

## RESEARCH

# Insecticidal, Fumigant, and Repellent Activities of Sweet Wormwood Oil and Its Individual Components Against Red Imported Fire Ant Workers (Hymenoptera: Formicidae)

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**ABSTRACT.** In total, 29 compounds from sweet wormwood (*Artemisia annua* L.) oil were identified using gas chromatography–mass spectrometry. The five active components were *D*-camphor, linalool, cineole,  $\alpha$ -terpineol, and  $\iota$ (–)-borneol. The effectiveness of *A. annua* oil, as well as *D*-camphor, linalool, cineole,  $\alpha$ -terpineol, and  $\iota$ (–)-borneol, as fumigants, contact insecticides, and repellents, were tested on the red imported fire ant *Solenopsis invicta* Buren. The results indicated that *A. annua* oil has no significant topical toxicity; however, the spray contact test revealed that it has strong insecticidal activity and the inhibitory effect is stronger during closed exposure than during open exposure. In the fumigant test, cineole and *D*-camphor exhibited strong fumigant toxicity on minor and major *S. invicta* workers. They also caused 100% mortality at 5, 3, 2, and 1 mg/centrifuge tube but not at 0.5 mg/centrifuge tube. The mortality rates of linalool,  $\alpha$ -terpineol, and  $\iota$ (–)-borneol exceeded 80% at 5, 3, and 2 mg/centrifuge tube. In the repellent test, cineole and *D*-camphor showed significant repellency at 100, 10, and 1 mg/kg. However, linalool,  $\alpha$ -terpineol, and  $\iota$ (–)-borneol significantly facilitated digging at 10 and 1 mg/kg.

**Key Words:** sweet wormwood oil, contact, fumigation, repellency

Red imported fire ants (*Solenopsis invicta* Buren) is a fierce omnivorous species found throughout Southern Brazil, Argentina, and Paraguay that was introduced into the United States, New Zealand, Australia, Taiwan, and China (Natrass and Vanderwoude 2001, Ascunce et al. 2011). The invading pest has outcompeted and eliminated many native ant species in rural, urban, and agricultural areas and it has disturbed the ecological balance. The native ant species in highly infested areas have declined by up to 90% through displacement by the invasive ants (Porter and Savignano 1990). Increasing populations of red imported fire ants negatively affect arthropods that they prey on at all life stages, including eggs, larvae, pupae, and adults (Stiles and Jones 2011). Moreover, these ants have caused serious damage to humans, animals, agriculture, and the environment. Traditional methods for managing *S. invicta* include using baits and conventional contact insecticides, such as chlorpyrifos and pyrethroid. However, these insecticides caused environmental contamination; thus, nontoxic or less-toxic treatments are receiving increasing attention (Vogt et al. 2002, Appel et al. 2004).

Essential oils (EOs) extracted from plants or secondary plant metabolites are widely used as fragrances and flavors in the perfume and food industries. EOs are also often used as repellents, contact insecticides, and fumigant insecticides in agriculture. Tripathi et al. (2002) investigated the leaf EOs of *Curcuma longa* L. for contact and fumigant toxicity on *Rhyzopertha dominica* F., *Sitophilus oryzae* L., and *Tribolium castaneum* Herbst. Cheng et al. (2008) reported the toxicity of EO from *Cinnamomum osmophloeum* leaves and its main chemical composition, *trans*-cinnamaldehyde, against *S. invicta* in open and closed exposure experiments. They found that the indigenous cinnamon leaf EO and *trans*-cinnamaldehyde have an excellent inhibitory effect in controlling *S. invicta*. *Hedychium* EOs, callicarpenal, and intermedeol isolated from the leaves of American beautyberry (*Callicarpa americana* L.)

and Japanese beautyberry (*C. japonica* Thunb.), as well as mint oil granules, are toxic and repellent to *S. invicta* (Appel et al. 2004, Chen et al. 2008, Sakhanokho et al. 2013).

The genus *Artemisia* is a member of family Asteraceae and consists of approximately 200 herb and shrub species distributed worldwide. *Artemisia annua* L. (annual wormwood, sweet wormwood, and sweet annie) is a highly aromatic annual herb of Asiatic and Eastern European origin. *A. annua* is rich in biologically active terpenoids (Lawrence 1982, Duke et al. 1988). It is traditionally grown in China as a medicinal plant. Our previous study found that *A. annua* oil has strong fumigant toxicity against *S. invicta* (Tang et al. 2013). Thus, the aim of this study is determine the chemical composition of sweet wormwood oil and evaluate fumigant, contact, and repellent activity of main active compounds against the red imported fire ant.

## Materials and Methods

***A. annua* Oil and Its Chemical Components.** *A. annua* was obtained from Kangshen Natural Medicinal Oil Refinery (Jiangxi, China). *D*-Camphor (purity > 98%), linalool (purity > 98%), cineole (purity > 99%),  $\alpha$ -terpineol (purity > 98%), and  $\iota$ (–)-borneol (purity > 98%) were purchased from Shanghai Jingchun Scientific Company (Shanghai, China).

*A. annua* oil and its chemical components were diluted with acetone to the desired concentration on the day of the experiments.

**Insects.** Workers from *S. invicta* colonies were collected from the suburbs of Guangzhou. The ants were reared in plastic boxes (38 by 28 by 17 cm), which were coated with Teflon emulsion on the top, at  $25 \pm 2^\circ\text{C}$  and 65–85% relative humidity (RH). A test tube (25 by 200 mm), which was partially filled with water and plugged with cotton, was used as a water source, and mealworms (*Tenebrio molitor*) were used as a food source.

*S. invicta* workers were classified as minor (2.8–3.0 mm body length, 0.6–0.7 mm head width), medium (3.5–3.7 mm body length, 0.8–0.9 mm head width), and major workers (4.3–4.5 mm body length, 1.0–1.1 mm head width).

**Open and Closed Exposure Toxicity Tests.** Contact activity was determined using the method proposed by Cheng et al. (2008), with some modifications. Glass cylinders (5 cm diameter by 10 cm) were used for the open and closed exposure toxicity tests. The vertical wall inside each glass cylinder was coated with Fluon emulsion and allowed to dry for 24 h to prevent the ants from escaping. In the open exposure toxicity tests, 20 workers were sprayed with 1 ml of acetone solution containing 2.0, 1.0, and 0.5% *A. annua* oil, and placed in a glass cylinder. The cylinders were uncovered and exposed to the air. In the closed exposure toxicity tests, 20 workers were confined in the cylinder and sprayed with acetone solution containing *A. annua* oil. The lid was then sealed so that the ants were not exposed to the air. The ants that were sprayed only with acetone solution were used as the control. All treatments were performed three times. The workers were maintained at  $25 \pm 1^\circ\text{C}$  and 80% RH.

Mortality was recorded every 30 min for 3 h for open exposure toxicity tests, and every 10 min for 90 min for closed exposure toxicity tests. During the tests, no food was given to the workers.  $LT_{50}$ , which is the estimated time (in minutes) to reach 50% mortality, was used to assess toxicity and activity.

**Topical Contact Toxicity.** The insecticidal activity of sweet wormwood oil was evaluated through topical application, as previously reported in the methods by Guerra et al. (2011) with slight modifications. A hand microapplicator (Burkard Manufacturing Co., Hertfordshire, United Kingdom) was used to topically apply 1  $\mu\text{l}$  of acetone solution containing 15.6  $\mu\text{g}/\text{head}$  of EOs onto the abdomen of each medium worker. The control groups were treated with acetone. Fifteen workers were placed in a plastic cup (5 cm by 7.5 cm) coated with Fluon emulsion and allowed to dry for 24 h to prevent the ants from escaping. Each test was performed three times. Mortality was recorded after 1, 3, 5, and 7 d.

**Chemical Analysis Using Gas Chromatography-Mass Spectrometry.** Sample isolation and cleanup were not needed, and the sample was detected directly using an Agilent 6890 gas chromatograph coupled with an Agilent mass spectrometer detector. A HP-5 capillary column (30.00 m by 0.25 mm by 0.25  $\mu\text{m}$ ) was used for gas chromatography–mass spectrometry (GC-MS). Mass spectra were obtained by electron ionization (EI) at 70 eV with a mass range of  $m/z$  50 to 500. The injector was maintained at  $250^\circ\text{C}$ . The oven temperature was set at  $50^\circ\text{C}$  for 5 min, and subsequently increased to  $220^\circ\text{C}$  ( $10^\circ\text{C}/\text{min}$ ) for 5 min. The detector was operated at  $300^\circ\text{C}$ . Helium was used as a carrier gas at a flow rate of 1 ml/min and a split ratio of 50:1. The components were identified by comparing their mass spectra with those of the computer mass libraries.

**Fumigant Toxicity Bioassay.** The insecticidal activity of the main constituents of *A. annua* oil were evaluated using a fumigant toxicity bioassay, as previously reported by Seo et al. (2009) with slight modifications. The compounds were placed in a 1.5-ml centrifuge tube. Eight pinholes were drilled into the tube to allow the compounds to vaporize outside. The tube was then placed at the bottom lid of a glass cylinder (5 cm diameter by 10 cm). The vertical wall inside each glass cylinder was coated with Fluon emulsion and allowed to dry for 24 h to prevent the ants from escaping. In total, 15 minor workers and 15 major workers were placed separately at the bottom of the cylinder, and the cylinder was sealed with a lid. Mortality was assessed after 24 and 48 h of exposure to the components at 0.5, 1, 2, 3, and 5 mg/centrifuge tubes. Each test was performed three times.

**Repellent Activity.** The five major ingredients were evaluated for repellency using a two-choice digging bioassay, as described by Chen 2009 with some modifications. The rationale for this bioassay is that ants will always express digging behavior whenever an adequate digging substrate, such as sand, is available. However, ants do not dig or

they dig less when the substrate contains a repellent. Therefore, repellency was defined as suppression of ant digging behavior. Four holes were drilled into a plastic dish (8.5 cm by 1.5 cm), and the vertical wall inside each glass cylinder was coated with Fluon emulsion and allowed to dry for 24 h to prevent the ants from escaping. Four 2-ml centrifuge tubes were fastened to the dish, two of which were filled with sand. One tube was filled with treated sand, whereas the other tube was filled with control sand. The remaining tubes were used to support the dish without any substrate. The treated and control tubes were pinned opposite each other about 5 cm apart in the center of the dish. A 3-mm diameter access hole was drilled into the caps of each of the two tubes. Sand was first sieved through a #40 testing sieve, washed with distilled water, and dried for 12 h at  $350^\circ\text{C}$ . Then, 3 ml of dichloromethane was added to 30 g of treated sand. The sand was stirred every 2 min to facilitate solvent evaporation under a fume hood. After the solvent evaporated, 1.92 ml of distilled water was mixed with the sand. In each tube,  $2.70 \pm 0.2$  g of sand was added. Twenty medium workers were introduced to the center of the dish.

In this study, *D*-camphor, linalool, cineole,  $\alpha$ -terpineol, and  $\iota$ (-)-borneol were prepared at 1.0, 10.0, and 100.0 mg/kg. Control sand was treated only with dichloromethane and water. Each experiment was replicated five times. All experiments were carried out at  $25 \pm 1^\circ\text{C}$  and 50% RH. After 24 h, the tubes and sand were weighed. Digging suppression index (DSI) was calculated to compare the repellency using the following formula:

$$\text{DSI} = (A_c - A_t)/(A_c + A_t)$$

where  $A_c$  is the amount of sand removed from the control tube and  $A_t$  is the amount of sand removed from the treated tube.

**Statistical Analysis.** The mortality and repellent rates were analyzed and transformed to arcsine square root values for analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT). All data are expressed as means  $\pm$  SE. Statistical analyses were carried out using SPSS 13.0 (SPSS Inc., Chicago, USA). Differences with  $P < 0.05$  were considered significant.

## Results

**Open and Closed Exposure Toxicity Tests.** Figures 1 and 2 show the open and closed exposure toxicity of *A. annua* oil to medium workers. In the open exposure tests, all *S. invicta* workers were killed after 180 min of exposure to 2.0% oil, and the  $LT_{50}$  value was 85.5 min (Table 1). The mortality rate was only 51.67% ( $LT_{50} > 180.0$  min) after 180 min of exposure to 1.0% oil and 48.33% ( $LT_{50} > 180.0$  min) after 180 min of exposure to 0.5% oil (Fig. 1; Table 1). Figure 2 shows the

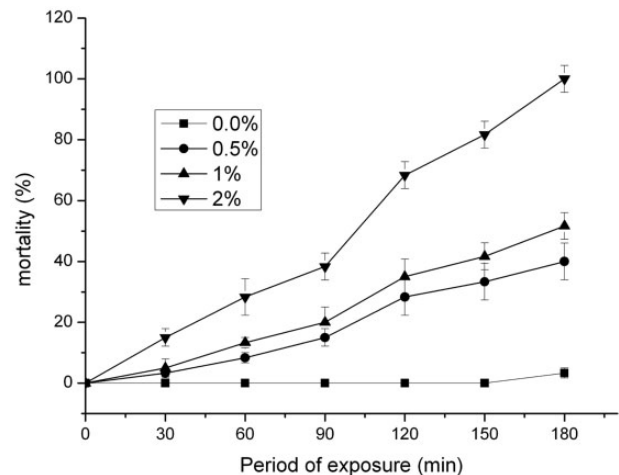


Fig. 1. Mortality of medium workers of red imported fire ants after open exposure to sweet wormwood oil.

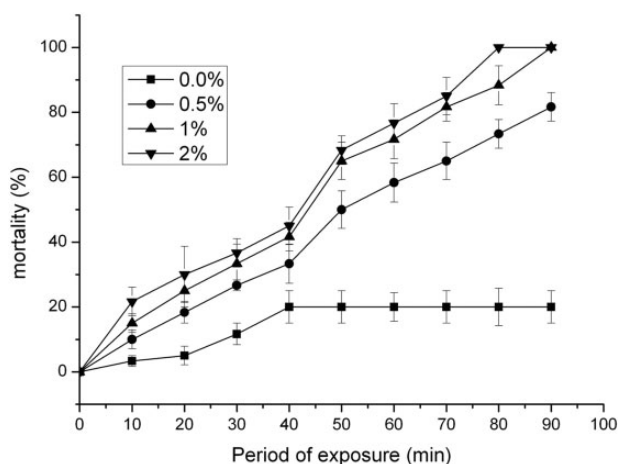


Fig. 2. Mortality of medium workers of red imported fire ants after closed exposure to sweet wormwood oil.

Table 1.  $LT_{50}$  (min) values of medium workers of red imported fire ants after open and closed exposures to sweet wormwood oil

Concentration (%)	Open		Closed	
	$LT_{50}$ (min)	$Cl_{95}$	$LT_{50}$ (min)	$Cl_{95}$
0	>180	-	>90	-
0.5	>180	-	49.26	37.24–65.17
1.0	>180	-	33.43	26.69–42
2.0	85.5	70.73–103.34	28.43	22.04–36.69

strong toxicity of *A. annua* oil against *S. invicta* at all concentrations under closed conditions. The mortality rate of medium workers treated with 2.0% *A. annua* oil reached 100% after 80 min. *A. annua* oil at 1.0% completely killed the workers after 90 min, and the mortality at 0.5% was 81.67% after 90 min. The  $LT_{50}$  values of 2.0%, 1.0%, and 0.5% *A. annua* oil were 28.43, 33.43, and 49.26 min, respectively (Table 1). These results indicate that *A. annua* oil exhibited higher inhibitory effect against *S. invicta* in closed exposure than in open exposure.

**Topical Contact Toxicity.** Topical application was used to test the toxicity of sweet wormwood oil on medium workers (Fig. 3). The results revealed that the oil exhibited a low inhibitory effect against the invasive ants. The mortality rate at 15.6  $\mu\text{g}/\text{head}$  was only  $15.58\% \pm 3.85\%$  after 7 d compared with the control group ( $8.89\% \pm 3.85\%$ ).

**Chemical Composition of *A. annua* Oil.** Figure 4 showed the GC-MS total ion chromatograms of *A. annua* oil. The results reveal that *A. annua* oil is a mixture of monoterpenoids, sesquiterpenes, aliphatic ethers, and alcohols, and up to 29 compounds were identified (Table 2), accounting for 95.89% of the total oil. The major compounds were *D*-camphor (21.63%), cineole (17.67%), *N,N'*-bis(2,6-dimethyl-6-nitrosohept-2-en-4-one) (13.51%), and (1*R*)-(+)- $\alpha$ -pinene (9.45%). In addition, linalool and *L*(-)-borneol were 0.36 and 0.99%, respectively.

**Fumigation Bioassay.** Table 3 shows the fumigant activity of linalool, cineole, *D*-camphor, *L*(-)-borneol, and  $\alpha$ -terpineol from *A. annua* oil against major and minor workers. In all cases, cineole and *D*-camphor exhibited excellent fumigation activity. The mortality of minor and major workers did not significantly differ in terms of component type, dosage, exposure time, and worker type ( $P < 0.05$ ).

Cineole and *D*-camphor achieved 100% mortality on minor workers after 24 h of exposure at 5 and 3 mg/centrifuge tube. Mortality of major workers was 100% after 24 h of exposure at the four concentrations, except at 0.5 mg/centrifuge tube. Linalool completely killed the minor workers at 5 mg/centrifuge tube within 24 h after treatment, followed

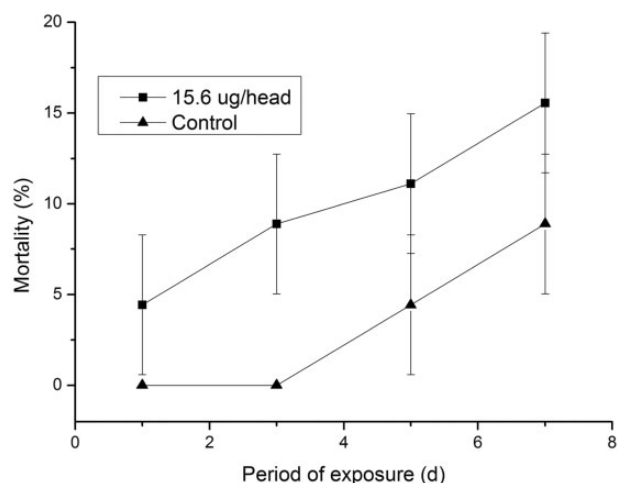


Fig. 3. Mortality of medium workers of red imported fire ants caused by sweet wormwood oil in topical contact bioassay at 1, 3, 5, and 7 d.

by 3 mg/centrifuge tube (48 h). Moreover,  $\alpha$ -terpineol terminated all minor and major workers at 5 mg/centrifuge tube after 24 and 48 h of exposure, respectively. After 24 h of exposure to *L*(-)-borneol at 5 and 3 mg/centrifuge tube, the fumigation mortality rates were 80% and 26.67% on minor workers, respectively. These values increased to 100% and 86.67%, respectively, when the exposure time was increased to 48 h. In addition, linalool, cineole, *D*-camphor, and  $\alpha$ -terpineol caused >80% mortality on minor workers at 2 mg/centrifuge tube. Only cineole, *D*-camphor, and  $\alpha$ -terpineol caused >80% mortality on major workers at 2 mg/centrifuge tube.

**Repellent Activity.** Compounds with DSIs greater than zero and *P* values less than 0.05 were considered repellent. By contrast, compounds with DSIs less than zero and *P* values less than 0.05 were considered attractant. Table 4 shows the repellent activity of five components of *A. annua* oil. Cineole and *D*-camphor completely suppressed the digging behavior of at 100 mg/kg level ( $DSI = 1.00 \pm 0.00$ ). The DSIs for cineole at 1 and 10 mg/kg, were  $0.28 \pm 0.034$  and  $0.52 \pm 0.041$  and those for *D*-camphor were  $0.27 \pm 0.084$  and  $0.36 \pm 0.14$ , respectively. These results indicate that cineole and *D*-camphor are significantly repellent. Moreover, the DSIs of 100 mg/kg *L*(-)-borneol and 100 mg/kg  $\alpha$ -terpineol were  $0.0077 \pm 0.069$  and  $0.013 \pm 0.11$ , respectively, and the *P* values were 0.94 and 0.83, respectively. Therefore, *L*(-)-borneol and  $\alpha$ -terpineol were not considered repellent. The DSIs for *L*(-)-borneol and  $\alpha$ -terpineol at 1 and 10 mg/kg were lower than zero; thus, they were considered as attractants. In addition, linalool did not indicate repellency because its DSIs were  $0.17 \pm 0.097$  and  $0.28 \pm 0.046$  at 10 and 100 mg/kg, respectively, and the *P* values were 0.46 and 0.091, respectively. At 1 mg/kg, the DSI was  $-0.19 \pm 0.09$ .

## Discussion

*A. annua* oil exhibited a higher mortality rate against red imported fire ants in closed rather than in open exposure, but it exhibited weak topical toxicity. This result may be attributed poor penetration of the oil through the ant integument and the volatility of the EOs, entering the body through the pores. In closed conditions, more of the sweet wormwood oil vapor entered the body of the ants. Tang et al. (2013) found that *A. annua* oil is strongly toxic to *S. invicta* using fumigation. Therefore, *A. annua* oil caused significant mortality even at the lowest dosage (0.5%;  $LT_{50} = 49.26$  min).

The dominant constituents in Folium artemisiae EO were *D*-camphor (21.63%), cineole, *N,N'*-bis(2,6-dimethyl-6-nitrosohept-2-en-4-one), and (1*R*)-(+)- $\alpha$ -pinene. This finding is similar to that reported by Mirjalili et al. (2007). However, the chemical components obtained in

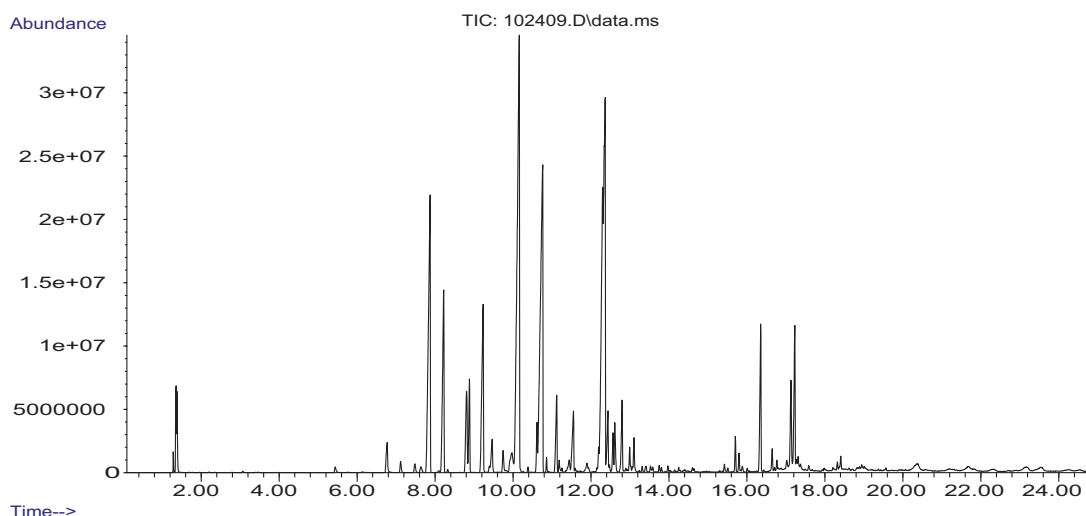


Fig. 4. GC-MS total ion chromatograms of sweet wormwood oil.

Table 2. Chemical composition of sweet wormwood oil

Composition	Relative retention time (min)	Percentage (%)
1 (1 <i>R</i> )-(+)- $\alpha$ -Pinene	7.872	9.45
2 Camphene	8.223	4.77
3 2-Thujene	8.812	2.19
4 $\beta$ -Pinene	9.233	6.43
5 3,3,6-Trimethyl-1,4-heptadiene-6-ol	9.465	0.87
6 2-Carene	9.746	0.62
7 <i>O</i> -Cymene	9.973	0.92
8 Cineole	10.162	17.67
9 $\gamma$ -Terpinene	10.616	0.87
10 <i>N,N'</i> -Bis(2,6-dimethyl-6-nitrosohept-2-en-4-one)	10.762	13.51
11 1 $\beta$ -Methyl-4 $\alpha$ -(1-methylethenyl)cyclohexanol	10.864	0.19
12 (Z)-Dihydro-5-(2-octenyl)furan-2(3H)-one	11.124	1.69
13 Linalool	11.442	0.36
14 1,4-Diethylbenzene	11.55	1.59
15 2-Methyl-6-methylene-1,7-octadien-3-one	12.199	0.40
16 <i>D</i> -Camphor	12.361	21.63
17 4-Octyne	12.436	1.34
18 ( $\pm$ )-2(10)-Pinen-3-one	12.566	0.71
19 <i>L</i> (-)-Borneol	12.615	0.99
20 Terpinene-4-ol	12.798	1.44
21 $\alpha$ -Terpineol	12.998	0.45
22 $\alpha$ -Thujenal	13.106	0.61
23 $\alpha$ -Cubebene	15.704	0.54
24 Benzyl pivalate	15.802	0.26
25 $\beta$ -Caryophyllene	16.353	2.96
26 ( <i>E</i> )- $\beta$ -Farnesene	16.65	0.36
27 $\alpha$ -Humulene	16.769	0.15
28 $\beta$ -Selinene	17.228	2.65
29 Caryophyllene oxide	18.411	0.27

RRT, relative retention time.

this study differed from those reported by Safaei-Ghomi et al. (2005) for 1-phenyl-penta-2,4-diyne,  $\beta$ -pinene, limonene, and (*E*)- $\beta$ -ocimene and by Basher et al. (1997) for methyl eugenol. These components were either absent or present in small amounts in this study. Oil composition varied greatly depending on differences in growth stages, plant parts, harvest time, variations in edaphic and climatic factors, and geographical region (Batish et al. 2006, 2008).

EOs easily volatilize and they are widely used as repellents and fumigants. Trongtokit et al. (2005) reported the repellent effect of 38 EOs against *Aedes aegypti* L. In total, 13 EOs were tested for repellency and contact toxicity against *Acanthoscelides obtectus* Say. They found that *Mentha microphylla* and *M. viridis* are toxic to female *A. obtectus*. *Lavandula hybrida* Rev. (Labiatae) and *Rosmarinus officinalis* L. are toxic to male *A. obtectus*

(Papachristos and Stamopoulos 2002). Callicarpenal and intermedeol from the leaves of *C. americana* and *C. japonica* are strongly repellent to *S. invicta* (Chen et al. 2008). Chen 2009 investigated the repellent effects against *S. invicta* workers of over-the-counter EO products from China Feng You Jing. They found that each of its six major components were repellent effects at various concentrations. Cineole and *D*-camphor exhibited excellent fumigant activity on minor and major workers at 5, 3, and 2 mg/centrifuge tube within 24 h of exposure. At 5 and 3 mg/centrifuge tube, linalool, *L*(-)-borneol, and  $\alpha$ -terpineol demonstrated good fumigant activity against minor workers. Cineole and *D*-camphor showed significant repellent activity at 100, 10, and 1 mg/kg. By contrast, linalool, *L*(-)-borneol, and  $\alpha$ -terpineol showed significant digging facilitation at 10 and 1 mg/kg. These results reveal that the mortality and

**Table 3. Mortality of minor and major red imported fire ant workers caused by the five main constituents of sweet wormwood oil at different concentrations in the fumigation bioassay for 24 and 48 h**

Chemical constituents	Milligram per centrifuge tube	Mortality of major workers (%)		Mortality of minor workers (%)	
		24 h	48 h	24 h	48 h
Linalool	5	20.00 ± 3.85 fg	66.67 ± 3.85 d	100.00 a	100.00 a
	3	13.33 ± 3.85 gh	46.67 ± 3.85 e	86.67 ± 3.85 b	100.00 a
	2	6.67 ± 3.85 hi	33.33 ± 3.85 f	60.00 ± 6.67 c	86.67 ± 3.85 bc
	1	0.00 i	20.00 ± 3.58 fgh	40.00 ± 6.67 d	53.33 ± 3.85 d
	0.5	0.00 i	13.33 ± 3.85 ghi	26.67 ± 3.85 e	33.33 ± 3.85 e
Cineole	5	100.00 a	100.00 a	100.00 a	100.00 a
	3	100.00 a	100.00 a	100.00 a	100.00 a
	2	100.00 a	100.00 a	100.00 a	100.00 a
	1	33.33 ± 3.85 e	80.00 ± 7.70 c	100.00 a	100.00 a
	0.5	13.33 ± 3.85 gh	53.33 ± 3.85 e	60.00 ± 6.67 c	80.00 ± 7.70 c
D-Camphor	5	100.00 a	100.00 a	100.00 a	100.00 a
	3	100.00 a	100.00 a	100.00 a	100.00 a
	2	80.00 ± 7.70 b	93.33 ± 7.70 ab	100.00 a	100.00 a
	1	46.64 ± 7.70 d	80.00 ± 7.70 c	100.00 a	100.00 a
	0.5	6.67 ± 3.85 hi	26.67 ± 3.85 fg	80.00 ± 7.70 b	100.00 a
L(-)-Borneol	5	13.33 ± 3.85 gh	53.33 ± 3.85 e	80.00 ± 3.85 b	100.00 a
	3	6.67 ± 3.85 hi	26.67 ± 3.85 fg	26.67 ± 3.85 e	86.67 ± 3.85 bc
	2	0.00 i	20.00 ± 3.85 fgh	13.33 ± 3.85 fg	53.33 ± 3.85 d
	1	0.00 i	0.00 j	6.67 ± 3.85 gh	26.67 ± 3.85 ef
	0.5	0.00 i	0.00 j	6.67 ± 3.85 gh	13.33 ± 3.85 gh
α-Terpineol	5	26.67 ± 3.85 ef	100.00 a	100.00 a	100.00 a
	3	13.33 ± 3.85 gh	80.00 ± 7.70 c	100.00 a	100.00 a
	2	6.67 ± 3.85 hi	33.33 ± 3.85 f	66.67 ± 3.85 c	86.67 ± 3.85 bc
	1	0.00 i	6.67 ± 3.85 ij	26.67 ± 3.85 e	53.33 ± 3.85 d
	0.5	0.00 i	0.00 j	0.00 h	20.00 ± 3.85 fg
Control Check	0	0.00 i	0.00 j	0.00 h	0.00 h

Means sharing the same letters are not significantly different from each other ( $P > 0.05$ , Duncan test).

**Table 4. Weight of sand removed by medium red imported fire ant workers 24 h after they were released in two-choice digging bioassay at difference dosages of individual compound in sweet wormwood oil**

Chemical constituents	Concentration (mg/kg)	Sand removed		Digging Suppression index	t value	P value
		Treatment (g)	Control (g)			
Linalool	1	0.52 ± 0.008	0.094 ± 0.029	-0.19 ± 0.09	0.78	0.23
	10	0.14 ± 0.037	0.099 ± 0.038	0.17 ± 0.097	1.92	0.46
	100	0.096 ± 0.016	0.13 ± 0.0093	0.28 ± 0.046	2.2	0.091
Cineole	1	0.089 ± 0.0024	0.16 ± 0.012	0.28 ± 0.034	5.81	0.0035
	10	0.067 ± 0.0058	0.21 ± 0.0081	0.52 ± 0.041	13.96	0.0001
	100	0.00 ± 0.00	0.25 ± 0.0013	1.00 ± 0.00	18.42	0.0001
D-Camphor	1	0.04 ± 0.0055	0.068 ± 0.0043	0.27 ± 0.084	4.22	0.0029
	10	0.47 ± 0.11	0.10 ± 0.0089	0.36 ± 0.14	3.81	0.0052
	100	0.00 ± 0.00	0.12 ± 0.017	1.00 ± 0.00	7.17	0.002
L(-)-Borneol	1	0.24 ± 0.061	0.14 ± 0.0084	-0.083 ± 0.19	1.5	0.2
	10	0.15 ± 0.042	0.11 ± 0.012	-0.058 ± 0.18	0.91	0.41
	100	0.11 ± 0.02	0.11 ± 0.014	0.0077 ± 0.069	0.083	0.94
α-Terpineol	1	0.23 ± 0.062	0.15 ± 0.01	-0.12 ± 0.18	1.19	0.3
	10	0.11 ± 0.026	0.098 ± 0.018	-0.04 ± 0.13	0.38	0.71
	100	0.11 ± 0.024	0.11 ± 0.012	0.013 ± 0.11	0.23	0.83

Means are significantly different from each other ( $P < 0.05$ ). Data are expressed as arithmetic means ± SE of the five independent experiments.

repellence rates of the *S. invicta* workers varied depending on component type, dosage, and exposure time.

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