee et al., 2006), and much effort has been focused on exploring bioactive chemical compounds from indigenous plants for mosquito control (Chang et al., 2003; Cheng et al., 2003, 2004, 2008; Kuo et al., 2007).

Lauraceae is an economically important family consisting mostly of trees. The genus *Cinnamomum* comprises about 250 species distributed in Asia and Australia (Jayaprakash et al., 2003). Indigenous cinnamon (*Cinnamomum*

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osmophloeum Kaneh.) (*Cinnamomum*) is an endemic tree that grows in Taiwan's natural hardwood forests at elevations between 400 and 1500 m. It has been of interest to researchers because the chemical constituents of its leaf essential oil are similar to those of *Cinnamomum cassia* bark oil (Chang et al., 2001; Hu et al., 1985). Our previous studies have demonstrated that leaf essential oils from *C. osmophloeum* had excellent antibacterial, antitermite, antimite, antimildew, antipathogenic, antifungal, and anti-inflammatory activities (Chang et al., 2001; Chang and Cheng, 2002; Chen et al., 2002; Chen and Chang, 2002; Cheng et al., 2006; Lee et al., 2005; Wang et al., 2005; Chao et al., 2005, 2008). In addition, we found that essential oil and cinnamaldehyde from the leaves of *C. osmophloeum* exhibited the strongest mosquito larvicidal activity against fourth-instar *Aedes aegypti* larvae (Cheng et al., 2004). It is interesting to know whether the essential oil and its constituents from the leaves of *C. osmophloeum* possess the inhibitory action against other mosquito species. Thus, in this study we present the chemical composition of the leaf essential oils from six *C. osmophloeum* provenances (Table 1, COA–COF) as well as their larvicidal activities against *Aedes albopictus*, *Culex quinquefasciatus*, and *Armigeres subalbatus* larvae. In addition, the mosquito larvicidal activity of cinnamaldehyde congeners was also examined in order to elucidate the effects of chemical structure on the mosquito larvicidal property.

2. Methods

2.1. Plant material and essential oil extraction

Mature leaf of one *C. osmophloeum* provenance (COA, CO2001) was collected in August, 2003 from Haw–Lin experimental forest located in Taipei. Mature leaf of one *C. osmophloeum* provenance (COB, CO1102) was collected in October, 2003 from the Da–Pin–Ting of the Taiwan Sugar Farm and mature leaves of four *C. osmophloeum* provenances (COC–COF, CO0901, CO1701, CO0602, CO0501) were collected from the Lien Hua–Chin Research Center in July 2003, both locations are situated in Nantou County of central Taiwan. The species were identified by Mr. Yen-Ray Hsui of the Taiwan Forestry Research Institute and the voucher specimens (provenances COA to COF) were deposited at the laboratory of wood chemistry, School of Forestry and Resource Conservation, National Taiwan University. The samples (200 g each), in triplicate, were subjected to hydrodistillation in a Clevenger-type apparatus for 6-h (Cheng et al., 2004), followed by determination of oil contents. The leaf essential oils obtained were separated from the aqueous phase and stored in airtight containers at 4 °C prior to further analysis.

2.2. GC–MS analysis

Analyses of volatile constituents were determined using a Polaris Q Ion Trap GC/FID/MSⁿ system (Thermo,

USA), equipped with a fused silica column (30 mm × 0.25 mm i.d.) and coated with 5% phenyl-methylpolysiloxane using a DB-5MS (df = 0.25 μm) (Agilent J&W Scientific, USA). The temperature program used for the analysis was as follows: initial temperature at 80 °C, held for 1 min, ramped at 4 °C/min to 200 °C and held for 5 min. Helium was the carrier gas at 1.0 ml/min; the sample (1.0 μl, 1/100, v/v, in ethyl acetate) was injected in the split mode (1:10). The mass spectrometer was equipped with a PoLaris Q mass selective detector in electron impact (EI) ionization mode (70 eV). The ion source temperature and the injector temperature were set at 230 °C and 270 °C, respectively. The sector mass analyzer was set to scan from 50 to 650 amu every 0.5 s. The Kovats retention indices were calculated for all volatile constituents using a homologous series of *n*-alkanes C₉–C₁₇ on DB-5MS column. Quantification was performed using percentage peak area calculations and the identification of individual components was done using the Wiley/NBS Registry of Mass Spectral Database and NIST MS Search 2.0, the literature (Adams, 2001), and several authentic reference compounds. The relative concentration of each compound in essential oil was quantified according to the peak area integrated by the analysis program.

2.3. Mosquito larvicidal test

The free-mating colonies of *A. albopictus*, *C. quinquefasciatus*, and *A. subalbatus* were established in the insectariums of the Department of Parasitology, Chang-Gung University. The mosquito colonies were maintained continuously at 27 °C and 80 ± 10% relative humidity under a photoperiod of 12:12 h (light/dark) cycle, with 10% yeast suspension used as the growth medium. The method of Momin and Nair (2001) and Cheng et al. (2004) was employed to conduct mosquito larvicidal activity tests. Ten fourth-instar mosquito larvae were placed in 24.5 ml of degassed distilled water, followed by the addition of 500 μl dimethyl sulfoxide (DMSO) solution containing the test essential oil or the known compound in a 30-ml cup, shaken lightly to ensure a homogeneous test solution, and incubated at the ambient temperature. A total of six essential oils and 26 known compounds were tested in this manner. Essential oils were tested at 400, 200, 100, 50, and 25 μg/ml and each compound was tested at 100, 50, 25, and 12.5 μg/ml. The control was prepared with 24.5 ml of degassed distilled water and 500 μl of DMSO solution to which larvae were added. Each test was replicated four times. For comparison, commercial chlorpyrifos (*o,o*-diethyl-*o*-3,5,6-trichloro-2-pyridyl phosphorothioate), an organophosphorus pesticide was used as the positive control. The toxicity of chlorpyrifos was determined at 6.25, 3.125, 1.56, 0.78, and 0.39 μg/ml.

Mortality was recorded after 24- and 48-h of exposure, during which no food was given to the larvae. Percent mortality was corrected for control mortality using Abbott's formula, and the results were plotted on log/probability

paper using the method of Finney (1971). Toxicity and activity were reported as LC₅₀ and LC₉₀, representing the concentration in µg/ml that caused 50 and 90% larval mortality, respectively, in 24-h or 48-h.

2.4. Cluster analysis

The cluster analysis was performed with multi-variate statistical package (MVSP) software to identify relatively

Table 1

Chemical compositions of leaf essential oils from six provenances of *C. osmophloeum*

No.	Compounds	R.t. ^a	KI ^b	Peak area (%)						Identification ^c
				COA	COB	COC	COD	COE	COF	
1	α-Pinene	3.74	942	– ^d	–	–	–	–	1.32	MS, KI, ST
2	Camphene	3.97	958	–	–	–	–	–	1.01	MS, KI, ST
3	Benzaldehyde	4.07	965	1.18	1.19	0.49	1.55	–	–	MS, KI, ST
4	Unidentified	4.40	985	1.45	–	–	–	–	–	
5	β-Myrcene	4.51	992	–	–	–	–	–	1.42	MS, KI, ST
6	(+)-Limonene	5.28	1034	–	–	–	–	0.40	7.39	MS, KI, ST
7	1,8-Cineole	5.35	1038	9.21	–	–	–	–	–	MS, KI, ST
8	Salicylaldehyde	5.55	1048	–	–	0.60	–	–	0.85	MS, KI, ST
9	trans-Linalool oxide	6.18	1077	1.11	–	–	–	–	–	MS, KI, ST
10	cis-Linalool oxide	6.53	1091	0.91	–	–	–	0.41	–	MS, KI, ST
11	Linalool	6.75	1100	5.16	–	–	–	95.13	–	MS, KI, ST
12	Camphor	7.99	1152	–	–	0.40	0.65	–	56.78	MS, KI, ST
13	Benzenepropanal	8.33	1164	–	3.30	–	1.61	–	–	MS, KI, ST
14	Borneol	8.53	1172	0.97	–	–	–	–	0.75	MS, KI, ST
15	2-Methylbenzofuran	8.76	1180	–	–	9.32	–	–	–	MS
16	(–)-Terpinen-4-ol	8.83	1182	0.74	0.46	–	–	–	2.01	MS, KI, ST
17	α-Terpineol	9.17	1194	2.78	–	–	–	0.43	1.89	MS, KI, ST
18	4-Allylanisole	9.34	1199	–	1.43	0.76	0.72	0.56	–	MS
19	cis-Cinnamaldehyde	9.92	1221	–	0.88	–	0.55	–	–	MS
20	Unidentified	10.19	1231	0.84	–	–	–	0.31	–	
21	Neral	10.52	1243	13.09	–	–	–	–	–	MS, KI, ST
22	Geraniol	10.91	1256	2.02	–	–	–	–	–	MS, KI, ST
23	Geranial	11.37	1271	24.13	–	3.19	–	–	0.89	MS, KI, ST
24	trans-Cinnamaldehyde	11.42	1273	–	91.09	–	36.50	1.33	–	MS, KI, ST
25	Unidentified	11.63	1279	1.11	–	–	–	–	–	
26	L-Bornyl acetate	11.91	1288	4.13	0.56	1.04	0.70	–	21.53	MS, KI, ST
27	Unidentified	12.20	1297	0.95	–	–	–	–	–	
28	Geranyl formate	12.31	1300	0.98	–	–	–	–	–	MS
29	Cinnamyl alcohol	12.40	1304	–	–	2.27	0.97	–	–	MS
30	Unidentified	13.27	1335	–	0.31	–	–	–	–	
31	Cinnamyl formate	13.79	1353	–	–	1.40	–	–	–	MS
32	Unidentified	13.84	1354	1.67	–	–	–	–	–	
33	Eugenol	13.98	1359	–	0.28	–	0.57	–	–	MS, KI, ST
34	Geranyl acetate	14.74	1383	4.64	–	4.71	–	–	1.01	MS, KI, ST
35	Unidentified	16.07	1428	0.93	–	–	0.41	0.60	–	
36	Coumarin	16.30	1436	–	–	2.67	–	0.48	–	MS, KI, ST
37	Cinnamyl acetate	16.60	1447	–	–	59.65	54.31	–	–	MS, KI, ST
38	δ-Cadinene	18.99	1527	1.09	–	1.43	0.40	–	–	MS, KI
39	Spathulenol	20.57	1582	12.55	–	0.98	–	–	–	MS, KI
40	Caryophyllene oxide	20.74	1587	1.89	0.48	3.00	0.50	–	1.27	MS, KI, ST
41	Unidentified	20.98	1595	–	–	0.53	–	–	–	
42	Unidentified	21.48	1613	–	–	0.45	–	–	–	
43	T-Cadinol	22.33	1645	0.72	–	2.34	–	0.34	1.11	MS, ST
44	α-Cadinol	22.70	1659	1.13	–	2.95	–	–	0.77	MS, KI, ST
	Monoterpene hydrocarbons (%)			9.21	0.00	0.00	0.00	0.40	11.14	
	Oxygenated monoterpenes (%)			60.66	1.02	9.34	1.35	95.97	84.86	
	Sesquiterpene hydrocarbons (%)			1.09	0.00	1.43	0.4	0.00	0.00	
	Oxygenated sesquiterpenes (%)			16.29	0.48	9.27	0.50	0.34	3.15	
	Other (%)			1.18	98.17	77.16	97.19	2.97	0.85	
	Identified components (%)			88.22	99.67	96.22	99.44	99.68	100.00	
	Unidentified components (%)			8.78	0.31	1.81	0.00	0.31	0.00	
	Oil yield (%)			1.03	1.11	1.59	1.64	4.69	0.82	

^a Retention time (min).

^b Kovats retention index relative to *n*-alkanes (C₉–C₁₇) on DB-5MS column.

^c Identification based on comparison of the mass spectrum, Kovats index on a DB-5MS column in reference (Adams, 2001) and co-injection with authentic compounds.

^d Not detected.

homogeneous groups of six provenances (COA–COF) of *C. osmophloeum* according to percent composition of their essential oil samples. Euclidean distance was selected as a measure of similarity, and the unweighted pair-group method with arithmetic average (UPGMA) was used for cluster definition (Pereira et al., 2003).

2.5. Statistical analysis

The Scheffe multiple comparison procedure of SAS statistical program was used to analyze the difference in mosquito larvicidal activities among six *C. osmophloeum* leaf essential oils and their constituents ($P < 0.05$). All results were obtained from four independent experiments and expressed as mean $\pm$ SD.

3. Results and discussion

3.1. Yields and chemical polymorphism of essential oils from different provenances

The essential oil contents of six provenances of *C. osmophloeum* leaves obtained by hydrodistillation ranged from a minimum of 0.82% dry weight to a maximum of 4.69% dry weight (Table 1). The highest oil yield was found in COE (4.69%), followed by COD (1.64%), COC (1.59%), COB (1.11%), COA (1.03%), and COF (0.82%).

Table 1 shows the constituents of the six essential oils identified their percentage composition as well as their Kovats index (KI) values listed in order of elution from the DB-5MS capillary column. A total of 36 compounds were identified in the leaf essential oils from the six provenances, representing 88.22% to 100.00% of the oils obtained. The compounds from the six essential oils could be assigned to five different classes: monoterpene hydrocarbons (0.00–11.14%), oxygenated monoterpenes (1.02–95.97%), sesquiterpene hydrocarbons (0.00–1.43%), oxygenated sesquiterpenes (0.34–16.29%), and others (0.85–98.17%). The leaf essential oils of provenance COA show the presence of 19 identified compounds accounting for 88.22% of the whole oil with geranial (24.13%), neral (13.09%), and spathulenol (12.55%) as its main constituents. Provenance COB leaf essential oil contained nine identified compounds, accounting for 99.67% of the whole oil and containing 91.09% *trans*-cinnamaldehyde as the major constituent. Provenance COC leaf essential oil contained 17 identified compounds, accounting for 96.22% of the whole oil with cinnamyl acetate as the major components (59.65%) followed by 2-methylbenzofuran (9.32%). Provenance COD leaf essential oil contained 12 identified compounds, accounting for 99.44% of the whole oil with cinnamyl acetate (54.31%) and *trans*-cinnamaldehyde (36.50%) as its main constituents. Provenance COE leaf essential oil contained eight identified compounds, accounting for 99.68% of the whole oil containing 95.13% linalool as its main constituents. Provenance COF leaf essential oil contained 15 identified compounds, accounting for 100.00% of the whole oil with camphor

(56.78%) and L-bornyl acetate (21.53%) as its main constituents.

The differences in the percent composition of *C. osmophloeum* essential oils among the six provenances were determined using cluster analysis (Fig. 1). Following the results obtained herein and the previous classification by Hu et al. (1985), Lee et al. (2003), and Cheng et al. (2004, 2006), we classified the leaf essential oils into six main types, cinnamaldehyde type (COB), cinnamaldehyde/cinnamyl acetate type (COD), cinnamyl acetate type (COC), camphor type (COF), and linalool type (COE), while the essential oil from COA was classified as a mixed type owing to the lack of a dominant compound. The present results plus those obtained by Cheng et al. (2004, 2006) shows that all the leaf essential oils extracted are similar.

3.2. Mosquito larvicidal activity of essential oils

A total of the six chemotypes of *C. osmophloeum* leaf essential oil were investigated for their mosquito larvicidal activities against fourth-instar *A. albopictus* larvae primarily. Fig. 2 shows that the cinnamaldehyde type (provenance COB) and cinnamaldehyde/cinnamyl acetate type (provenance COD) induced 100% larval mortality of *A. albopictus* after 24 h with a dosage of 100 $\mu\text{g/ml}$. However, a dosage of 200 $\mu\text{g/ml}$ of mixed type (provenance COA), cinnamyl acetate type (provenance COC), camphor type (provenance COF) and a dosage of 400 $\mu\text{g/ml}$ of linalool type (provenance COE) were required to reach 100% larval mortality (Fig. 2).

The efficacy of larvicidal performance of six chemotypes of *C. osmophloeum* leaf essential oil against *A. albopictus* in 24 h can be recognized from the comparison of their LC_{50} and LC_{90} values (Table 2). Since the essential oils of mixed type (provenance COA), cinnamyl acetate type (provenance COC), linalool type (provenance COE), and camphor type (provenance COF) showed an $\text{LC}_{50} > 50 \mu\text{g/ml}$ against fourth-instar *A. albopictus* larvae in 24 h (Table 2),

Fig. 1. Dendrogram obtained by cluster analysis of the percentage composition of essential oils from six provenances of *C. osmophloeum*.

Fig. 2. Mosquito larvicidal activities of leaf essential oils from six chemotypes of *C. osmophloeum* against *A. albopictus* larvae after 24 h. Means ($n = 4$) using 10 fourth-instar mosquito larvae per replicate. Numbers followed by different letters (a–c) are significantly different at level of $P < 0.05$ according to Scheffe's test.

they were consequently considered inactive. Both the cinnamaldehyde type (provenance COB) and cinnamaldehyde/cinnamyl acetate type (provenance COD) leaf essential oils showed excellent toxicity against *A. albopictus* larvae in 24 h, and the LC_{50} values were 40.8 and 46.5 $\mu\text{g/ml}$ with corresponding LC_{90} values of 81.7 and 83.3 $\mu\text{g/ml}$, respectively (Table 2). When incubation was extended to 48 h, the LC_{50} values of these two chemotypes were 30.5 $\mu\text{g/ml}$ ($LC_{90} = 65.6 \mu\text{g/ml}$) and 45.4 $\mu\text{g/ml}$ ($LC_{90} = 81.3 \mu\text{g/ml}$), respectively. Since cinnamaldehyde type (provenance COB) and cinnamaldehyde/cinnamyl acetate type (provenance COD) leaf essential oils contained a higher amount of cinnamaldehyde (91.09%) and cinnamyl acetate (54.31%), respectively (Table 1), which also displayed strong mosquito larvicidal activities, it is clear that the larvicidal activities of these two leaf essential oils are directly affected by the cinnamaldehyde and cinnamyl acetate content. A similar observation was noted in our previous study on the mosquito larvicidal activity of *C. osmophloeum* leaf essential oil against *A. aegypti* larvae ranging between 36 and 44 $\mu\text{g/ml}$ (Cheng et al., 2004). In addition, Cavalcanti

et al. (2004) reported that the larvicidal activity of essential oils from Brazilian plants with LC_{50} values against the larvae of *A. aegypti* ranged from 60 to 69 $\mu\text{g/ml}$. Essential oil from commonly used herbs in Lebanon against *Ochlerotatus caspius* larvae ranged between 15 and 156 $\mu\text{g/ml}$ (Knio et al., 2008). Pitasawat et al. (2007) also found that the larvicidal activity of five aromatic plant essential oils with LC_{50} values ranging between 24.6 and 54.6 $\mu\text{g/ml}$ against *A. aegypti* larvae and ranging from 29.7 to 72.3 $\mu\text{g/ml}$ against *Anopheles dirus* larvae. Comparisons of the aforesaid results revealed that the leaf essential oils of both cinnamaldehyde type (provenance COB) and cinnamaldehyde/cinnamyl acetate type (provenance COD) have excellent mosquito larvicidal activities.

3.3. Mosquito larvicidal activity of main compounds in essential oils

To confirm the relationship between the constituents of *C. osmophloeum* leaf essential oils and mosquito larvicidal activity, 14 main compounds in the leaf essential oils were selected to test their mosquito larvicidal activity against fourth-instar *A. albopictus*. Chlorpyrifos, well known for its mosquito larvicidal activity, was used in parallel as the positive control. As shown in Table 3, among the 14 compounds tested for 24 h, *trans*-cinnamaldehyde, cinnamyl acetate, benzaldehyde, eugenol, caryophyllene oxide, citral (mixture of geranial and neral), and α -terpineol exhibited the strongest activity ($LC_{50} < 100 \mu\text{g/ml}$). The LC_{50} values of these seven compounds were 48.1, 52.7, 47.0, 67.4, 65.6, 70.7, and 92.7 $\mu\text{g/ml}$, respectively. It is clear that benzaldehyde, *trans*-cinnamaldehyde, and cinnamyl acetate have the best mosquito larvicidal activity, with LC_{50} values of 47.0, 48.1, and 52.7 $\mu\text{g/ml}$ ($LC_{90} = 85.5$, 89.1, and 99.3 $\mu\text{g/ml}$), respectively. When the test was extended to 48 h, the LC_{50} and LC_{90} values of benzaldehyde, *trans*-cin-

Table 2
 LC_{50} and LC_{90} values ($\mu\text{g/ml}$) of leaf essential oils of six chemotypes of *C. osmophloeum* against *A. albopictus* larvae

Chemotypes ^a	24 h			48 h		
	LC_{50}	LC_{90}	RP ^b	LC_{50}	LC_{90}	RP
COA	69.0	154.0	0.026	64.6	147.0	0.015
COB	40.8	81.7	0.044	30.5	65.6	0.033
COC	69.8	156.9	0.026	59.5	149.8	0.017
COD	46.5	83.3	0.039	45.4	81.3	0.022
COE	144.4	>400.0	0.012	124.3	368.7	0.001
COF	77.7	183.5	0.023	71.7	167.1	0.014
Chlorpyrifos ^c	1.8	2.8	1	1.0	2.3	1

^a COA, mixed type; COB, cinnamaldehyde type; COC, cinnamyl acetate type; COD, cinnamaldehyde/cinnamyl acetate type; COE, linalool type; COF, camphor type.

^b RP, relative potency (LC_{50} standard/ LC_{50} test substance).

^c Positive control.

Table 3
 LC_{50} and LC_{90} values ($\mu\text{g/ml}$) of 14 compounds in *C. osmophloeum* leaf essential oils against *A. albopictus* larvae

Compounds	24 h		48 h	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}
<i>trans</i> -Cinnamaldehyde	48.1	89.1	47.4	89.1
Cinnamyl acetate	52.7	99.3	45.0	84.8
Benzaldehyde	47.0	85.5	45.4	81.3
Camphor	>100.0	>100.0	>100.0	>100.0
Benzenepropanal	>100.0	>100.0	>100.0	>100.0
1,8-Cineole	>100.0	>100.0	>100.0	>100.0
L-Bornyl acetate	>100.0	>100.0	>100.0	>100.0
Eugenol	67.4	>100.0	54.8	>100.0
Caryophyllene oxide	65.6	>100.0	58.3	>100.0
α -Terpineol	>100.0	>100.0	>100.0	>100.0
Citral	70.7	>100.0	65.4	>100.0
Geranyl acetate	>100.0	>100.0	>100.0	>100.0
T-cadinol	92.7	>100.0	74.5	>100.0
Linalool	>100.0	>100.0	>100.0	>100.0
Chlorpyrifos ^a	1.8	2.8	1.0	2.3

^a Positive control.

namaldehyde, and cinnamyl acetate were 45.0–47.4 µg/ml and 81.3–89.1 µg/ml, respectively (Table 3). Whereas, the LC₅₀ and LC₉₀ values for chlorpyrifos were 1.8 µg/ml (LC₅₀ = 1.0 µg/ml for 48 h) and 2.8 µg/ml (LC₉₀ = 2.3 µg/ml for 48 h), respectively.

Although benzaldehyde, *trans*-cinnamaldehyde, and cinnamyl acetate all possess stronger mosquito larvicidal activity, benzaldehyde exists in only small quantity in the leaf essential oils of all *C. osmophloeum* provenances (COA to COD). In addition, cinnamyl acetate was not found in provenances COA–COB and COE–COF except in provenances COC (59.65%) and COD (54.31%). Therefore, cinnamaldehyde is clearly responsible for the excellent mosquito larvicidal activity of provenances COB and COD essential oils. Similar results in previous studies on the antibacterial, antitermite, antimitic, antimildew, antipathogenic, antimosquito, antifungal, and anti-inflammatory activities of *C. osmophloeum* were obtained by Chang et al. (2001), Chang and Cheng (2002), Chen et al. (2002), Chen and Chang (2002), Cheng et al. (2004, 2006), Lee et al. (2005), Wang et al. (2005), and Chao et al. (2008).

It has been reported that eugenol, thymol, and carvacrol from commonly used herbs in Lebanon were effective against fourth-instar larvae of *O. caspius* with LC₅₀ values of 7.53, 33.65, and 35.48 µg/ml, respectively (Knio et al., 2008). Rahuman et al. (2000) also found that *n*-hexadecanoic acid in *Feronia limonia* dried leaves was effective against fourth-instar larvae of *C. quinquefasciatus*, *Anopheles stephensi*, and *A. aegypti* with LC₅₀ values of 129.24, 79.58, and 57.23 µg/ml, respectively. In another investigation, Kiran et al. (2006) found that pregeijerene, geijerene, and germacrene D isolated from *Chloroxylon swietenia* DC had LC₅₀ values of 28.3, 43.4, and 63.6 µg/ml when tested against fourth-instar *A. aegypti* larvae and LC₅₀ values of 25.8, 41.2, and 59.5 µg/ml when tested against fourth-instar *A. stephensi* larvae at 24 h. Comparisons of these data revealed that benzaldehyde, *trans*-cinnamaldehyde, and cinnamyl acetate examined in this study exhibited great larvicidal performance.

3.4. Mosquito larvicidal activity of cinnamaldehyde type essential oil and *trans*-cinnamaldehyde against *C. quinquefasciatus* and *A. subalbatus* larvae

The above-mentioned results show that the cinnamaldehyde type leaf essential oil and *trans*-cinnamaldehyde have significant mosquito larvicidal activities against fourth-instar *A. albopictus* larvae. Thus, two mosquito larvae of *C. quinquefasciatus* and *A. subalbatus* were selected to test the mosquito larvicidal activities of cinnamaldehyde type leaf essential oil and *trans*-cinnamaldehyde. As shown in Table 4, the LC₅₀ values of cinnamaldehyde type leaf essential oil against *C. quinquefasciatus* and *A. subalbatus* larvae in 24 h were 31.3 and 22.1 µg/ml with corresponding LC₉₀ values of 82.8 and 62.3 µg/ml, respectively. When incubation was extended to 48 h, the LC₅₀ values of these two samples were 14.0 µg/ml (LC₉₀ = 69.7 µg/ml) and 21.8

Table 4

LC₅₀ and LC₉₀ values (µg/ml) of cinnamaldehyde type in *C. osmophloeum* leaf essential oil and *trans*-cinnamaldehyde against *C. quinquefasciatus* and *A. subalbatus* larvae

Specimens	Time (h)	<i>C. quinquefasciatus</i>		<i>A. subalbatus</i>	
		LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀
Cinnamaldehyde type oil	24	31.3	82.8	22.1	62.3
	48	14.0	69.7	21.8	36.5
<i>trans</i> -Cinnamaldehyde	24	37.5	80.6	27.6	70.2
	48	18.3	68.4	23.8	65.7

µg/ml (LC₉₀ = 36.5 µg/ml), respectively. On the other hand, the LC₅₀ values of *trans*-cinnamaldehyde against *C. quinquefasciatus* and *A. subalbatus* larvae in 24 h were 37.5 and 27.6 µg/ml with corresponding LC₉₀ values of 80.6 and 70.2 µg/ml, respectively. When incubation was extended to 48 h, the LC₅₀ values of these two samples were 18.3 µg/ml (LC₉₀ = 68.4 µg/ml) and 23.8 µg/ml (LC₉₀ = 65.7 µg/ml), respectively (Table 4). In addition, it is obvious that *A. subalbatus* larvae proved to be more sensitive to *trans*-cinnamaldehyde than *C. quinquefasciatus* and *A. albopictus* larvae.

Our results showed that cinnamaldehyde type leaf essential oil was more potent than *trans*-cinnamaldehyde against *A. albopictus*, *C. quinquefasciatus*, and *A. subalbatus* larvae. The observation may be attributable to the synergistic effect of other components of the cinnamaldehyde type leaf essential oil. The results obtained herein suggest that cinnamaldehyde type leaf essential oil should have a strong potential as larvicide against different mosquitoes.

3.5. Structure-activity relationship study of *trans*-cinnamaldehyde's congeners

According to the results obtained in this study, *trans*-cinnamaldehyde is the strongest mosquito larvicidal compound in the leaf essential oils of *C. osmophloeum*. Thus, to examine the structure-activity relationships of *trans*-cinnamaldehyde, 15 compounds whose chemical structures are similar to cinnamaldehyde were selected for this study. Table 5 presents the results of LC₅₀ and LC₉₀ values of these compounds against fourth-instar *A. albopictus* larvae, showing that *trans*-cinnamaldehyde, benzaldehyde, methyl cinnamate, α -methyl cinnamaldehyde were the most active against *A. albopictus* larvae amongst the 16 compounds tested in 24 h, with LC₅₀ values lower than 50.0 µg/ml. They are ranked as α -methyl cinnamaldehyde (LC₅₀ = 31.2 µg/ml; LC₉₀ = 46.4 µg/ml) > benzaldehyde (LC₅₀ = 47.0 µg/ml; LC₉₀ = 85.5 µg/ml) > *trans*-cinnamaldehyde (LC₅₀ = 48.1 µg/ml; LC₉₀ = 89.1 µg/ml) > methyl cinnamate (LC₅₀ = 50.0 µg/ml; LC₉₀ = 89.6 µg/ml). These results indicated that *trans*-cinnamaldehyde or α -methyl cinnamaldehyde, having an aldehyde group, a conjugated double bond and a length of CH chain outside the ring, possesses much stronger mosquito larvicidal activity. A similar observation was also noted in our previous studies on the

Table 5

LC₅₀ and LC₉₀ values (μg/ml) of cinnamaldehyde congeners in mosquito larvicidal assay against *A. albopictus* larvae

Compounds	24 h		48 h	
	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀
<i>trans</i> -Cinnamaldehyde	48.1	89.1	47.4	89.1
Benzenepropanal	>100.0	>100.0	>100.0	>100.0
Cinnamyl acetate	52.7	99.3	45.0	84.8
Cinnamic acid	82.0	>100.0	75.6	>100.0
Cinnamyl alcohol	>100.0	>100.0	79.0	>100.0
Benzaldehyde	47.0	85.5	45.4	81.3
4-Hydroxybenzaldehyde	80.1	128.4	74.4	122.0
Methyl cinnamate	50.0	89.6	43.7	78.4
α-Methyl cinnamaldehyde	31.2	46.4	30.1	46.2
2-Methyl-3-phenyl-2-propen-1-ol	>100.0	>100.0	61.7	>100.0
2-Hydroxycinnamic acid	>100.0	>100.0	>100.0	>100.0
3-Hydroxycinnamic acid	>100.0	>100.0	>100.0	>100.0
4-Hydroxycinnamic acid	>100.0	>100.0	>100.0	>100.0
2-Methylcinnamic acid	64.7	95.1	61.7	94.1
3-Methylcinnamic acid	64.1	98.9	58.3	96.4
4-Methylcinnamic acid	66.2	95.2	59.7	93.3
Chlorpyrifos ^a	1.8	2.8	1.0	2.3

^a Positive control.

antibacterial (Chang et al., 2001), antitermitic (Chang and Cheng, 2002), antimosquito (Cheng et al., 2004), and antifungal (Lee et al., 2005; Wang et al., 2005) activities of *C. osmophloeum*. In addition, on the basis of the mosquito larvicidal activities of the seven cinnamic acid derivatives, the hydroxyl group on the cinnamic acid structure has no significant influence (LC₅₀ > 100.0 μg/ml) on mosquito larvicidal activity, but the presence of the methyl group may have a considerable influence on the inhibitory action against fourth-instar *A. albopictus* larvae (Table 5). The results differ from those reported by Kim et al. (2004), who suggested that the presence of the hydroxyl moiety in the *ortho* position may have a considerable influence on the *Tyrophagus putrescentiae* toxicity. We assume that the discrepancy might be due to the different mechanisms involved in the inhibition of mosquito larvae and mites.

4. Conclusions

The use of natural products may be considered as an important alternative insecticide for the control of mosquito larvae. Results from this study demonstrated that the cinnamaldehyde type and cinnamaldehyde/cinnamyl acetate type of *C. osmophloeum* leaf essential oils have excellent mosquito larvicidal activity and that *trans*-cinnamaldehyde is responsible for such activity. Furthermore, *trans*-cinnamaldehyde as well as the cinnamaldehyde type and cinnamaldehyde/cinnamyl acetate type essential oils are natural and more selective larvicides against *A. albopictus*, *C. quinquefasciatus*, and *A. subalbatus* larvae. However, further investigations for the insecticidal action mode of *trans*-cinnamaldehyde, effects on non-target organisms and field evaluation are needed. Accordingly, these results could be useful in the research for selecting

newer, more selective, biodegradable and natural larvicidal compounds.

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