JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Chemical Composition and Mosquito Larvicidal Activity of Essential Oils from Leaves of Different *Cinnamomum osmophloeum* Provenances

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Chemical compositions of leaf essential oils from eight provenances of indigenous cinnamon (*Cinnamomum osmophloeum* Kaneh.) were compared. According to GC-MS and cluster analyses, the leaf essential oils of the eight provenances and their relative contents were classified into five chemotypes—cinnamaldehyde type, linalool type, camphor type, cinnamaldehyde/cinnamyl acetate type, and mixed type. The larvicidal activities of leaf essential oils and their constituents from the five chemotypes of indigenous cinnamon trees were evaluated by mosquito larvicidal assay. Results of larvicidal tests demonstrated that the leaf essential oils of cinnamaldehyde type and cinnamaldehyde/cinnamyl acetate type had an excellent inhibitory effect against the fourth-instar larvae of *Aedes aegypti*. The LC_{50} values for cinnamaldehyde type and cinnamaldehyde/cinnamyl acetate type against *A. aegypti* larvae in 24 h were 36 ppm (LC_{90} = 79 ppm) and 44 ppm (LC_{90} = 85 ppm), respectively. Results of the 24-h mosquito larvicidal assays also showed that the effective constituents in leaf essential oils were cinnamaldehyde, eugenol, anethole, and cinnamyl acetate and that the LC_{50} values of these constituents against *A. aegypti* larvae were <50 ppm. Cinnamaldehyde had the best mosquito larvicidal activity, with an LC_{50} of 29 ppm (LC_{90} = 48 ppm) against *A. aegypti*. Comparisons of mosquito larvicidal activity of cinnamaldehyde congeners revealed that cinnamaldehyde exhibited the strongest mosquito larvicidal activity.

KEYWORDS: ***Cinnamomum osmophloeum*; *Aedes aegypti*; leaf; essential oils; GC-MS; mosquito larvicidal activity; cinnamaldehyde; eugenol**

INTRODUCTION

Mosquito not only is the most important vector for the transmission of malaria, filariasis, and viral diseases (*1*) but also is an important pests to humans, causing allergic responses that include local skin reaction and systemic reactions, such as angioedema and urticaria (*2*). For instance, *Aedes aegypti* is one of the mosquito species responsible for the transmission of dengue fever and dengue hemorrhagic fever. Constant applications of organophosphates such as temephos and fenthion and insect growth regulators such as diflubenzuron and methoprene are generally used for the control of mosquito larvae (*3*). Although effective, repeated use of these controlling agents has fostered several environmental and health concerns, including disruption of natural biological control systems, outbreaks of other insect species, widespread development of resistance, and undesirable effects on nontarget organisms (*3*). These problems have highlighted the need for new strategies for mosquito larval control. Essential oils recently have received much attention as potential bioactive compounds against insects (*4*). Dengue fever cases have increased significantly in Taiwan recently, so much effort has been focused on exploring bioactive chemical compounds from indigenous plants for mosquito control (*5*, *6*).

Lauraceae is an economically important family consisting mostly of trees. The genus *Cinnamomum* comprises ~250 species that are distributed in Asia and Australia (*7*). *Cinnamomum cassia* bark oil is generally used in food and beverages and is very valuable in commerce. In addition, its antimicrobial activity has also attracted great attention from many researchers (*8*−*11*). The main constituents of *C. cassia* bark oils are cinnamaldehyde and eugenol. Cinnamaldehyde is recognized as a safe food additive and widely used as a flavoring agent. It has also been demonstrated that *C. cassia* bark-derived materials could be of practical use as new preventive agents against *Mechoris ursulus* (*12*), and *Cinnamomum zeylanicum* oil has an inhibitory effect against meat spoilage organisms (*11*). Singh

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10.1021/jf0497152 CCC: $27.50
Published on Web 06/10/2004

and co-workers have also demonstrated that *C. zeylanicum* bark oil has fungitoxic properties against fungi involved in respiratory tract mycoses, such as *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus nidulans*, and *Aspergillus flavus* (*13*). Huang and Ho (*14*) reported that a methylene chloride extract of cinnamon, *Cinnamomum aromaticum* Nees, was shown to be insecticidal to *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch.

Indigenous cinnamon (*Cinnamomum osmophloeum* kaneh.) (*Cinnamomum*) is an endemic tree that grows in Taiwan's natural hardwood forests at elevations between 400 and 1500 m. Chemical constituents of its leaf essential oil are similar to those of the famous *C. cassia* bark oil (*8*, *15*). Hu et al. (*15*) analyzed the composition of essential oils of *C. osmophloeum* leaves collected randomly from 21 provenances in central, southern, and eastern Taiwan and found that cinnamaldehyde was the major constituent of some *C. osmophloeum* provenances and that eugenol was present in other *C. osmophloeum* provenances. On the basis of differences in the chemical composition of leaf essential oil, *C. osmophloeum* was classified into nine types: cassia type, cinnamaldehyde type, coumarin type, linalool type, eugenol type, camphor type, 4-terpinenol type, linalool−terpinenol type, and mixed type (*15*).

Chang et al. (*8*, *16*−*19*) found that the leaf essential oil of the cinnamaldehyde type *C. osmophloeum* has an excellent inhibitory effect against bacteria, termites, mites, mildew, and fungi. However, there are no prior studies of mosquito larvicidal activity of leaf essential oils and their composition from *C. osmophloeum*. In light of the existence of chemical polymorphism of leaf essential oil from different provenances of *C. osmophloeum* (*20*), it is interesting to study differences in the bioactivity of varieties of indigenous cinnamon leaf oils. In this study leaf essential oils from eight *C. osmophloeum* provenances (**Table 1**, A−H) were analyzed by GC-MS for their chemical composition, and the mosquito larvicidal activities of these leaf essential oils were investigated. In addition, the mosquito larvicidal activity of cinnamaldehyde congeners was also examined to elucidate the effects of chemical structure on the mosquito larvicidal property.

MATERIALS AND METHODS

Isolation of Essential Oils. Leaves of eight *C. osmophloeum* provenances (A−H) were collected in July 2001 from the Lien Hua-Chin Research Center and the Da-Pin-Ting of Taiwan Sugar Farm located in Nantou County in central Taiwan. The species were identified by Yen-Ray Hsui of the Taiwan Forestry Research Institute, and voucher specimens (provenances A−H) were deposited at the Laboratory of Wood Chemistry, School of Forestry and Resource Conservation, National Taiwan University. The samples (150 g each), in triplicate, were subjected to hydrodistillation in a Clevenger-type apparatus for 6 h (*8*), followed by determination of oil contents. Leaf essential oils were stored in airtight containers prior to analysis by gas chromatography−mass spectrometry (GC-MS).

GC-MS Analysis. The mass spectrometer was equipped with a PoLaris Q mass selective detector in the electron impact (EI) ionization mode (70 eV). A Trace gas chromatograph was used and operated under the following conditions: RTx-5 capillary column (30 m × 0.25 mm; film thickness = 0.25 μm); held at 80 °C for 1 min, raised to 200 °C at a rate of 4 °C/min, and held for 5 min; 250 °C injector temperature; carrier helium at a flow rate of 10 mL/min; 1:10 split ratio. Diluted samples (1.0 μL, 1/100, v/v, in ethyl acetate) were injected manually in the splitless mode. Identification of the major components of *C. osmophloeum* leaf oils was confirmed by comparison with standards, by spiking, and on the basis of their mass spectral fragmentation using the Wiley GC-MS library. The quantity of compounds was obtained by integrating the peak area of the spectrograms.

Mosquito Larvicidal Test. Larvae of *A. aegypti* (Kaohsiung strain) were reared in the Department of Parasitology, Chang-Gung University, at 27 °C with a photoperiod of 12-h light and 12-h dark and 80 ± 10% relative humidity. A 10% yeast suspension was used as the growth medium. The method of Rafikali and Nair (*21*) was modified and used 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 of 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 room temperature. A total of 8 essential oils and 14 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 50, 25, 12.5, 6.25, and 3.15 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.

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 (*22*). Toxicity and activity were reported as LC_{50} and LC_{90}, representing the concentration in micrograms per milliliter that caused 50 and 90% larval mortality, respectively, in 24 or 48 h.

Cluster Analysis. Percent composition of the essential oil samples was used to determine differences among the eight provenances (A−H) of *C. osmophloeum* by cluster analysis using multivariate statistical package (MVSP) software. Euclidean distance was selected as a measure of similarity, and the unweighted pair-group method with arithmetic average (UPGMA) was used for cluster definition.

Statistical Analyses. Percent of mortality was determined and transformed to arcsine square-root values for analyses of variance (ANOVA). Significant differences ($P < 0.05$) were determined by the Scheffe test.

RESULTS AND DISCUSSION

Yields and Chemical Compositions of Essential Oils. Distillation of eight provenances of *C. osmophloeum* leaves yielded from 0.13 to 4.69% (v/w) essential oils based on dry weight (**Table 1**). The highest oil content was found in provenance F (4.69%), followed by C (2.19%), G (1.11%), A and B (1.02%), H (0.89%), E (0.82%), and D (0.13%).

Compositions of the eight essential oils also are reported in **Table 1**. A total of 54 compounds were identified in the eight leaf essential oils, constituting 86.4−100% of the oils. The leaf essential oils of provenances A, G, and H show the presence of 19, 19, and 10 identified compounds accounting for 99.6, 99.3, and 100% of the whole oil and containing 60.22, 85.32, and 74.57% *trans*-cinnamaldehyde, respectively, as the major constituent. Provenance B leaf essential oil contained 20 identified compounds, accounting for 100% of the whole oil, and its main constituents were *trans*-cinnamaldehyde (50.93%) and cinnamyl acetate (28.48%). Provenance C leaf essential oil contained 19 identified compounds, accounting for 100% of the whole oil, with cinnamyl acetate (44.94%) and *trans*-cinnamaldehyde (33.93%). Provenance D leaf essential oil contained 27 identified compounds, accounting for 86.4% of the whole oil, with T-cadinol (17.46%) and α-cadinol (11.68%) as its main constituents. Provenance E leaf essential oil contained 22 identified compounds, accounting for 100% of the whole oil, with camphor (43.99%) and bornyl acetate (20.81%) as its main constituents. Provenance F leaf essential oil contained 11 identified compounds, accounting for 99.2% of the whole oil and containing 90.61% linalool.

On the basis of their main constituents, cluster analysis of the identified compounds classified the leaf oils into five main groups (**Figure 1**): *trans*-cinnamaldehyde (provenances A, G, and H), *trans*-cinnamaldehyde/cinnamyl acetate (provenances B and C), T-cadinol/α-cadinol (provenance D), camphor (provenance E), and linalool (provenance F). On the basis of

Table 1. Chemical Compositions of Leaf Essential Oils from Eight Provenances of *C. osmophloeum*

no.	compound	RT[a]	A	B	C	D	E	F	G	H
1	benzaldehyde	4.12	1.74	2.07	0.87	–[b]	–	–	1.75	5.92
2	camphene	4.33	–	–	9.01	–	0.63	–	–	–
3	β-pinene	4.65	–	–	–	–	2.24	–	–	–
4	*p*-cymene	5.38	–	–	–	–	–	–	0.26	–
5	limonene	5.45	–	–	–	–	8.70	–	–	–
6	salicylaldehyde	5.83	–	–	–	–	1.79	0.77	–	–
7	α-terpinene	6.74	–	–	–	–	0.81	–	–	–
8	linalool oxide	6.77	–	–	–	–	–	0.59	–	–
9	linalool	6.93	–	–	–	0.50	–	90.61	–	–
10	camphor	8.29	–	–	0.85	–	43.99	–	0.26	–
11	benzyl alcohol	8.32	–	0.44	–	–	–	–	–	–
12	benzenepropanal	8.71	4.50	3.69	1.22	–	–	–	3.39	8.41
13	borneol	8.80	–	–	–	0.26	0.92	–	–	–
14	terpinene-4-ol	9.11	–	–	–	–	4.22	–	0.44	–
15	α-terpineol	9.42	–	–	0.21	0.80	2.18	0.55	–	–
16	*p*-allylanisole	9.60	–	–	–	3.80	–	–	–	–
17	anethole	9.64	1.78	1.25	0.38	–	–	1.02	1.45	2.75
18	*cis*-cinnamaldehyde	10.33	1.10	1.00	0.62	–	–	–	1.42	1.32
19	geraniol	11.13	–	–	–	0.59	1.39	–	–	–
20	4-allyphenol	11.29	–	–	0.32	–	–	–	0.31	–
21	*trans*-cinnamaldehyde	11.66	60.22	50.93	33.93	0.24	0.96	–	85.32	74.57
22	citral	11.78	–	–	–	–	–	1.93	–	–
23	bornyl acetate	12.15	4.69	1.61	0.48	9.75	20.81	–	0.17	–
24	cinnamyl alcohol	12.77	–	–	0.23	–	–	–	–	–
25	eugenol	14.29	2.40	1.63	4.59	–	2.32	–	0.63	3.02
26	cyclosativene	14.57	–	–	–	0.38	–	–	–	–
27	α-cubebene	14.84	0.57	–	–	2.95	–	–	0.41	–
28	α-fenchene	14.94	–	–	–	–	1.53	–	–	–
29	humulen	15.30	–	–	–	0.18	–	–	–	–
30	β-caryophyllene	16.19	4.71	1.09	1.07	–	1.60	1.02	0.66	0.80
31	coumarin	16.79	–	–	–	2.01	–	1.09	–	–
32	cinnamyl acetate	16.83	7.54	28.48	44.94	–	0.74	–	0.43	1.89
33	α-caryophyllene	17.23	0.78	–	–	–	–	–	–	–
34	aromadendrene	17.42	–	–	–	3.03	–	–	–	–
35	T-murrolene	17.82	–	–	–	1.77	–	–	–	–
36	valencene	17.95	–	–	–	0.47	–	–	–	–
37	α-murrolene	18.51	–	–	–	1.21	–	–	–	–
38	γ-murrolene	18.94	–	–	–	1.78	–	–	0.20	–
39	β-cadinene	19.19	0.62	0.61	0.65	1.44	0.56	–	0.61	–
40	γ-elemene	20.23	0.68	0.50	0.23	0.51	0.85	–	0.32	0.54
41	isoledene	20.80	0.54	–	–	2.24	–	0.50	0.24	–
42	caryophyllene oxide	20.98	3.59	0.91	0.26	8.02	0.75	0.49	0.68	0.79
43	guaiol	21.32	0.72	–	–	0.68	0.46	–	–	–
44	α-guaiene	21.54	–	–	–	0.80	–	–	–	–
45	unknown	22.20	–	–	–	10.32	–	–	–	–
46	copaene	22.25	–	0.35	–	–	–	–	0.34	–
47	T-cadinol	22.55	0.89	0.74	0.43	17.46	1.56	0.63	–	–
48	α-cadinol	22.94	–	0.61	0.51	11.68	0.97	–	–	–
49	azunol	23.48	–	–	–	2.94	–	–	–	–
50	β-cadinol	26.67	–	–	–	0.61	–	–	–	–
51	rimuen	30.03	1.41	1.75	–	–	–	–	–	–
52	labda-8(20),12,14-triene	30.39	–	0.38	–	–	–	–	–	–
53	verticiol	31.79	1.11	1.57	–	–	–	–	–	–
54	kaur-16-ene	32.82	–	0.39	–	–	–	–	–	–
identified components (%)			**99.6**	**100.0**	**100.0**	**86.4**	**100.0**	**99.2**	**99.3**	**100.0**
monoterpenes (%)			0	0	9.01	0	13.91	0	0.26	0
oxygenated monoterpenes (%)			4.69	1.61	1.54	11.90	73.51	93.68	0.87	0
sesquiterpenes (%)			7.36	2.55	1.95	14.52	3.01	1.02	2.54	1.34
oxygenated sesquiterpenes (%)			5.74	2.26	1.20	51.01	3.74	1.62	0.92	0.79
diterpenes (%)			1.41	2.52	0	0	0	0	0	0
oxygenated diterpenes (%)			1.11	1.57	0	0	0	0	0	0
other (%)			79.28	89.49	87.10	8.99	5.81	2.88	94.70	97.88
oil yield (%, v/dry wt)			**1.02**	**1.02**	**2.19**	**0.13**	**0.82**	**4.69**	**1.11**	**0.89**

[a] Retention time (minutes). [b] Not detected.

the classification by Hu et al. (*15*) and the results obtained from cluster analysis in this study, we classified the leaf essential oils of provenances A, G, and H as the cinnamaldehyde type, those of provenances B and C as the cinnamaldehyde/cinnamyl acetate type, that of provenance E as the camphor type, and that of provenance F as the linalool type. However, the essential oil from provenance D was classified as a mixed type because of the lack of a dominant compound.

Mosquito Larvicidal Activity of Essential Oils. Mosquito larvicidal activities of the five chemotypes of *C. osmophloeum* leaf essential oil against fourth-instar yellow fever mosquito (*A. aegypti*) larvae are shown in **Figure 2**. Results showed that the

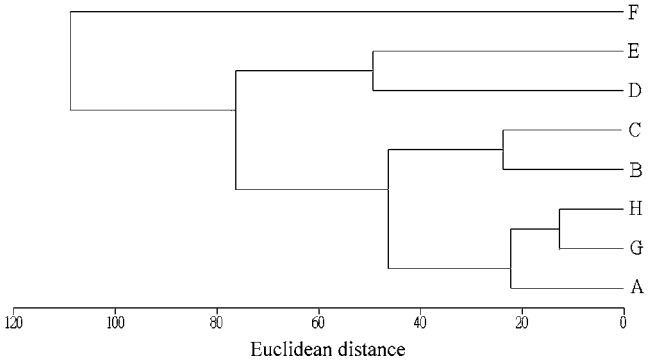

Figure 1. Dendrogram obtained by cluster analysis of the percentage composition of essential oils from eight provenances of *C. osmophloeum*.

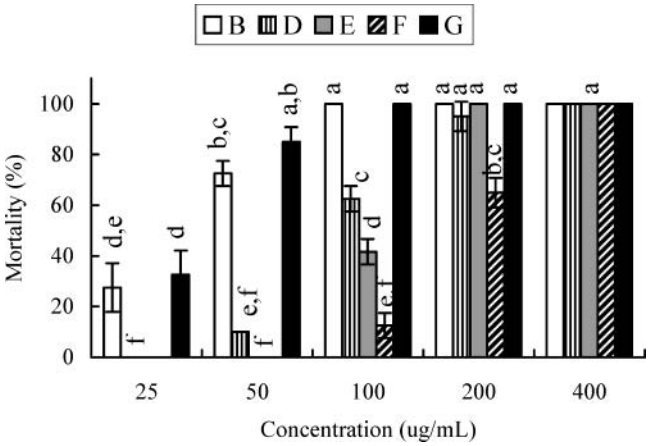

Figure 2. Mosquito larvicidal activities of leaf essential oils from five chemotypes of *C. osmophloeum* against the yellow fever mosquito *A. aegypti* after 24 h. Means ($n = 4$) were determined using 10 fourth-instar mosquito larvae per replicate. Numbers followed by different letters (a–f) are significantly different at the level of $P < 0.05$ according to the Scheffe test.

Table 2. LC_{50} and LC_{90} Values of Leaf Essential Oils of Five Chemotypes of *C. osmophloeum* against Yellow Fever Mosquito *A. aegypti* Larvae

	24 h		48 h	
chemotype	LC_{50} (μg/mL)	LC_{90} (μg/mL)	LC_{50} (μg/mL)	LC_{90} (μg/mL)
cinnamaldehyde type (provenance G)	36	79	23	43
cinnamaldehyde/cinnamyl acetate type (provenance B)	44	85	34	76
mixed type (provenance D)	108	180	94	168
camphor type (provenance E)	115	189	109	182
linalool type (provenance F)	177	296	144	318

cinnamaldehyde type (provenance G) and cinnamaldehyde/cinnamyl acetate type (provenance B) induced 100% larval mortality of *A. aegypti* after 24 h with a dosage of 100 μg/mL. However, a dosage of 200 μg/mL of camphor type (provenance E) and a dosage of 400 μg/mL of mixed type (provenance D) and linalool type (provenance F) were required to reach 100% larval mortality (**Figure 2**). Because the essential oils of mixed type (provenance D), camphor type (provenance E), and linalool type (provenance F) showed an $LC_{50} > 100$ μg/mL against fourth-instar *A. aegypti* larvae in 24 h (**Table 2**), they consequently were considered to be not active. Both the cinnamaldehyde type (provenance G) and cinnamaldehyde/cinnamyl acetate type (provenance B) essential oils showed an excellent

Table 3. LC_{50} and LC_{90} Values of 11 Compounds in *C. osmophloeum* Leaf Essential Oils against Yellow Fever Mosquito *A. aegypti* Larvae

	24 h		48 h	
compound	LC_{50} (μg/mL)	LC_{90} (μg/mL)	LC_{50} (μg/mL)	LC_{90} (μg/mL)
cinnamaldehyde	29	48	21	42
cinnamyl acetate	33	>50	26	48
benzaldehyde	>50	>50	33	>50
camphor	>50	>50	>50	>50
benzenepropanal	>50	>50	>50	>50
eugenol	33	>50	13	37
bornyl acetate	>50	>50	48	>50
β-caryophyllene	>50	>50	34	>50
caryophyllene oxide	>50	>50	>50	>50
anethole	42	>50	16	38
linalool	>50	>50	>50	>50

toxicity against *A. aegypti* larvae in 24 h, the LC_{50} values being 36 and 44 μg/mL with corresponding LC_{90} values of 79 and 85 μg/mL, respectively (**Table 2**). When incubation was extended to 48 h, the LC_{50} values of these two chemotypes were 23 μg/mL ($LC_{90} = 43$ μg/mL) and 34 μg/mL ($LC_{90} = 76$ μg/mL), respectively. Chang et al. (*6*) examined *Calocedrus formosana* leaf essential oil against *A. aegypti* larvae and found complete mortality at a concentration of 200 ppm. Araujo et al. (*23*) also found that *Hyptis martiusii* leaf essential oils induced 100% mortality of *A. aegypti* larvae after 1 day at a dosage of 500 mg/L. From comparisons of the results mentioned above, the essential oils of both cinnamaldehyde type (provenance G) and cinnamaldehyde/cinnamyl acetate type (provenance B) have excellent mosquito larvicidal activities.

Mosquito Larvicidal Activity of Main Compounds in Essential Oils. Eleven main compounds in indigenous cinnamon leaf essential oils were tested for mosquito larvicidal activity against fourth-instar *A. aegypti*. As shown in **Table 3**, among the 11 compounds tested for 24 h, cinnamaldehyde, cinnamyl acetate, eugenol, and anethole exhibited the strongest activities ($LC_{50} < 50$ μg/mL). The LC_{50} values of these four compounds were 29, 33, 33, and 42 μg/mL, respectively. It is clear that cinnamaldehyde has the best mosquito larvicidal activity, with an LC_{50} of 29 μg/mL ($LC_{90} = 48$ μg/mL). When the test was extended to 48 h, the LC_{90} values of cinnamaldehyde, cinnamyl acetate, eugenol, and anethole were 42, 48, 37, and 38 μg/mL, respectively (**Table 3**). Although cinnamaldehyde, cinnamyl acetate, eugenol, and anethole all possess a stronger mosquito larvicidal activity, eugenol and anethole exist in only minor quantities in the leaf essential oils of all *C. osmophloeum* provenances (A−H). In addition, cinnamyl acetate also exists as a minor essential oil constituent in most provenances except in provenances B (28.48%) and C (44.94%). Therefore, cinnamaldehyde clearly is responsible for the excellent mosquito larvicidal activity of provenance G and B essential oils.

Araujo et al. (*23*) demonstrated that 1,8-cineole caused a high mortality rate of *A. aegypti* larvae after 1 day of test at a dosage as low as 100 mg/L. Rahuman et al. (*24*) also found that *n*-hexadecanoic acid in *Feronia limonia* dried leaves was effective against fourth-instar larvae of *Culex quinquefasciatus*, *Anopheles stephensi*, and *A. aegypti* with LC_{50} values of 129.24, 79.58, and 57.23 ppm, respectively. In another investigation, Ramsewak et al. (*25*) found that both linoleic acid and oleic acid isolated from the hexane of *Dirca palustris* seeds had an LC_{50} value of 100 μg/mL when tested against fourth-instar *A. aegypti* larvae at 24 h. The results of the present study suggest that cinnamaldehyde is a potential natural mosquito larvicide.

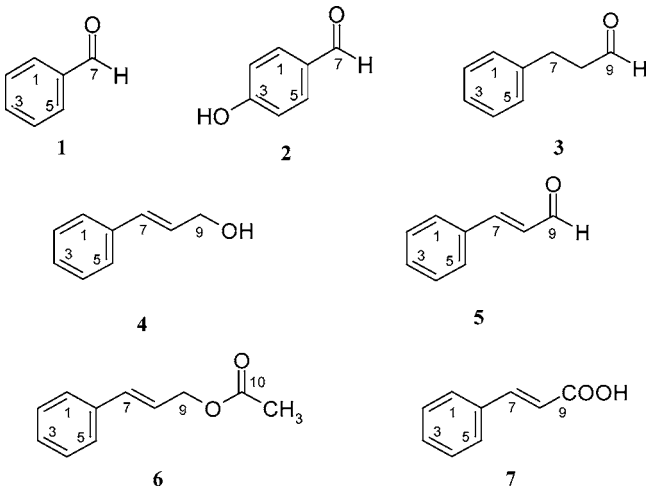

Figure 3. Chemical structures of cinnamaldehyde congeners: (**1**) benzaldehyde, (**2**) 4-hydroxybenzaldehyde, (**3**) benzenepropanal, (**4**) cinnamyl alcohol, (**5**) cinnamaldehyde, (**6**) cinnamyl acetate, and (**7**) cinnamic acid.

Table 4. LC_{50} and LC_{90} Values of Cinnamaldehyde Congeners in the Mosquito Larvacidal Assay against the Yellow Fever Mosquito *A. aegypti*

	24 h		48 h	
compound	LC_{50} (μg/mL)	LC_{90} (μg/mL)	LC_{50} (μg/mL)	LC_{90} (μg/mL)
cinnamyl acetate	33	>50	26	48
cinnamic acid	>50	>50	>50	>50
cinnamyl alcohol	>50	>50	>50	>50
cinnamaldehyde	29	48	21	42
4-hydroxybenzaldehyde	>50	>50	>50	>50
benzaldehyde	>50	>50	33	>50
benzenepropanal	>50	>50	>50	>50

Mosquito Larvicidal Activity of Cinnamaldehyde Congeners. To examine the structure−mosquito larvicidal activity relationships, cinnamic acid, cinnamyl alcohol, and cinnamyl acetate (**Figure 3**) with their chemical structures similar to cinnamaldehyde were studied for mosquito larvicidal activity. **Table 4** presents the LC_{50} and LC_{90} values of these compounds against yellow fever mosquito *A. aegypti*, showing that cinnamaldehyde had the strongest mosquito larvicidal activity. The 24-h mosquito larvicidal activity of these four cinnamaldehyde congeners in order was cinnamaldehyde (LC_{50} = 29 μg/mL; LC_{90} = 48 μg/mL) > cinnmyl acetate (LC_{50} = 33 μg/mL; LC_{90} > 50 μg/mL) > cinnamic acid (LC_{50} > 50 μg/mL; LC_{90} > 50 μg/mL) = cinnamyl alcohol (LC_{50} > 50 μg/mL; LC_{90} > 50 μg/mL). It is noted that cinnamaldehyde with an aldehyde group has the best mosquito larvicidal activity. Thus, the mosquito larvicidal activities of 4-hydroxybenzaldehyde, benzenepropanal, and benzaldehyde (**Figure 3**) also were studied. The LC_{50} values of all three of these compounds were >50 μg/mL. These results suggest that a compound having a conjugated double bond and a long CH chain outside the ring, such as cinnamaldehyde, has a much stronger mosquito larvicidal activity. A similar observation also was noted in our previous study on the antibacterial (*8*), antitermitic (*16*), and antifungal activities (*19*) of *C. osmophloeum*.

In conclusion, this study demonstrates that the cinnamaldehyde type and cinnamaldehyde/cinnamyl acetate type of *C. osmophloeum* leaf essential oils have excellent mosquito larvicidal activities and that cinnamaldehyde is responsible for such activity. Therefore, cinnamaldehyde as well as the cinnamaldehyde type and cinnamaldehyde/cinnamyl acetate type essential oils are natural and more selective larvicides against *A. aegypti* larvae.

ACKNOWLEDGMENT

We thank Yen-Ray Hsui, Dr. Han-Ming Yu, and Dr. Zenn-Zong Chen of the Taiwan Forestry Research Institute and the Taiwan Da-Pin-Ting Sugar Farm for providing *C. osmophloeum* materials and Dr. Sheng-Yang Wang and Pi-Wen Tsai of the Institute of BioAgricultural Sciences, Academia Sinica, Taiwan, for assistance in the GC-MS analysis.

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Received for review February 20, 2004. Revised manuscript received April 26, 2004. Accepted May 8, 2004. This study was supported by a grant from the Council of Agriculture (COA) of the Executive Yuan, Taiwan. We thank the COA for its financial support.

JF0497152