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Repellent and fumigant activities of *Eucalyptus globulus* and *Artemisia carvifolia* essential oils against *Solenopsis invicta*

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Abstract

The repellent and fumigant activities plant essential oils of *Eucalyptus globulus* Labill. and *Artemisia carvifolia* Buch.-Ham. ex Roxb. on red imported fire ants (RIFAs) *Solenopsis invicta* Buren (Hymenoptera Formicidae) were evaluated by using digging bioassay and feeding/climbing behavior tests. Oil of *Eucalyptus* and *Artemisia* (mugwort) showed significant repellency at 100 mg/kg. However, slight digging facilitation was observed at 1 mg/kg. The attack and feeding rates of RIFAs on *Tenebrio molitor* L. (Coleoptera Tenebrionidae) daubed with 5 µl of the two essential oils were evaluated. *Eucalyptus* oil showed a complete repellent effect and prevented RIFA attacks on *T. molitor*. The time for complete repellency was 16 h. Mugwort oil showed weaker results than *Eucalyptus* oil. The feeding ability of RIFAs treated with the two essential oils decreased. The feeding, attack, and climbing rates of RIFA were observed 30 min after fumigation with the essential oils. *Eucalyptus* oil showed a greater effect on the climbing rate of RIFAs than mugwort oil. The feeding and attack rates after fumigation with the two essential oils were significantly lower than the control. *Eucalyptus* and mugwort oils could be used as alternatives to commercial repellents and fumigants.

Key words: *Eucalyptus* oil, mugwort oil, *Solenopsis invicta*, repellent, fumigant.

Introduction

The red imported fire ant (RIFA) *Solenopsis invicta* Buren (Hymenoptera Formicidae) causes severe damage to humans, animals, and the environment. Nevertheless, RIFAs are important medical and agricultural pests in the Southern United States, Australia, Philippines, Taiwan, and China (Zhang *et al*., 2007). Chemical treatment is a major component of RIFA management (Drees and Gold, 2003), which is usually accomplished by broadcast or individual mound treatments by using baits or traditional contact insecticides. Individual mound treatments generally result in the effective and rapid control of RIFA colonies (Vogt and Appel, 1996). However, traditional contact insecticides can be a source of environmental pollution. Homeowners have expressed concerns about traditional insecticides and have shown considerable interest in the use of less toxic or "natural" materials such as fire ant repellents and fumigants for RIFA control (Drees and Lennon, 1998).

Most repellents and fumigants are derived from secondary plant substances, such as alkaloids, quinones, essential oils (e.g., terpenoids), glycosides, and flavonoids. These compounds are often used in cosmetics, food, and pharmacological additives to enhance flavor or fragrance. The repellent actions of terpenoids, callicarpenal, and intermedeol from leaves of American beautyberry (*Callicarpa americana* L.) and Japanese beautyberry (*Callicarpa japonica* Thunb.) against RIFA workers were evaluated by using digging bioassays (Chen, 2008). Anderson *et al*. (2002) found that sage (*Salvia* spp.) leaves, pine (*Pinus* spp.) needles, and a water suspension of cedar shavings repel RIFAs. Chen (2005) discovered that dimethyl and diethyl phthalates repel RIFA. The essential oil from horseradish plant *Armoracia rusticana* (L.) was active against *Plodia interpunctella* (Hubner) at different life stages and against *Sitophilus zeamais* Motschulsky adults (Chen *et al*., 2011).

Fumigation and repellency are the major strategies for pest management in stored products and quarantined containers. Studies on the toxicity of botanical essential oils on RIFA show that various essential oils are repellent and toxic to such ants.

This study evaluated the repellent and fumigant activities of *Eucalyptus* and *Artemisia* (mugwort) oils on red fire ants by using digging bioassay and feeding/climbing behavior tests.

Materials and methods

Plant essential oils

Essential oils were purchased from Kangshen Natural Medicinal Oil Refinery (China) which is engaged in the R&D and production of effective monomer and effective components of plants. *Eucalyptus* and mugwort oils were extracted from *Eucalyptus globulus* Labill. and *Artemisia carvifolia* Buch.-Ham. ex Roxb., respectively.

Insects

RIFA colonies were collected from the suburb of Zengcheng, Guangdong Province, and were stored in the laboratory for bioassays at 25 ± 2 °C and 60 to 80% RH. A test tube (25 mm × 200 mm) that was partially filled with 10% honey water and plugged with cotton was used as the water source. A Petri dish (8.5 × 1.5 cm) containing the larvae of *Tenebrio molitor* L. (Coleoptera Tenebrionidae) was used for the food source. *T. molitor* were obtained from the insect-fish market in Guangzhou, fed with wheat bran, and kept in a dry indoor environment at 25 ± 2 °C until use.

Digging bioassay

The repellent activities of *Eucalyptus* and mugwort oils against RIFA were evaluated by using the two-way selection digging bioassay method developed by Chen *et al*., 2008. Four 2-ml centrifuge tubes were mounted under a (8.5 × 2.0 cm) Petri dish and positioned 3.0 cm away from the center of the Petri dish and at equal distance from each other. Only two tubes with access holes were filled with sand: one was sand treated with *Eucalyptus* or mugwort oil and the other with solvent as a control. The other two tubes without access holes were used to support the bioassay device. The inner side of the Petri dish was coated with Fluon. Sand was first sieved through a #35 USA standard testing sieve (Thomas Scientific, Swedesboro, NJ) and then washed with distilled water and dried at 350 °C for 12 h. A 3 ml dichloromethane solution was mixed with 30 g of sand in an aluminum pan. The sand was stirred every 2 min to facilitate the evaporation of the solvent under a fume hood. After the dichloromethane evaporated (5 min), 1.92 ml of distilled water was added and mixed with the sand. Sand in the control tube was treated only with dichloromethane. A total of 2.70 ± 0.2 g wet sand was added in each tube. The experiment was conducted at 25 ± 1 °C and 50% RH. After 24 h, the sand in each vial was collected, dried at 250 °C for at least 4 h, and weighed. Three essential oil concentrations (1, 10, and 100 mg/kg) were tested. Twenty medium-sized RIFA workers were placed in the center of the Petri dish. The experiment was replicated five times for each treatment. The digging suppression index (DSI) was used to compare the repellency, and DSI was calculated by using the following formula:

$$I = (A_c - A_t)/(A_c + At),$$

where I is the DSI, and A_c and A_t are the amounts of sand removed from the control and treatment tubes, respectively.

Attacking and feeding activities on *T. molitor* daubed with essential oils

One *T. molitor* larva was daubed with 5 µl essential oils on the body and then placed on the bottom of a beaker in a cup. The *T. molitor* larva untreated was the control. The wall of the cup was coated with Fluon before the experiment. A total of 50 medium-sized RIFA workers (body length of 3.5 mm to 3.7 mm, head width of 0.8 to 0.9 mm) were selected and starved for 2 days and subsequently placed inside the beaker. The attacks on the *T. molitor* were recorded at 1, 5, 10, 15, 20, 25, and 30 min by using the following formula:

$$A(\%) = A_n/T_n \times 100,$$

where A is the attack rate, A_n is the number of RIFAs attacking the *T. molitor*, and T_n is the total number of RIFAs. The feeding activities were observed and recorded at 24 and 48 h by using the following formula:

$$F(\%) = (I_w - R_w)/I_w \times 100,$$

where F is the feeding rate, I_w is the initial weight of *T. molitor*, and R_w is the remaining weight of *T. molitor*. A camera was used to film the whole process for 24 h and to observe the deterring time. Each treatment was replicated 3 times.

Attacking, feeding, and climbing behavior after fumigation

According to the fumigation bioassay method (Seo *et al*., 2009), 50 RIFA medium-sized workers were placed in a disposable plastic cup 30 min after fumigation treatment, which was conducted at a concentration of 2 mg/centrifuge tube. The attacking and feeding experiment involved placing one *T. molitor* of consistent size inside a cup with one water tube placed at the bottom. The frequency of attacks and feeding activities of RIFA on the *T. molitor* were recorded. In the climbing experiment, the cup containing fumigated RIFAs was gently flipped with the mouth facing downward on a piece of paper for 3 s. The cup was then flipped gently back upward. The behavior of the tested ants was observed, and climbing activity (from the paper back to the cup) was recorded at 1, 30, 60, 90, 120, 150, and 180 min. Control ants were kept under the same conditions without fumigation. Each treatment was replicated three times. The climbing rate was calculated as follows:

$$C(\%) = (T_n - F_n)/T_n \times 100,$$

where C is the climbing rate, T_n is the total number of RIFA, and F_n is the falling number of RIFA.

Chemical analysis by using gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry (GC-MS) analysis was performed by using Agilent 6890N (Agilent Technologies, USA) coupled with a 5975 mass selective detector (Agilent Technologies, USA). *Eucalyptus* and mugwort oils were analyzed by GC-MS. Gas chromatography was equipped with a 30 m HP-5 capillary column with 0.25 mm internal diameter and 0.25 µm film thickness. The helium carrier gas had a delivery rate of 1 ml/min (controlled constant flow). The injector temperature was 250 °C, and the detector temperature was 300 °C. The injection volume was 1 µl. The split ratio was 50:1. The temperature program was as follows: the initial temperature was maintained at 50 °C for 5 min and then increased to 220 °C at a rate of 10 °C/min for 5 min. Most compounds were identified by comparison of their relative retention index (RI) to series of n-alkanes, MS Library search (NIST, 2005), and/or by comparison with the literature data (Adams, 2007).

Statistical analysis

For the repellency analysis, a paired t-test was used to compare the mean amount of removed sand between the treatment and control groups. Feeding rates were recorded, analyzed, and transformed to arcsine square root values for ANOVA by using Duncan's new multiple range and multiple comparison (i.e., Duncan's multiple range test). The data was expressed by average number ± standard error and evaluated by Data Processing System statistics.

Table 1. Weight of sand removed by medium-sized RIFA workers 24 h after they were tested in two-way digging bioassay at difference dosages of the two essential oils.

Essential oil	Concentration (mg/kg)	Sand removed (g) Treatment	Control	Digging suppression index (DSI)	*T*-value	*P*-value
Eucalyptus	1	0.10 ± 0.03	0.09 ± 0.04	−0.15 ± 0.27	0.35	0.75
	10	0.05 ± 0.07	0.04 ± 0.01	−0.12 ± 0.13	0.50	0.64
	100	0.00 ± 0.00	0.13 ± 0.03	1.00 ± 0.00	3.79	0.02
Mugwort	1	0.14 ± 0.04	0.10 ± 0.02	−0.19 ± 0.09	1.59	0.19
	10	0.10 ± 0.02	0.18 ± 0.03	0.27 ± 0.07	3.13	0.04
	100	0.04 ± 0.02	0.16 ± 0.02	0.59 ± 0.26	4.84	0.008

Results

Repellency of *Eucalyptus* oil and mugwort oil

The carrying quantity of treated sand was significantly less than the control sand (table 1). *Eucalyptus* oil showed significant repellency at 100 mg/kg (DSI was 1.00 ± 0.00) but not at the other two concentrations. The DSI at 10 and 1 mg/kg was −0.12 ± 0.13 and −0.15 ± 0.27, respectively. The negative DSI indicated digging facilitation. The results for the mugwort oil were similar. Mugwort oil showed repellency at 100 and 10 mg/kg, with DSI values at 0.59 ± 0.26 and 0.27 ± 0.07, respectively. Digging facilitation was observed at 1 mg/kg (DSI value of −0.19 ± 0.09). The repellency of *Eucalyptus* oil was better than mugwort oil.

Feeding and attack rates on daubed *T. molitor*

The attack rate of RIFA treated with the two essential oils was lower than the attack rate of the control. The attack rate of RIFA treated with *Eucalyptus* oil was 0.00 ± 0.00 (figure 1), thus indicating that *Eucalyptus* oil completely prevented attacks on *T. molitor*. The time for complete repellency was 16 h. The effect of mugwort oil on the attack rate was weaker than *Eucalyptus* oil. The feeding rate at 24 h was 12.4 ± 0.3, 23.49 ± 1.33, and 38.94 ± 0.95 for the *Eucalyptus* oil, mugwort oil, and control, respectively. At 48 h, the feeding rate was 43.79 ± 0.85, 43.57 ± 0.89, and 53.86 ± 1.00 for the *Eucalyptus* oil, mugwort oil, control, respectively (table 2). Thus, feeding ability in two treatments were different at 24 h.

Attack rate on *T. molitor* and feeding rate after fumigation

The attack rate of RIFA treated with two essential oils was lower than the control. Mugwort oil showed weaker effects on the attack rate after 5 min than *Eucalyptus* oil (figure 2). For the feeding rate, the results from table 3 indicated that both *Eucalyptus* and mugwort oils could reduce the feeding rate of RIFA compared with the control. The feeding ability of RIFA treated with *Eucalyptus* oil was also lower than RIFA treated with mugwort oil.

Effect on climbing ability

The conditions of RIFA survival in the natural environment are complex. The ability of RIFA to climb plants, soil, stones, and nest walls is crucial to its survival. *Eucalyptus* and mugwort oils had obvious effects on climbing rate. At 1 min, the climbing rates were

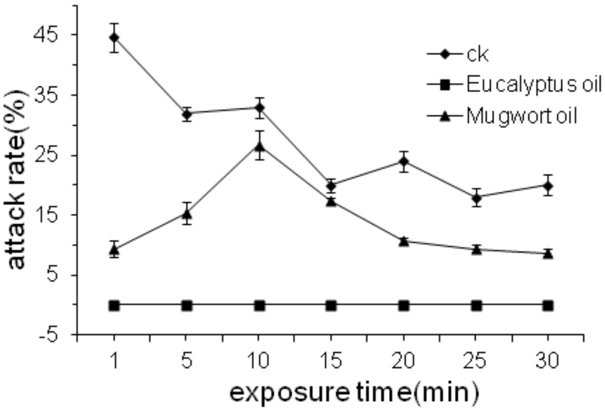

Figure 1. Attack rate of medium-sized RIFA workers on *T. molitor* treated with essential oils at 5 µl/*T. molitor*.

Table 2. Feeding rate of medium-sized RIFA workers on *T. molitor* treated with essential oils at 5 µl/*T. molitor*.

Essential oil	Feeding rate % (mean ± SE)* 24 h	48 h
Eucalyptus	12.4 ± 0.3 c	43.79 ± 0.85 b
Mugwort	23.49 ± 1.33 b	43.57 ± 0.89 b
CK	38.94 ± 0.95 a	53.86 ± 1.00 a

* Means within a column followed by the same letter are not significantly different (P > 0.05, Duncan test).

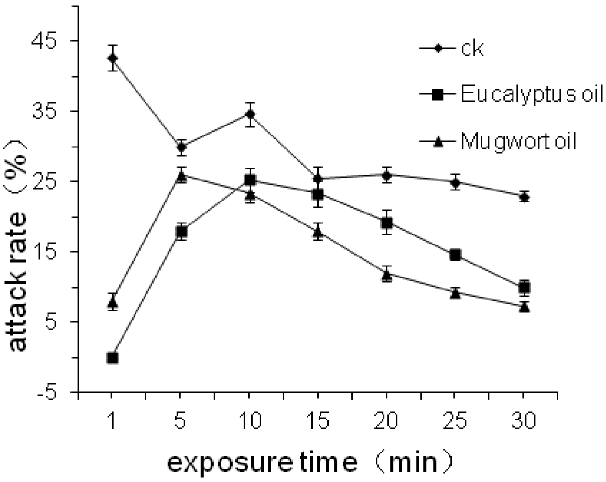

Figure 2. Attack rate of medium-sized RIFA workers 30 min after fumigation treatment with essential oils at 2 mg/centrifuge tube on *T. molitor*.

Table 3. Feeding rate of medium-sized RIFA workers 30 min after fumigation treatment with essential oils at 2 mg/centrifuge tube on *T. molitor*.

Essential oil	Feeding rate % (mean ± SE)*	
	24 h	48 h
Eucalyptus	22.04 ± 0.78 c	42.41 ± 0.56 c
Mugwort	31.51 ± 0.57 b	50.93 ± 0.20 b
CK	39.89 ± 0.70 a	54.38 ± 0.56 a

* Means within a column followed by the same letter are not significantly different (P > 0.05, Duncan test).

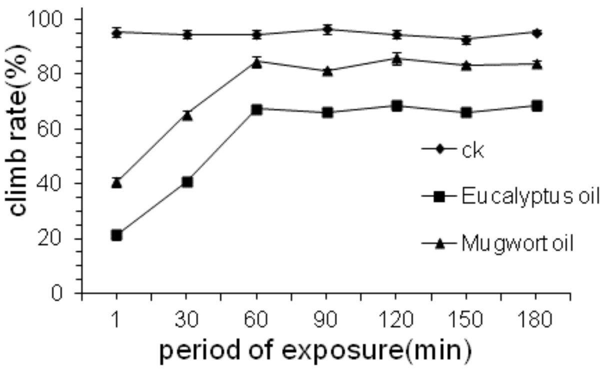

Figure 3. Climbing rate of medium-sized RIFA workers 30 min after Fumigation treatment with essential oils at 2 mg/centrifuge tube.

Table 4. Main chemical composition of *Eucalyptus* oil.

	RI*	Composition	Percentage (%)
1	930	2-N-propylpyridine	1.12
2	946	m-cymene	1.04
3	957	1,8-cineole	94.30
4	964	γ-terpinene	1.10

*RI means retention index as determined on a HP-5 capillary column using the homologous series of *n*-hydrocarbons.

Table 5. Main chemical composition of mugwort oil.

	RI	Composition	Percentage (%)
1	898	α-pinene	1.93
2	922	β-pinene	1.77
3	930	β-phellandrene	3.05
4	955	1,8-cineole	4.30
5	1005	camphor	12.90
6	1012	borneol	0.83
7	1016	4-terpineol	1.39
8	1021	α - terpineol	2.03
9	1056	safrole	2.50
10	1093	methyl eugenol	1.08
11	1096	longifolene	1.20
12	1100	α-santalene	7.89
13	1101	caryophyllene	1.54
14	1104	α-bergamotene	2.85
15	1112	β-santalene	6.39
16	1131	δ-cadinene	3.23
17	1142	nerolidol	10.81

95.33 ± 0.67, 21.33 ± 0.67, and 40.67 ± 0.67 in the control, *Eucalyptus* oil, and mugwort oil treatments, respectively (figure 3). After 60 min, the climbing rates were 94.67 ± 1.67, 67.33 ± 1.33, and 84.67 ± 2.07 in the control, *Eucalyptus* oil, and mugwort oil treatments, respectively. This trend indicated that mugwort oil had lower inhibitory effects on the climbing capacity of RIFA than *Eucalyptus* oil.

Chemical analysis by GC-MS

Ten compounds from *Eucalyptus* oil and 50 compounds from mugwort oil were identified by GC-MS. The main components of the *Eucalyptus* oil were 1,8-cineole (94.30%), 2-N-propylpyridine (1.12%), γ-terpinene (1.10%), and m-cymene (1.04%) (table 4). The main components of mugwort oil were camphor (12.90%), nerolidol (10.81%), α-santalene (7.89%), β-santalene (6.39%), 1,8-cineole (4.30%), δ-cadinene (3.23%), β-phellandrene (3.05%), α-bergamotene (2.85%), safrole (2.50%) and α-terpineol (2.03%) (table 5). The other compounds in the oils were not shown because the concentrations of these compounds were less than 1%.

Discussion

The insecticidal activity of the oil of *E. globulus* has already been observed for some insect pests (Kumar *et al*., 2012), but we believe that the higher insecticidal activity of the *Eucalyptus* oil to RIFA workers is due to its major compound 1,8-cineole (94.30%), as this molecule is highly active against housefly (*Musca domestica* L.) (Kumar *et al*., 2014). Yang *et al*. (2004) evaluated the toxic effects of 1,8-cineole on eggs and females of *Pediculus humanus capitis* De Geer through contact toxicity and fumigation bioassays and found it to be highly effective, comparable to chemical insecticides. Fumigation assay with 1,8-cineole showed best larvicidal activity against *Aedes aegypti* (L.) larvae (Lucia *et al*., 2009).

Many *Artemisia* species are rich in polyacetylenes, flavonoids, terpenoids, and cyanogenic glycosides and are well-known medicinal plants. The essential oil of *Artemisia rupestris* L. exhibited stronger contact toxicity and repellency against the booklice *Liposcelis bostrychophila* Badonnel. The principal compounds in the essential oil were α-terpineol, α-terpinyl acetate, 4-terpineol and linalool. The essential oil of *A. rupestris* (LC_{50} = 6.67 mg/l air) also possessed fumigant toxicity against *L. bostrychophila* while the four constituents, 4-terpineol, α-terpineol, α-terpinyl acetate and linalool had LC_{50} values of 0.34, 1.12, 1.26 and 1.96 mg/l air, respectively. α-Terpineol and α-terpinyl acetate showed strong repellency against *L. bostrychophila*, while linalool and 4-terpineol exhibited weak repellency (Liu *et al*., 2013). Camphor, menthol, and eucalyptol were the main components of the essential oil product, named as Feng Yu Jing in Chinese (FYJ). At 100 mg/kg, FYJ and its main components showed significant repellency against RIFA workers (Chen *et al*., 2009). Caryophyllene was highly active against eggs and females of *Pe-*

diculus capitis De Geer (Yang *et al*., 2003). The components santalene, δ-cadinene and nerolidol, characteristic of many other compounds derived from secondary metabolism of plants, traditionally used as botanical insecticides (Li *et al*., 2011; Perumalsamy *et al*., 2014). Phenylpropanoids, particularly safrole, isosafrole, eugenol, isoeugenol, and methyleugenol were shown to be toxic and/or repellent to species belonging to different orders of insects (Ngoh *et al*., 1998; Huang *et al*., 2002; Souto *et al*., 2012).

Fumigation treatment significantly decreased the feeding and climbing abilities of RIFA. Liu *et al*. (2011) found that the α-terthienyl possessed the lethal effect and knock-down ability against the RIFA, and could significantly decrease the aggregation, cling ability and normal walking ability. Feeding and climbing abilities directly determine the RIFA survival. To conclude, mugwort and *Eucalyptus* oils could be used as natural repellents and natural fumigants because of their inhibitory effects on RIFA feeding and climbing abilities, which could indirectly lead to the decline of the entire colony. Further studies should be conducted on the composition and dose range of these essential oils.

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