Short Communication

Anti-termite activities of essential oils from coniferous trees against *Coptotermes formosanus*

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Abstract

In this study, the anti-termite activities of 11 essential oils from three species of coniferous tree against *Coptotermes formosanus* Shiraki were investigated using direct contact application. Results demonstrated that at the dosage of 10 mg/g, the heartwood and sapwood essential oils of *Calocedrus macrolepis* var. *formosana* and *Cryptomeria japonica* and the leaf essential oil of *Chamaecyparis obtusa* var. *formosana* had 100% mortality after 5 d of test. Among the tested essential oils, the heartwood essential oil of *C. macrolepis* var. *formosana* killed all termites after 1 d of test, with an LC_{50} value of 2.6 mg/g, exhibiting the strongest termiticidal property. The termiticidal effect of heartwood essential oil was due to its toxicity and its repellent action.

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Keywords: *Calocedrus macrolepis* var. *formosana*; *Cryptomeria japonica*; *Chamaecyparis obtusa* var. *formosana*; Heartwood; Essential oil; *Coptotermes formosanus*; Anti-termite activity

1. Introduction

Biodegradation of wood caused by termites is recognized as one of the most serious problems for wood utilization. It is also known that termites damage a variety of materials ranging from paper fabrics to even non-cellulosic materials such as asbestos, asphalt bitumen, lead, and metal foils (Bultman et al., 1979). Damage to wooden structures and other cellulosic materials by termites has been estimated to exceed \$3 billion annually worldwide (Su and Scheffrahn, 1990). *Coptotermes formosanus* Shiraki is the termite species responsible for most wood destruction in countries such as Taiwan, Japan, and parts of the United States (Chang and Cheng, 2002).

It is also well known that extractives have a significant effect on the durability of wood (Chang et al., 1999), and

certain extractives from wood tissues can provide protection against harmful insects. For nondurable woods, it may be necessary to use inorganic compounds or synthetic pesticides to preserve the woods and prolong their application life. To avoid environmental pollution and health problems caused by the use of traditional wood preservatives or synthetic pesticides, there is increasing interest in naturally occurring toxicants from plants (Chang et al., 2001). Many plant extracts and essential oils (Arihara et al., 2004; Chang et al., 2001; Chang and Cheng, 2002; Cheng et al., 2004; Park and Shin, 2005; Sakasegawa et al., 2003; Sogabe et al., 2000) may be the alternative sources of termite control agents because they constitute rich sources of bioactive chemicals. Therefore, the purpose of this study is to determine the bioactivity of 11 essential oils from three coniferous trees against *C. formosanus* Shiraki. In addition, the related anti-termite functions of *Calocedrus macrolepis* var. *formosana* heartwood and sapwood essential oils were also examined.

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2. Methods

2.1. Plant materials

Heartwood, bark and leaf of *Chamaecyparis obtusa* var. *formosana* were collected from the Dasi working circle located in Ilan. Heartwood, sapwood, bark and leaf of both *Cryptomeria japonica* and *C. macrolepis* var. *formosana* were collected from the Experimental Forest of National Taiwan University. The species were identified and voucher specimens (COHO01, COBO01, COLO01, CJHO01, CJSO01, CJBO01, CJLO01, CFHO01, CFSO01, CFBO01 and CFLO01) were deposited in the laboratory of wood chemistry (School of Forestry and Resource Conservation, National Taiwan University).

2.2. Essential oil distillation

The samples (200 g each), in triplicate, were subjected to hydrodistillation in a Clevenger-type apparatus for 6 h and the contents of the essential oils were determined (Chang et al., 2001). Eleven essential oils were stored in airtight containers prior to anti-termite tests.

2.3. Termite

The test termite species, *C. formosanus* Shiraki, was collected from Taipei in Northern Taiwan. The colony was reared in an incubator at 26.5 °C and 80% relative humidity (RH) for more than one year. Water and newspaper were used as the food source.

2.4. Anti-termite activity

The no-choice bioassay method of Kang et al. (1990) was employed to evaluate the anti-termite activity of the 11 essential oils. Samples of 1, 2.5, 5 and 10 mg of the essential oils dissolved in 600 µl of ethanol were applied to 1 g filter paper samples (Whatman No. 3, 8.5 cm in diameter). A piece of filter paper treated with solvent only was used as a control. After the solvent was removed from the treated filter papers by air-drying at ambient temperature, 33 active termites (30 workers and 3 soldiers) above the third instar were put on each piece of filter paper in a Petri dish (9 cm in diameter × 1.5 cm in height). The dishes with covers were then placed in an incubator at 26.5 °C and 80% RH. A few drops of water were periodically dripped onto the bottom edge of each Petri dish. Three replicates were made for each test sample, and the mortality of the termites was counted daily for 14 d.

2.5. Anti-termite function of essential oil

The method of Ohtani et al. (1996) was employed to distinguish the termiticidal, repellent and respiratory poisoning functions of essential oils. For testing method A, filter

paper was impregnated with essential oils and distilled water. For testing method B, one half of the filter paper was impregnated with essential oils and distilled water and the other half of the filter paper was impregnated with distilled water. For testing method C, essential oils were applied into 2.5 cm Petri dish and filter paper was impregnated with distilled water. The procedures used were the same as those described in Section 2.4.

2.6. Statistical analysis

The Scheffe multiple comparison procedure from the SAS statistical program was employed to evaluate differences in percent mortality for the anti-termite tests. Results with $P < 0.05$ were considered statistically significant. All results were obtained from three independent experiments and expressed as mean ± SD.

3. Results and discussion

3.1. Essential oil yields by water distillation

The 11 essential oils were distilled from various parts of three coniferous tree species, and analysis indicated that the yields of essential oils ranged from 0.2 to 27.4 ml/kg. The yields of heartwood, bark and leaf essential oils of *C. obtusa* var. *formosana* were 9.5, 2.6 and 14.1 ml/kg, respectively. The yields of heartwood, sapwood, bark and leaf essential oils of *C. japonica* and *C. macrolepis* var. *formosana* were 3.8, 1.3, 6.3 and 27.4 ml/kg and 5.8, 0.2, 5.5 and 3.4 ml/kg, respectively.

3.2. Anti-termite activities of essential oils

As can be seen from Table 1, three of the 11 essential oils extracted from the three coniferous tree species were incapable of killing all termites at a dosage of 10 mg/g after 14 d. Thus, the essential oils of bark of *C. japonica*, and bark and leaf of *C. macrolepis* var. *formosana* were considered inactive. Eight essential oils showed toxicity against *C. formosanus*: heartwood, bark and leaf essential oils of *C. obtusa* var. *formosana*; heartwood and sapwood essential oils of *C. macrolepis* var. *formosana*; and heartwood, sapwood and leaf essential oils of *C. japonica*. The leaf essential oil of *C. japonica* as well as heartwood and bark essential oils of *C. obtusa* var. *formosana* killed all termites within 12 d, 10 d and 14 d, respectively; the heartwood and sapwood essential oils of *C. macrolepis* var. *formosana* and *C. japonica* and the leaf essential oil of *C. obtusa* var. *formosana* showed 100% mortality after 5 d of test. Among the eight essential oils, heartwood essential oil of *C. macrolepis* var. *formosana* killed all termites after 1 d of test, indicating that it had the strongest toxicity against *C. formosanus*. In our previous studies, the termite mortalities of heartwood and sapwood essential oils of *Taiwania cryptomerioides* were 56% and 32% at a dosage of 10 mg/g after 14 d (Chang et al., 2001).

Table 1
Anti-termite activities of 11 essential oils extracted from three coniferous trees against *C. formosanus* at a dosage of 10 mg/g

Essential oils ^a	Termite mortality (%) ^b		
	1 d	5 d	14 d
COH	5.3 ± 2.3d,e,f	88.0 ± 8.0a	100.0 ± 0.0a
COB	2.7 ± 1.2f	40.0 ± 4.0b,c	100.0 ± 0.0a
COL	4.0 ± 0.0f	100.0 ± 0.0a	100.0 ± 0.0a
CJH	6.0 ± 1.7d,e,f	100.0 ± 0.0a	100.0 ± 0.0a
CJS	4.7 ± 5.0e,f	100.0 ± 0.0a	100.0 ± 0.0a
CJB	2.7 ± 2.3f	11.0 ± 2.3d,e,f	41.3 ± 1.2b,c
CJL	24.0 ± 5.3c,d	82.0 ± 12.2a	100.0 ± 0.0a
CFH	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
CFS	52.0 ± 3.5b,c	100.0 ± 0.0a	100.0 ± 0.0a
CFB	2.7 ± 1.2f	21.3 ± 3.1c,d,e,f	55.3 ± 5.0b
CFL	3.3 ± 3.1f	14.7 ± 2.0d,e,f	26.7 ± 4.6c,d,e
Control	0.0 ± 0.0f	1.3 ± 1.2f	2.7 ± 1.2f

Numbers followed by different letters (a–f) are significantly different at the level of $P < 0.05$ according to the Scheffe test.

^a CO: *Chamaecyparis obtuse* var. *formosana*, CJ: *Cryptomeria japonica*, CF: *Calocedrus macrolepis* var. *formosana*, H: heartwood, S: sapwood, B: bark, L: leaf.

^b Means ($n = 3$) using 33 termites per replicate.

Table 2
LC₅₀ values of 5 essential oils extracted from three coniferous trees against *C. formosanus*

Essential oils ^a	LC ₅₀ (mg/g)		
	1 d	5 d	7 d
COL	>10	3.1	2.8
CJH	>10	2.8	2.3
CJS	>10	4.7	2.8
CFH	2.6	1.7	1.6
CFS	4.9	2.1	1.9

^a See Table 1.

Comparison with the LC₅₀ values (Table 2) of the essential oils examined after 5 and 7 d of test against *C. formosanus* showed that *C. macrolepis* var. *formosana* heartwood essential oil (LC₅₀ = 1.7 and 1.6 mg/g) exhibited the highest anti-termite activity, followed by *C. macrolepis* var. *formosana* sapwood essential oil (LC₅₀ = 2.1 and 1.9 mg/g), *C. japonica* heartwood essential oil (LC₅₀ = 2.8 and 2.3 mg/g), *C. obtusa* var. *formosana* leaf essential oil (LC₅₀ = 3.1 and 2.8 mg/g) and then *C. japonica* sapwood essential oil

(LC₅₀ = 4.7 and 2.8 mg/g). In addition, the LC₅₀ values were 2.6, 4.9, >10.0, >10.0 and >10.0 mg/g even after 1 d of test for *C. macrolepis* var. *formosana* heartwood and sapwood essential oils, *C. japonica* heartwood and sapwood essential oils and *C. obtusa* var. *formosana* leaf essential oil, respectively. These results demonstrated that both heartwood and sapwood essential oils of *C. macrolepis* var. *formosana* have excellent anti-termite activities.

3.3. Anti-termite functions of *C. macrolepis* var. *formosana* heartwood and sapwood essential oils

Fig. 1 shows the results obtained by applying 2.5 mg of heartwood and sapwood essential oils of *C. macrolepis* var. *formosana* dissolved in 600 µl of ethanol to 1 g filter paper samples. The heartwood essential oil displayed termiticidal activity not only in termite test A, but also in termite test B. The termite mortality of heartwood essential oil in termite tests A and B after 7 d was 100% and 95%, respectively. In addition, the dead termites in termite test B were observed to be lying on the side of the filter paper impregnated with distilled water. Therefore, the anti-termite functions of heartwood essential oil could be attributed to its toxicity and its repellent action. In addition, the sapwood essential oil displayed high activity in termite test A, followed by termite test C and termite test B (termite mortality 62, 46 and 13%, respectively) after 7 d. This suggested that the anti-termite function of sapwood essential oil was due to its toxicity. The results demonstrated that both heartwood and sapwood essential oils of *C. macrolepis* var. *formosana* did not have respiratory poisoning functions for *C. formosanus*.

4. Conclusions

From the results it was concluded that heartwood and sapwood essential oils of *C. macrolepis* var. *formosana* and *C. japonica* and the leaf essential oil of *C. obtusa* var. *formosana* were termiticidal, showing 100% mortality after 5 d of test. The anti-termite function of heartwood essential oil was due to its toxicity and its repellent action. Thus, the essential oil from *C. macrolepis* var. *formosana* heartwood could be used as a potential natural termiticide.

Fig. 1. Anti-termite activities of essential oils extracted from *C. macrolepis* var. *formosana* heartwood (CFH) and sapwood (CFS) against *C. formosanus* at a dosage of 2.5 mg/g using three testing methods (A, B and C).

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