Terminating red imported fire ants using *Cinnamomum osmophloeum* leaf essential oil

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Abstract

Eleven compounds from indigenous cinnamon (*Cinnamomum osmophloeum*) leaf essential oil were identified by GC–MS and the dominant constituent was *trans*-cinnamaldehyde (79.85%). The toxicity of leaf essential oil and *trans*-cinnamaldehyde were then determined to study their effectiveness in controlling the red imported fire ant, *Solenopsis invicta* Buren. The results of the toxicity tests indicated that both the indigenous cinnamon leaf essential oil and *trans*-cinnamaldehyde had an excellent inhibitory effect in controlling the red imported fire ant. The LT₅₀ values for both 2% leaf essential oil and 2% *trans*-cinnamaldehyde after open exposure were 105.0 min and 32.2 min; after close exposure were 18.5 min and 21.2 min, respectively.

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1. Introduction

The red imported fire ant, *Solenopsis invicta* Buren, is a voracious consumer of numerous other arthropod species and often is the most abundant predaceous arthropod in crop fields throughout United States, New Zealand, and Australia (Natrass and Vanderwoude, 2001). It causes almost \$5 billion losses a year in urban and agricultural areas in the USA. In a large-scale invasion that has never been seen before, the spread of red imported fire ant in Taiwan increased at an alarming rate in the year of 2004. In general, the control of red imported fire ants is usually accomplished by using baits or traditional insecticides, such as pyrethroid or organophosphate, both contribute significantly to human health and environmental pollution. These problems have called for the need for new strategies

in red imported fire ant control. As a result, the search for natural, safe and none polluting insecticides is starting to attract a lot of attention, and many efforts have been focused on exploring bioactive chemical compounds from plants (Appel et al., 2004; Vogt et al., 2002; Cheng et al., 2004a).

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 (Liu et al., 1988). *C. osmophloeum* has been of interest to researchers because the chemical constituents of its leaf essential oil are similar to those of the famous *Cinnamomum cassia* bark oil (Chang et al., 2001; Hu et al., 1985). Cinnamon oil is commonly used in the food industry for its special aroma. In addition, its antimicrobial and antifungal properties have also drawn great attention from many researchers (Chang et al., 2001; Hili et al., 1997; Kim et al., 2004; Singh et al., 1995). Our previous studies demonstrated that leaf essential oils from cinnamaldehyde type of *C. osmophloeum* had excellent antitermite,

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antibacterial, antimitic, antimosquito, antipathogenic and antifungal properties (Chang et al., 2001; Chang and Cheng, 2002; Chen et al., 2002; Cheng et al., 2004b; Cheng et al., 2006; Lee et al., 2005; Wang et al., 2005). In this study, leaf essential oil from *C. osmophloeum* was analyzed using GC–MS for its chemical composition and the toxicity of indigenous cinnamon leaf essential oil and *trans*-cinnamaldehyde were determined to evaluate the effectiveness of both in controlling the red imported fire ant.

2. Methods

2.1. Insect

Workers of red imported fire ants were reared in the Department of Entomology, National Taiwan University in Taiwan. Workers and nest material were placed in glass column bottle (20 cm diameter $\times$ 30 cm) and the bottle was then coated with Teflon emulsion on the top. The nest material was held at 25–27 °C and a photoperiod alternating between 12-h in light and 12-h in the dark at $80 \pm 5\%$ RH. A 10% sucrose solution, dead crickets, and peanut butter were used as the growth media.

2.2. Collection of essential oil from *C. osmophloeum* leaf

The leaves from a 12-year-old *C. osmophloeum* were collected in September, 2002 from Da-Pin-Ting of the Taiwan Sugar Farm located in Nantou county in central Taiwan. The species was identified by Mr. Yen-Ray Hsui of the Taiwan Forestry Research Institute, and the voucher specimen (CO1001) was deposited at the laboratory of wood chemistry, School of Forestry and Resource Conservation, National Taiwan University. Fresh leaf essential oil from *C. osmophloeum* was obtained using water distillation in a Clevenger-type apparatus for 6 h (Chang et al., 2001) and their constituents were determined by gas chromatography–mass spectrometry.

2.3. Gas chromatography–mass spectrometry analysis

The mass spectrometer was equipped with a PoLaris Q mass selective detector in electron impact (EI) ionization mode (70 eV). A Trace GC Ultra was used and operated under the following conditions: a DB-5MS capillary column (5% phenyl methylpolysiloxane, 30 m $\times$ 0.25 mm; film thickness 0.25 μ m) was held at 50 °C for 2 min, then raised to 250 °C at the rate of 5 °C/min, and held for 5 min; helium carrier gas was used and held at the flow rate of 1.0 ml/min; the split ratio was kept at 1:60. Diluted samples (1.0 μ l, 1/100, v/v, in ethyl acetate) were injected manually in split mode. The ion source temperature was set at 200 °C, and the injector temperature was set at 270 °C. 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–C₁₅ on DB-5MS column.

Quantification was performed using percentage peak area calculations and the identification of individual components was done using the NIST MS Search 2.0, literature (Adams, 2001), and several authentic reference compounds.

2.4. Isolation and identification

One active component (9.04 min retention time) was isolated and purified from *C. osmophloeum* leaf essential oil by semipreparative HPLC on a model L-7150 instrument (Hitachi, Japan) with a 250 $\times$ 10 mm i.d., 5 μ m Luna Silica (2) (Phenomenex, American) column, ethyl acetate/hexane (15:85) mobile phase, 4.0 ml/min flow rate, and Hitachi L-7490 RI detector. Structural determination of the compound isolated was made by spectroscopic analyses, including UV spectrum was recorded with a Jasco V-550 spectrometer, IR spectrum was obtained from a Bio-Rad FTS-40 spectrometer, Electron impact mass spectrometer (EIMS) data was obtained on a Finnigan MAT-95S mass spectrometer, ¹H and ¹³C NMR spectra were recorded on a Bruker Avance 500 spectrometer at 500 MHz, and by direct comparison with an authentic reference compound, and it was characterized as *trans*-cinnamaldehyde on the basis of the data from the literature (Lee et al., 2002). The compound was identified on the basis of the following evidence: UV (EtOH), λ_{max} : 285 nm. IR (KBr), cm^{−1}: 2925, 2816, 2744, 1682, 1625, 1574, 1450, 1123, 748, 690. EIMS (70 eV), *m/z* (relative intensity) for C₉H₈O found 132, M⁺ 132 (59), 131 (100), 103 (50), 77 (35), 51 (23). ¹H NMR (CD₃OD, 500 MHz) δ 6.67 (dd, 1H, *J* = 7.7 and 16 Hz, H-8), 7.45 (d, 1H, *J* = 16 Hz, H-7), 7.41 (dd, 1H, H-4), 7.40–7.41 (m, 2H, H-2 and H-6), 7.53–7.55 (m, 2H, H-3 and H-5), 9.67 (d, 1H, *J* = 7.7 Hz, H-9); ¹³C NMR (CD₃OD, 500 MHz) δ 193.6 (C-9), 152.7 (C-7), 133.9 (C-1), 131.2 (C-4), 129.0 (C-2 and C-6), 128.5 (C-8), 128.4 (C-3 and C-5).

2.5. Constituents of essential oil

The following compounds of essential oil constituents were purchased from Acros (Belgium): benzaldehyde, benzalpropanal, eugenol, L-bornyl acetate, and cinnamyl acetate. *p*-Allylanisole and β -caryophyllene were purchased from TCI (Japan). *trans*-Cinnamaldehyde was isolated from *C. osmophloeum* leaf essential oil.

2.6. Open and close exposure toxicity tests

The method of Appel et al. (2004) was modified slightly and used to determine the effectiveness of controlling the red imported fire ant. Beakers of 10 cm diameter bottoms were used for both the open and close exposure toxicity tests. The inside vertical wall of each beaker was coated with Teflon emulsion and allowed to dry to prevent the red imported fire ants from escaping and to force the red imported fire ants onto granules placed on the bottom.

For the open exposure toxicity test, 20 workers of red imported fire ants were transferred into a beaker, followed by spraying EtOH solution containing *C. osmophloeum* leaf essential oil or *trans*-cinnamaldehyde into the beaker. The red imported fire ants sprayed with *C. osmophloeum* leaf essential oil or *trans*-cinnamaldehyde were uncovered and exposed to the air. For the close exposure toxicity tests, groups of 20 workers were placed in a beaker, followed by spraying EtOH solution containing essential oil or *trans*-cinnamaldehyde in the beaker. The beakers were covered so they were not exposed to the air. Essential oil or *trans*-cinnamaldehyde was tested at the concentrations of 2%, 1% and 0.5% in EtOH. The ants in the control beaker were only sprayed by EtOH solution that did not contain any essential oil or *trans*-cinnamaldehyde. Each test was replicated three times.

Mortality was recorded every 10 min for a total of 90 min for the close exposure toxicity tests and every 30 min for 3 h for the open exposure toxicity tests. During the tests no food was given to the fire ants. 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 LT_{50} , the time it took, in min, to cause 50% fire ant mortality.

2.7. Statistical analyses

Percent of fire ant mortality was determined and transformed to arcsine square-root values for analyses of variance (ANOVA). Treatment means were compared and separated using the Scheffe test at $P = 0.05$. The means ($\pm$ SE) of untransformed data are reported.

3. Results and discussion

3.1. Chemical compositions of essential oil

Distillation of *C. osmophloeum* leaves yielded about 1.02% (w/w) essential oil based on dry weight. The results of GC–MS analyses of the *C. osmophloeum* leaves essential oil are compiled and shown in Table 1. It shows that the essential oil contain eleven compounds representing 99.16% of the essential oils. *trans*-Cinnamaldehyde with a yield of 79.85% is the dominant constituent that matches the finding in our previous classification (Cheng et al., 2004b) that the leaf essential oil from the *C. osmophloeum* was in the class of cinnamaldehyde and the percentage distribution of essential oil in the leaves of *C. osmophloeum* are similar to that reported by Fang et al. (1989) and Cheng et al. (2004b, 2006).

3.2. Open and close exposure toxicity of essential oil

The toxicities of indigenous cinnamon leaf essential oil after close exposure and open exposure were determined for controlling the red imported fire ant (Figs. 1 and 2).

Table 1

Chemical compositions of leaf essential oil from *C. osmophloeum*

No.	Compounds	RT ^a	KI ^b	Peak area (%)	Identification ^c
1	Benzaldehyde	8.12	961	5.35	MS, KI, CO
2	Benzenepropanal	14.00	1163	7.00	MS, CO
3	<i>p</i> -Allylanisole	15.07	1198	1.61	MS, CO
4	<i>cis</i> -Cinnamaldehyde	15.63	1219	1.91	MS, KI
5	4-Allylphenol	16.68	1258	0.22	MS
6	<i>trans</i> -Cinnamaldehyde	17.06	1271	79.85	MS, KI, CO
7	L-Bornyl acetate	17.56	1289	1.11	MS, KI, CO
8	Eugenol	19.43	1360	1.13	MS, KI, CO
9	α -Cubebene	20.03	1382	0.17	MS, KI
10	β -Caryophyllene	21.18	1426	0.19	MS, KI, CO
11	Cinnamyl acetate	21.66	1446	0.62	MS, KI, CO
Identified components (%)				99.16	
Yield (%)				1.02	

^a Retention time (min).

^b Kovats retention index relative to *n*-alkanes (C₉–C₁₅) on DB-5MS column.

^c MS, by comparison of the mass spectrum with those of the computer mass libraries; KI, by comparison of Kovats retention index with those from the literature (Adams, 2001); CO, identified by co-injection of an authentic sample.

Fig. 1. Mortality of red imported fire ant after close exposure to *C. osmophloeum* leaf essential oil. Each data point represents mean $\pm$ SE of three replicate cups each containing 20 ants.

Fig. 1 shows that 2.0% indigenous cinnamon leaf essential oil terminated red imported fire ant completely after 40.0 min and the LT_{50} value was 18.5 min (Table 2) in the close exposure tests, indicating that it has the strongest toxicity against red imported fire ant. When concentration decreased to 1.0% and 0.5%, the indigenous cinnamon leaf essential oil induced 100% mortality of red imported fire ant after 60.0 min and 90.0 min, respectively. Their LT_{50} values were 24.2 min and 31.3 min, respectively (Table 2). The mortality of the control was 28.3% after 90.0 min against red imported fire ant, showing that EtOH did have a low toxicity effect. In the open exposure tests, the mortality of cinnamon leaf essential oil against red imported fire ant after 180 min was 3.3% ($LT_{50} > 180.0$ min), 6.7%

Fig. 2. Mortality of red imported fire ant after open exposure to *C. osmophloeum* leaf essential oil. Each data point represents mean $\pm$ SE of three replicate cups each containing 20 ants.

Table 2

LT₅₀ (min) values of red imported fire ant after close and open exposures to *C. osmophloeum* leaf essential oil and *trans*-cinnamaldehyde

Concentration (%)	Leaf essential oil		<i>trans</i> -Cinnamaldehyde	
	Open	Close	Open	Close
0	>180.0	>90.0	>180.0	>90.0
0.5	>180.0	31.3	>180.0	39.5
1.0	>180.0	24.2	64.8	31.0
2.0	105.0	18.5	32.2	21.2

(LT₅₀ > 180.0 min), 33.3% (LT₅₀ > 180.0 min), and 61.7% (LT₅₀ = 105.0 min) at the concentration of 0%, 0.5%, 1.0%, and 2.0%, respectively (Fig. 2; Table 2). Similar results have been observed in the Appel et al. (2004) study; the LT₅₀ value of 2.0% mint oil granules (164.81 mg/cm²) was 1.2 h in continuous exposure. These results indicate that leaf essential oil provided higher inhibitory performance against red imported fire ant in the closed exposure than in the open exposure.

3.3. Open and close exposure toxicity of *trans*-cinnamaldehyde

Since leaf essential oil contained a higher amount of *trans*-cinnamaldehyde (Table 1) and it also displayed strong toxicity, it is clear that the mortality of red imported fire ant of leaf essential oil is directly affected by the *trans*-cinnamaldehyde content. The results of *trans*-cinnamaldehyde after close exposure and open exposure were reported in Figs. 3 and 4. In the close exposure tests, 0.5%, 1.0%, and 2.0% of *trans*-cinnamaldehyde killed all red imported fire ants after 90.0 min, 70 min, and 50.0 min, respectively (Fig. 3), indicating that *trans*-cinnamaldehyde has the strongest toxicity against red imported fire ant. As seen in Fig. 4, 2.0% *trans*-cinnamaldehyde terminated all red imported fire ant in the open exposure tests after 80.0 min, followed by 1.0% *trans*-cinnamaldehyde (180 min). The mortality of 0.5% *trans*-cinnamaldehyde was only 18.3% after 180.0 min

Fig. 3. Mortality of red imported fire ant after close exposure to *trans*-cinnamaldehyde. Each data point represents mean $\pm$ SE of three replicate cups each containing 20 ants.

Fig. 4. Mortality of red imported fire ant after open exposure to *trans*-cinnamaldehyde. Each data point represents mean $\pm$ SE of three replicate cups each containing 20 ants.

showing that 0.5% is too diluted to have an effective termination effect.

Comparing the LT₅₀ values of *trans*-cinnamaldehyde after open and close exposures as shown in Table 2, it indicated that 2.0% *trans*-cinnamaldehyde exhibited the strongest termination activity with a LT₅₀ value of 32.2 min (open exposure) and 21.2 min (close exposure), followed by 1.0% *trans*-cinnamaldehyde (LT₅₀ = 64.8 min and 31.0 min), and 0.5% *trans*-cinnamaldehyde (LT₅₀ > 180.0 min and =39.5 min). It is therefore proven that *trans*-cinnamaldehyde is responsible for the excellent termination effect against red imported fire ant using leaf essential oil.

4. Conclusions

The leaf essential oil of *C. osmophloeum* exhibits effective toxicity in both open exposure and close exposure against red imported fire ant. The major component in the essential oil is *trans*-cinnamaldehyde and it plays the key role in controlling the red imported fire ant. As a conclusion, both indigenous cinnamon leaf essential oil and *trans*-cinnamaldehyde are effective and environmentally benign agents in red imported fire ant control.

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