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Insecticidal activity of essential oil from *Chamaecyparis formosensis* Matsum

Pei-Min Kuo[1], Fang-Hua Chu[2], Shang-Tzen Chang[2], Wen-Feng Hsiao[3] and Sheng-Yang Wang[1,*]

[1] Department of Forestry, National Chung-Hsing University, Taichung, Taiwan

[2] School of Forestry and Resource Conservation, National Taiwan University, Taipei, Taiwan

[3] Department of Biological Resources, National Chiayi University, Chiayi, Taiwan

*Corresponding author.
Department of Forestry, National Chung-Hsing University, Taichung 402, Taiwan
Phone: +886-4-22840345 ext. 138
E-mail: taiwanfir@dragon.nchu.edu.tw

Abstract

Chamaecyparis formosensis is an endemic precious tree species grown in Taiwan. Based on the results obtained in our current study, the essential oil of *C. formosensis* wood possesses significant insecticidal activities. In an assay to assess mosquito larvicidal properties, the essential oil completely killed the larvae of *Aedes aegypti* and *Aedes albopictus* at a dosage of 100 $\mu g\ ml^{-1}$ in water. Concentrations of the essential oil at a dosage of 0.16 $mg\ cm^{-3}$ in air resulted in 100% mortality of silverfish after 2 h. It was found that the extractives of *C. formosensis* possess multiple bioactivities against insects. There is great potential for the development of insecticides for domestic application to kill mosquito larvae and silverfish based on this raw material.

Keywords: *Chamaecyparis formosensis*; essential oil; mosquito; silverfish; SPME.

Introduction

Plant-based products have been used to control domestic pests for a very long time. The search for and investigation of natural and environmentally friendly insecticidal substances are ongoing worldwide. Mosquitoes are responsible for transmitting more diseases than any other group of arthropods, being the major vectors for malaria, filariasis, dengue fever, yellow fever, and several viral diseases (Cheng et al. 2004; Hemingway 2004; Wandscheer et al. 2004). In Taiwan, dengue fever cases have increased significantly (Lee et al. 2006). Thus, the investigation of bioactive compounds from indigenous plants for mosquito control is being widely revisited (Cheng et al. 2003, 2004). Common silverfish (*Lepisma saccharina*) is another pest that is widely distributed in island-climate areas. Silverfish eat foods and consume materials rich in protein or carbohydrate, including paper, wallpaper glue, bookbinding, cereals, and dried meats. They can also damage some natural and synthetic fibers and they stain linens (Sloderbeck 2004).

Taiwan red cypress (*Chamaecyparis formosensis*) is endemic and grows at elevations of 1500–2150 m in Taiwan's central mountains (Liu et al. 1988). It is renowned for its excellent wood quality, beautiful texture and attractive fragrance. Investigation of the chemical ingredients in *C. formosensis* can be traced back to Kafuka and Ichikawa (1931), who studied the volatile constituents of its leaves and identified α-pinene as the major constituent (85%) in leaf oil. In addition, several terpenoids, including camphene, dipentene, cineol, α-terpinene, β-terpinene, borneol, bornyl acetate, bornyl formate, humulene, and cadinene, were identified. Later, Fang et al. (1986a) reanalyzed the leaf oil of *C. formosensis* and identified 41 terpenes. Besides the essential oil composition, constituents of root, bark, wood, and cones have also been studied (Nozoe et al. 1966; Fang et al. 1986b; Hsu et al. 1995). However, little research has been performed on the bioactivity of essential oil from *C. formosensis*. Wang et al. (2005) found strong antifungal activity of essential oil from *C. formosensis* leaves against wood decay fungi. Complete growth inhibition of *Laetiporus sulphureus* and *Trametes versicolor* was observed at concentrations as low as 50 and 100 $\mu g\ ml^{-1}$, respectively, in an agar plate assay.

To date, there is no related study on mosquito larvae and silverfish. In the present study, a larvicidal assay developed by Rafikali and Nair (2001) and Cheng et al. (2004) was applied. Moreover, a mortal activity test developed by Wang et al. (2006b) was used to assay the anti-silverfish activity.

Materials and methods

Extraction of oil from heartwood

Heartwood samples of 80-year-old *C. formosensis* were collected from the Experimental Forest of Nation Taiwan University and were identified by Yen-Hsueh Tseng (Department of Forestry, Nation Chung Hsing University). Voucher specimens (CF001-CF004) were deposited in the herbarium of the same university. A sample of 1 kg of air-dried wood chips was subjected to hydro-distillation for 8 h using a Clevenger-type apparatus, yielding 1.6% (vol/wt) of essential oil. Moisture-free essential oil was obtained by treatment with anhydrous Na_2SO_4.

Mosquito larvicidal test

Larvae of *Aedes albopictus* and *Aedes aegypti* were incubated at 27°C with a photoperiod of 12 h of light and 12 h of dark in

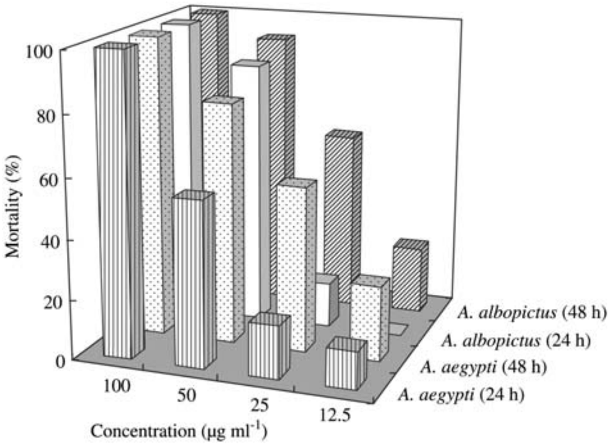

Figure 1 Activity of essential oil from *C. formosensis* against *A. aegypti* and *A. albopictus* larvae after 24 and 48 h.

80±10% relative humidity. A 10% yeast suspension was the growth medium. The methods of Rafikali and Nair (2001) and Cheng et al. (2004) were applied for mosquito larvicidal activity tests. However, the concentration of essential oil was different. Ten fourth instar mosquito larvae were placed in 24.5 ml of distilled water, then 500 μl of DMSO solution containing different concentrations of essential oil was added and incubated at room temperature. The control contained 24.5 ml of distilled water and 500 μl of DMSO. Each test was replicated four times and mortality was recorded after 24 and 48 h. Percentage mortality was corrected for control mortality using the Abbott (1925) formula: Corrected $\% = (1 - n_T$ after treatment$/n_{Co}$ after treatment$) \times 100$, where n is the insect population, T is treated, and Co is control. The results were plotted on log/probability paper as recommended by Finney (1971). Larvicidal activity was reported as LC_{50} and LC_{90}, representing the concentration in μg ml^{-1} that caused 50% and 90% larval mortality, respectively, in 24 or 48 h.

Insecticidal activity against silverfish

Adult silverfish (*Lepisma saccharina*) were collected and identified by Wen-Feng Hsiao (Department of Biological Resources, National Chiayi University). The silverfish were reared in plastic containers ($15\times20\times10$ cm^3) containing diet material (cellulose/mixed feed 1:1, with mixed feed of milk powder/oatmeal/yeast 1:9:1). Other parameters were a temperature of 25±3°C, 90% relative humidity, and darkness (Wang et al. 2006a). Various amounts of oil (0.085, 0.170, 0.340, 0.680 mg) were applied to filter paper squares (2 cm×2 cm), which were then inserted with 10 healthy adult silverfish into 9-cm-diameter Petri dishes (volume 4.26 cm^3). A few drops of water were put onto the bottom of the dishes. The test dishes were placed in a growth chamber maintained at 26°C and 90% relative humidity. Mortality was recorded periodically up to 8 h. Mortality (%) = (number of dead silverfish/total number of test silverfish)×100. Three replicates were performed for each sample.

Analysis of volatiles

The volatiles emitted in test Petri dishes were detected by solid-phase micro extraction (SPME). SPME holders and carboxen-poly-dimethylsiloxane (75 μm) fibers were purchased from Supelco (Bellefonte, PA, USA). SPME fibers were conditioned by heating in the hot injection port of a gas chromatograph at 250°C for 20 min. Volatiles were collected by placing an SPME holder containing fibers into the Petri dish for 10 min during the insecticidal assay. After extraction, the SPME fiber was inserted into the injection port of a GC equipped with an SPME liner and thermal desorption was allowed to occur at 250°C for 30 s.

A HP G1800A GC-MS system with a DB-5 column (30 m×0.25 mm internal Ø, 0.25 μm film thickness, J & W Scientific) was used for analysis. The temperature was held at 40°C for 1 min, then increased at 4°C min^{-1} to 260°C and held for a further 4 min. The other parameters were as follows: injection temperature, 250°C; ion source temperature, 280°C; EI, 70 eV; carrier gas, He at 1 ml min^{-1}; split ratio, 1:50; mass range, 45–425 *m/z*. Quantification was by percentage peak area. Volatiles were identified by comparison to Wiley/NBS Registry of Mass Spectral Databases and NIST search, or to authentic reference compounds. The Kováts indices (KI) were calculated and compared for identification. All area percentages were calculated using a response factor of 1.0.

Results

Mosquito larvicidal activity of essential oil

Figure 1 shows the effects of essential oil from *C. formosensis* against *A. aegypti* and *A. albopictus* after 24 and 48 h of exposure. At an oil concentration of 100 μg ml^{-1}, the mortality for both larvae was 100%. Even when the dosage was reduced to 50 μg ml^{-1}, the larvicidal activity was 55.0% and 87.5% for *A. aegypti* and *A. albopictus*, respectively. This activity was enhanced when the exposure time was increased to 48 h: the mortality was 80.0% for *A. aegypti* and 92.5% for *A. albopictus* at the dosage of 50 μg ml^{-1}. Table 1 presents LC_{50} and LC_{90} data for the larvae. After 24 h of exposure, 50% of *A. aegypti* and *A. albopictus* mosquito larvae were killed by essential oil dosages of 38.6 and 34.9 μg ml^{-1}, respectively. As expected, the lethal concentrations were lowered by an increase in exposure time to 48 h (Table 1).

Anti-silverfish activity of volatile compounds of essential oil

As illustrated in Figure 2, 70% of silverfish were killed at a concentration of 0.16 mg cm^{-3} after 2-h treatment; 8 h later, all silverfish were killed at this concentration of essential oil in air.

Table 1 Lethal concentrations of heartwood essential oil of *C. formosensis* causing 50% and 90% mortality (LC_{50} and LC_{90}) in larvae of *Aedes aegypti* and *A. albopictus*.

	A. aegypti		*A. albopictus*	
	24 h	48 h	24 h	48 h
LC_{50} (μg ml^{-1})	38.6	23.3	34.9	21.7
LC_{90} (μg ml^{-1})	97.2	70.7	73.6	61.6

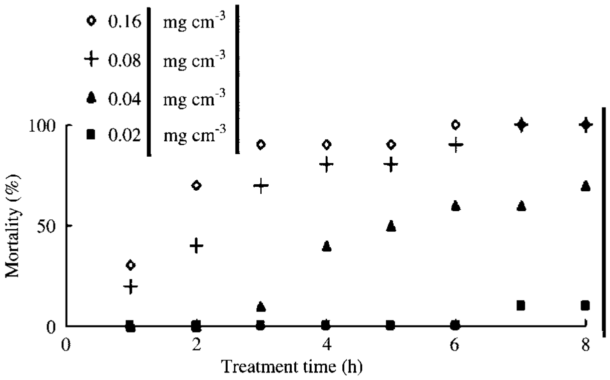

Figure 2 Silverfish mortality after 8-h treatment with essential oil from *C. formosensis*.

Composition of volatiles in anti-silverfish Petri dishes

The SPME technique was used to collect the compounds probably responsible for the anti-silverfish activity. The results are presented in Table 2. In total, 23 compounds were identified, with myrtenol predominant and accounting for 48.9% of total volatile constituents emitted. Myrtenal (13.2%), α-copaene (6.4%), T-muurolol (4.3%), camphene (3.4%), α-terpineol (2.8%), α-pinene (2.4%), β-myrcene (2.0%) and limonene (2.0%) were the other most abundant constituents emitted from the essential oil-impregnated paper.

Discussion

A large number of plant products have been reported to have mosquito repellent activity. For example, essential oils from citronella and lemon eucalyptus are effective and popular phytocompounds that provide the active ingredients of commercial repellents. Such substances are sold under several brand names (Trongtokit et al. 2005). In this study, we focus on the larvicidal activity of essential oil from *C. formosensis*. First, we demonstrated that the essential oil completely killed larvae of *A. aegypti* and *A. albopictus* at a dosage of 100 μg ml^{-1} in water. Compared with others plant extracts, the essential oil of *C. formosensis* exhibits significant activity against mosquito larvae. For example, Cheng et al. (2004) reported results for mosquito larvicidal activity of essential oils from leaves of *Cinnamomum osmophloeum*. Oils of the cinnamaldehyde and cinnamaldehyde/cinnamyl acetate types exhibited an excellent inhibitory effect against larvae of *A. aegypti* and *A. albopictus*. The LC_{50} doses were 36 and 44 μg ml^{-1}, respectively, in 24 h. According to our results, the LC_{50} doses of *C. formosensis* essential oil were 38.6 and 34.9 μg ml^{-1}, respectively. Chang et al. (2003) reported that essential oil of *Calocedrus formoseana* leaf killed *A. aegypti* larvae at a concentration of 200 μg ml^{-1}. Araujo et al. (2003) examined the essential oil of *Hyptis martiusii*, which could induce 100% mortality against *A. aegypti* larvae after 24 h at a dosage of 500 μg ml^{-1}.

Wang et al. (2006a) were the first to report that essential oils from the leaves of *Cryptomeria japonica* possess good silverfish repellency and caused high mortality. According to the results obtained in the present study, essential oil from *C. formosensis* is even more effective against silverfish than *C. japonica* essential oil. It thus has great potential as a natural insecticidal agent to inhibit silverfish activity.

The chemical composition of the essential oil of *C. formosensis* heartwood was determined by GC/MS

Table 2 Volatile constituents of essential oil from heartwood of *Chamaecyparis formosensis*.

Peak	KI	Compound	RT (min)	Conc. (%)	Identification
1	939	α-Pinene	9.76	2.43	MS, KI, ST
2	953	Camphene	10.50	3.43	MS, KI, ST
3	975	Sabinene	11.87	0.69	MS, KI
4	991	β-Myrcene	13.06	2.03	MS, KI, ST
5	1035	Limonene	13.21	1.97	MS, KI, ST
6	1060	γ-Terpinene	15.47	1.65	MS, KI, ST
7	1143	Camphor	17.17	0.95	MS, KI, ST
8	1169	Borneol	18.34	0.54	MS, KI, ST
9	1185	α-Terpineol	18.57	2.83	MS, KI, ST
10	1196	Myrtenol	19.65	48.89	MS, KI, ST
11	1193	Myrtenal	21.74	13.17	MS, KI, ST
12	1285	α-Bornyl acetate	22.50	0.90	MS, KI, ST
13	1351	α-Cubebene	23.14	1.66	MS, KI
14	1375	α-Copaene	26.09	6.37	MS, KI
15	1482	Geranyl acetate	26.40	1.65	MS, KI, ST
16	1490	β-Elemene	28.66	1.13	MS, KI, ST
17	1499	α-Muurolene	29.01	1.30	MS, KI
18	1506	β-Bisabolene	29.27	1.09	MS, KI, ST
19	1511	γ-Cadinene	29.39	1.25	MS, KI
20	1520	δ-Cadinene	29.81	0.61	MS, KI
21	1647	T-Muurolol	30.11	4.27	MS, KI, ST
22	1653	α-Cadinol	31.61	0.56	MS, KI, ST
23	2357	Ferruginol	52.42	0.62	MS, KI, ST

KI, Kováts index on a DB-5 column in reference to *n*-alkanes. RT, retention time. MS, mass spectra in NIST and Wiley libraries and in the literature; ST, authentic standard compounds.

(Wang et al. 2005). The most abundant components were α-eudesmol (18.1%), β-guaiene (8.0%), (–)-β-cadinene (7.9%), γ-costal (7.0%), α-muurolol (6.5%), 4α-hydroxy-4β-methyldihydrocastol (5.5%), humulene (4.1%), and myrtenol (4.1%). It has been demonstrated that a number of these components have bioactive properties. α-Eudesmol, for example, is a termite repellent (Watanabe et al. 2005) and has antimicrobial properties (Aboutabl et al. 2000). Humulene, (–)-β-cadinene, and α-muurolol are also bioactive (Cakir et al. 2005; Wu et al. 2005; Yonzon et al. 2005; Magwa et al. 2006). The mosquito larvicidal activity of these essential oils may be the result of individual compounds or may be due to synergism involving many of them.

α-Eudesmol, β-guaiene, β-cadinene, and γ-costal are the most abundant compounds in the essential oil of *C. formosensis* (Wang et al. 2005). However, these compounds were not detected when the volatiles were flushed with air. According to the SPME results of Wang et al. (2006b), the dominant aromatic compounds in the wood of *C. formosensis* are myrtenol (27.0%), myrtenal (19.2%), and γ-cadinene (11.4%). Myrtenol and myrtenal were also abundant in the volatiles detected in the present study. Fewer volatile compounds were detected in the silverfish assay (Table 2). There may be different mechanisms involved in mortality of mosquito larvae and silverfish. Less volatile compounds, which may be retained in the culture medium for a longer time, may contribute to mosquito larvicidal activity. In contrast, volatile compounds emitted from paper impregnated with essential oil may be the active compounds involved in silverfish mortality. Further studies are necessary to investigate the contribution of individual compounds and their synergism.

Natural products from plants are biodegradable. Thus, they are eco-friendly and attractive substitutes for synthetic chemicals (Shaalan et al. 2005). However, it should be emphasized that many naturally occurring bioactive compounds are extremely toxic and would not be deemed safe for domestic application. Thus, in future research, the safety of essential oil from the wood of *C. formosensis* will be further investigated. Nevertheless, it has great potential against mosquito larvae and silverfish.

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