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Repellency of an Over-the-Counter Essential Oil Product in China against Workers of Red Imported Fire Ants

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Repellency of an over-the-counter essential oil product from China, and its major components against workers of red imported fire ants, *Solenopsis invicta* Buren, was evaluated using an ant digging bioassay. Three concentrations (1.0, 10.0, and 100.0 mg/kg in sand) of the product were tested. At 100.0 mg/kg, the digging suppress index (DSI) was 1.0 ± 0.00 (mean ± SE) for all six test colonies, indicating this product produced a complete digging suppression; at 10 mg/kg, DSI was 0.22 ± 0.089 to 0.75 ± 0.12 and significant repellency occurred against five of six colonies; and at 1.0 mg/kg, DSI was 0.21 ± 0.091 to 0.38 ± 0.14 and significant repellency occurred against four of six colonies. The chemical components of this product were analyzed using gas chromatography−mass spectrometry (GC-MS). Camphor, eucalyptol, eugenol, menthol, methyl salicylate, and phenylethanol were identified. A digging bioassay was also conducted on each of those identified compounds at concentrations of 1.0, 10.0, and 100.0 mg/kg. Based on pooled data from three colonies, each component significantly suppressed the digging behavior at 100 mg/kg. Eugenol, menthol, and methyl salicylate significantly suppressed the digging at 10 mg/kg. At 1.0 and 10.0 mg/kg, DSI for eucalyptol was −0.039 ± 0.032 and −0.050 ± 0.021, respectively. The negative DSI indicated a digging facilitation. However, only at 10.0 mg/kg, was such facilitation statistically significant.

KEYWORDS: *Solenopsis invicta*; digging bioassay; camphor; eucalyptol; eugenol; menthol; methyl salicylate; phenylethanol

INTRODUCTION

The red imported fire ant, *Solenopsis invicta* Buren, is a serious problem to human health, agriculture, and wildlife (*1*). Synthetic contact insecticides are commonly used in its management (*2*), which can be a source of environmental pollution (*3*). In order to reduce the use of the synthetic contact insecticides, there has been increasing interest in the research and development of alternative control tactics, such as fire ant repellents (*4*−*9*).

Repellents could potentially be used to exclude red imported fire ants from some sensitive areas, such as schools and hospitals (*4*). In the United States, a federal quarantine has been enforced to prevent imported fire ants from further spread into noninfested areas (http://www.aphis.usda.gov/ppq/ispm/fireants/index.html). Repellents are also potentially useful to treat some regulated articles to push ants away and/or prevent them from re-entering treated articles. Such articles include nursery stocks and soil-moving equipments.

A number of chemical compounds have been identified as fire ant repellents by Vander Meer et al. (*5*, *6*). Chen (*7*) found that dimethyl and diethyl phthalates were repellents to red imported fire ants. Compounds of natural sources were also reported as fire ant repellents (*8*). Two terpenoids, callicarpenal and intermedeol, from American beautyberry (*Callicarpa americana* L., Verbenaceae) and Japanese beautyberry (*Callicarpa japonica* Thunb.) were found to be repellents against fire ant workers (*9*). Anderson et al. (*10*) found that sage (*Saliva sp.*), pine needle, and a cedar shaving water suspension were repellents to *S. invicta*. Appel et al. (*11*) tested repellency and toxicity of mint oil granules and found that all red imported fire ant mounds which were treated with mint oil granules were abandoned. All these previous reports demonstrated that natural products may be a valuable source of fire ant repellents.

In China, essential oil-based products are often used as insect repellents and pain relievers. Many such products are sold as over-the-counter traditional Chinese herb medicines. One over-the-counter essential oil product from China has been examined recently in our laboratory for its impact on red imported fire ants (Chen, unpublished data). We observed that this product strongly repelled red imported fire ants. The objectives of this study were (1) to identify the components that had contributed to the repellency in this product and (2) to evaluate the repellency of each identified compound on red imported fire ant workers using a digging behavior bioassay.

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MATERIALS AND METHODS

Chemicals. Essential oil product, named as Feng Yu Jing in Chinese (Dragon & Tiger, Zhonghua Pharmaceutical Company Affiliated with Shanghai Pharmaceutical Group Co., Ltd.), was purchased from a Chinese Traditional Herb Medicine Store in the city of JingHua, Zhejiang Province, The People's Republic of China, on January 12, 2005. This product will hereafter be referred to as FYJ in the text for simplicity. The cost of FYJ was about $0.5/bottle (size: 6 mL). The standards of six major components of this product, including camphor, eucalyptol, eugenol, menthol, methyl salicylate, and phenylethanol, were obtained from Sigma-Aldrich (St, Louis, MO). Dimethyl phthalate, used as an internal standard, was also purchased from Sigma-Aldrich.

Ants. Six monogyne red imported fire ant colonies were collected on February 22, 2007, in Sharkey County, MS. Workers were not amicable among colonies, indicating they were not from the same colony. Colonies were collected by shoveling the ant mound into a 19-L plastic bucket. The inside wall of the bucket was then coated with baby powder (Cumberland Swan Holdings, Inc., Smyrna, TN) to stop ants from climbing out of the bucket. Ants were separated using the method developed by Banks et al. (*12*) and modified by Chen and Wei (*13*). After being separated from the mound soil, ants were placed in a 44.5 × 60.0 × 13.0 cm^3 plastic tray. Fluon (Ag Fluoropolymers, Chadds Ford, PA) was used to coat the tray to prevent ant escape. Distilled water and 10% sugar water solution were provided in separate test tubes (2 cm × 15 cm). Each test tube was plugged with a cotton ball, which served as a feeding platform for the fire ants. Pupae of the corn earworm, *Heliocoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (Fabricius), were used as food sources. Petri dishes (14.0 cm × 2.0 cm) with 1.0 cm of hardened dental plaster (Castone, Dentsply International Inc., York, PA) on the bottom were used as artificial nests. There was a 5.0-cm diameter brood chamber at the center of the Petri dish. Two 8-mm access holes were made on the wall of the Petri dish above the dental plaster. The Petri dish lid was painted black (1302 Gloss Black Spray Enamel, Louisville, KY) to block the light. All colonies were maintained at 25−30 °C.

Chemical Analysis Using Gas Chromatography−Mass Spectrometry. The chemical identities of components in FYJ were achieved by matching the retention times and mass spectra of the samples with those of the standards. Dimethyl phthalate was used as internal standard in quantifying each component in the samples. Samples were soluble in acetone, and all dilutions needed in this experiment were made using acetone as the solvent. No sample cleanup was needed. A Varian GC-MS system was used for this study. It consisted of a CP-3800 gas chromatograph and a Saturn 2000 mass selective detector, which were controlled by Mass Spectrometry WorkStation version 6.41 (Varian, Walnut Creek, CA). A 30 m × 0.25 mm DB-1 capillary column with 0.25 μm film thickness was used (J & W Scientific, Folsom CA). The GC temperature program was as follows: initial temperature was 50 °C, held for 1 min, increased to 250 °C at a rate of 20 °C /min, and held for 40 min. The split ratio was 1:10, injection temperature was 250 °C, and transfer line temperature was 200 °C. Helium was used as the carrier gas, and the flow rate was 1.0 mL/min. The mass spectrometer was operated at 70 eV in the electron impact mode.

Digging Bioassay. The repellencies of FYJ and its six major ingredients were evaluated using a two-choice digging bioassay (*9*). This method takes advantage of the fact that a group of worker ants will always show digging behavior whenever an adequate digging substrate, such as sand, is available. The rationale for this bioassay is that ants would not dig or would dig less in sand containing the repellent, so the repellency in this study was defined as a suppression of ant digging behavior. The bioassay apparatus and sample preparation were the same as those used in a two choice digging bioassay by Chen et al. (*9*). Four 2-mL centrifuge tubes were mounted under a (8.7 cm × 2.3 cm) Petri dish using glue (Arrow Fastener Co., Inc., Saddle Brook, NJ). Only two tubes were filled with sand: one with treated sand and the other with control sand. The other two tubes were merely used to support the Petri dish. Two tubes with sand were 3.0 cm away from each other, located on a straight line that went through the center of the Petri dish, and at equal distances from the center of the Petri dish. A 3-mm diameter access hole was drilled for each centrifuge tube, which went through the bottom of the Petri dish and the cap of the tube. The inner side of the Petri dish was coated with Fluon. Sand (Premium Play Sand, Plassein International, Longview, TX) 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. In each tube a mean (±SD) 2.76 g (±0.06 g) of wet sand was added. There was no open space inside the tube. Twenty fire ant workers were introduced into the center of the Petri dish. The experiment was conducted at 22 ± 0.8 °C (mean ± SD) temperature and 45.4% ± 11.87% relative humidity. After 24 h, the sand in each vial was collected, dried at 250 °C for at least 4 h, and weighed. Three concentrations (1.0, 10.0, and 100.0 mg/kg) were tested for FYJ and each of its six major components. The experiment was replicated 10 times for each of six colonies. Digging suppression index (DSI) was used to compare the repellency, which was calculated using the formula $I = (A_c - A_t)/(A_c + A_t)$, where I is the digging suppression index and A_c and A_t are the amounts of sand removed from the control tube and treatment tube, respectively. For each concentration, a paired *t*-test was used to compare the mean amount of removed sand between the treatment and the control. For FYJ, a paired *t*-test was conducted for each colony; for each individual compound, the pooled data from six colonies were used for analysis. The analysis of variance followed by an LSD mean comparison at $\alpha = 0.05$ (SAS PROC GLIMMIX) (*14*) was used to compare digging suppression indices among concentrations and colonies.

RESULTS

Chemical Analysis. Typical GC-MS total ion chromatograms of FYJ were shown in **Figure 1**. In addition to camphor, eucalyptol, menthol, methyl salicylate, and eugenol, which were reported on the label as components of the product, phenylethanol was also identified and quantified (**Figures 2** and **3**). The percentage of each compound was 4.24 ± 0.15% (mean ± SE) for camphor, 2.44 ± 0.10% for eucalyptol, 51.78 ± 1.39% for menthol, 33.51 ± 1.23% for methyl salicylate, 4.78 ± 0.07% for eugenol, and 3.24 ± 0.08% for phenylethanol.

Repellency of FYJ and Their Components. At 100.0 mg/kg, the DSI of FYJ was 1.0 ± 0.00 (mean ± SE) for all six test colonies, indicating a complete digging suppression (**Table 1**). At 10 mg/kg, DSI was 0.22 ± 0.089 to 0.75 ± 0.12 and

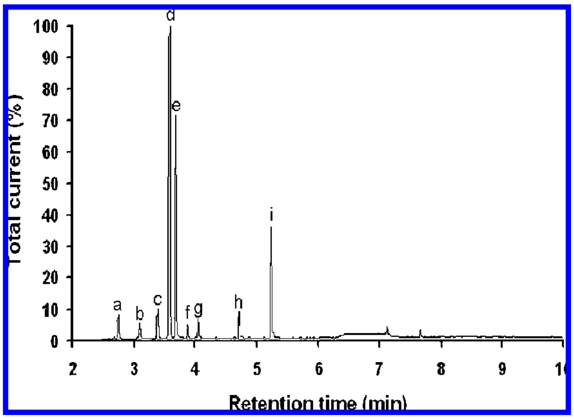

Figure 1. Typical GC-MS total ion chromatograms of FYJ: (a) eucalyptol; (b) phenylethanol; (c) camphor; (d) menthol; (e) methyl salicylate; (f) unkown; (g) unknown; (h) eugenol; (i) dimethyl phthalate (internal standard).

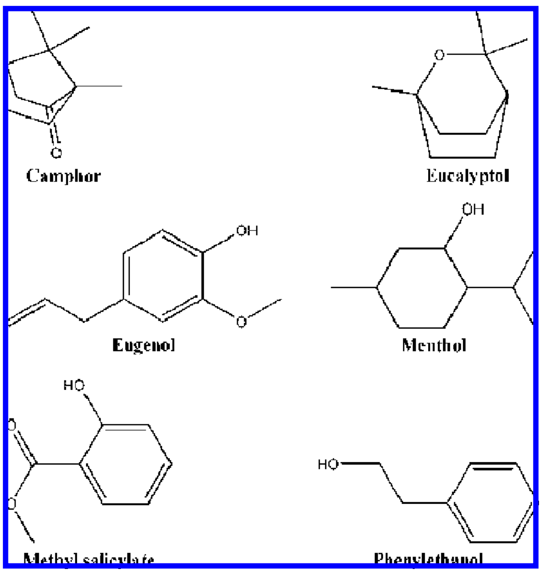

Figure 2. Chemical structures of identified compounds in FYJ.

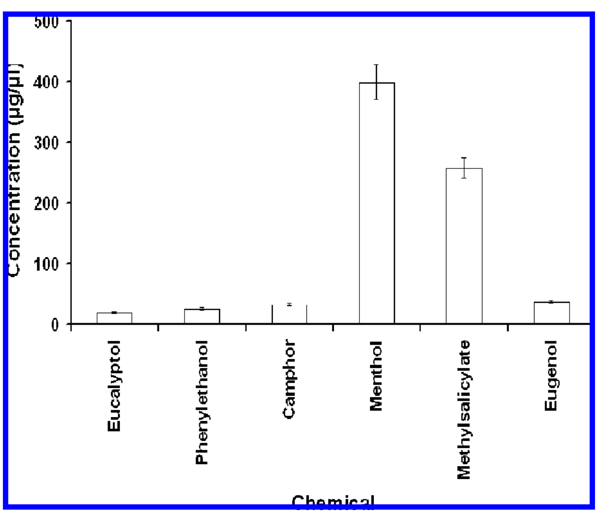

Figure 3. Concentration of identified chemicals in FYJ (mean ± SE, n = 3).

significant repellency occurred against five of six colonies. At 1.0 mg/kg, DSI was 0.21 ± 0.091 to 0.38 ± 0.14 and significant repellency occurred against four of six colonies. There was a significant difference in DSI among concentrations (F = 78.9, df = 2, 162, P < 0.0001) and among colonies (F = 3.44, df = 5, 162, P = 0.006). There was no significant interaction among colony and concentration (F = 1.73, df = 10, 162, P = 0.079). For individual compounds, there was a significant difference in DSI among chemicals (F = 60.92, df = 5, 1056, P < 0.0001), concentrations (F = 347.91, df = 2, 1056, P < 0.0001), and colonies (F = 4.64, df = 5, 1056, P = 0.0003). There was a significant interaction between colony and concentration (F = 3.32, df = 10, 1056, P = 0.0003). Camphor and phenylethanol showed significant repellency at the concentration 100 mg/kg but not the other two lower concentrations (**Table 2**). Menthol, methyl salicylate, and eugenol show significant repellency at 10.0 and 100.0 mg/kg concentrations but not at 1.0 mg/kg. Eucalyptol showed significant repellency at 100.0 mg/kg; however, at 1.0 and 10.0 mg/kg, DSI for eucalyptol was −0.039 ± 0.032 and −0.050 ± 0.021 respectively. The negative DSI indicated a digging facilitation; however, only at 10.0 mg/kg, was such facilitation statistically significant (t = 2.70, df = 59, P = 0.009). At the 100.0 mg/kg level, all six tested compounds showed significant repellency; however, only eugenol and menthol completely suppressed digging behavior (average DSI = 1.0) for three and two colonies, respectively, not like FYJ, which showed complete digging suppression for all six tested colonies at 100 mg/kg.

Table 1. Mean (SE) Weight (g) of Sand Removed by Worker Ants 24 h after They Were Released in the Two Choice Digging Bioassay at Three Concentrations of FYJ

conc (mg/kg)	colony	sand removed (SE) treatment	sand removed (SE) control	digging suppression index (SE)	t-value	P-value
1.0	1	0.32 (0.069)	0.51 (0.068)	0.30 (0.13)	2.32	0.045
	2	0.24 (0.051)	0.32 (0.063)	0.22 (0.14)	1.73	0.12
	3	0.55 (0.126)	0.95 (0.039)	0.36 (0.14)	2.95	0.016
	4	0.71 (0.17)	1.20 (0.070)	0.38 (0.14)	3.14	0.012
	5	0.80 (0.15)	1.08 (0.064)	0.25 (0.13)	1.95	0.081
	6	0.83 (0.05)	1.20 (0.063)	0.21 (0.091)	2.61	0.028
10.0	1	0.15 (0.057)	0.47 (0.064)	0.61 (0.12)	4.29	0.002
	2	0.35 (0.071)	0.51 (0.038)	0.26 (0.12)	2.11	0.064
	3	0.17 (0.071)	0.68 (0.073)	0.70 (0.12)	5.58	0.0003
	4	0.24 (0.11)	1.01 (0.095)	0.75 (0.12)	5.84	0.0002
	5	0.85 (0.12)	1.22 (0.066)	0.22 (0.089)	3.67	0.0052
	6	0.77 (0.092)	1.21 (0.067)	0.24 (0.070)	3.86	0.0038
100.0	1	0.00 (0.00)	0.59 (0.049)	1.00 (0.00)	11.98	<0.0001
	2	0.00 (0.00)	0.44 (0.024)	1.00 (0.00)	18.61	<0.0001
	3	0.00 (0.00)	0.81 (0.044)	1.00 (0.00)	18.13	<0.0001
	4	0.00 (0.00)	1.12 (0.068)	1.00 (0.00)	16.47	<0.0001
	5	0.00 (0.00)	1.07 (0.061)	1.00 (0.00)	17.48	<0.0001
	6	0.00 (0.00)	1.06 (0.046)	1.00 (0.00)	22.99	<0.0001

DISCUSSION

This study showed that FYJ is a repellent against workers of red imported fire ants. Each of its six major components also showed repellency at varied concentrations. Those components can be found in various natural sources. For example, camphor is found in wood of the camphor laurel (*Cinnamomum camphora*) and some other related trees in the family of Lamiaceae (*15*, *16*); eucalyptol in Tasmanian Blue Gum, *Eucalyptus globulus* (*17*), and in mugwort (*Artemisia vulgaris*) (*18*), rosemary (*Rosamarinus officinalis* L.) (*19*), Dalmatian sage (*Salvia officinalis* L.) (*20*), and other plants (*21*, *22*); eugenol in clove oil (*Syzygium aromaticum* (L.) Merr. & Perr.) (*23*), nutmeg (*Myristica fragrans* van Houtte) (*24*), indigenous cinnamon (*Cinnamomum osmophloeum* Kaneh) (*25*), and bay leaf (*Laurus nobilis* L.) (*26*); menthol in peppermint (*Mentha* × *piperita* L.) (*27*) and other essential oils (*28*); methyl salicylate

Table 2. Mean (SE) Weight (g) of Sand Removed by Worker Ants 24 h after They Were Released in the Two Choice Digging Bioassay at Three Concentrations of Individual Compound in FYJ

chemical	conc (mg/kg)	sand removed (SE) treatment	sand removed (SE) control	digging suppression index (SE)	*t*-value	*P*-value
camphor	1.0	0.78 (0.041)	0.74 (0.040)	−0.015 (0.033)	−0.58	0.56
	10.0	0.80 (0.049)	0.83 (0.036)	0.046 (0.030)	0.57	0.57
	100.0	0.65 (0.047)	0.80 (0.039)	0.14 (0.035)	3.32	0.0016
eucalyptol	1.0	0.77 (0.037)	0.75 (0.052)	−0.039 (0.032)	−0.33	0.74
	10.0	0.97 (0.052)	0.87 (0.044)	−0.05 (0.021)	−2.70	0.009
	100.0	0.60 (0.043)	0.75 (0.033)	0.15 (0.038)	3.56	0.0007
eugenol	1.0	0.66 (0.053)	0.84 (0.11)	0.059 (0.046)	1.817	0.074
	10.0	0.47 (0.054)	0.81 (0.060)	0.34 (0.054)	5.41	<0.0001
	100.0	0.018 (0.0058)	0.89 (0.054)	0.97 (0.038)	16.53	<0.0001
menthol	1.0	0.69 (0.051)	0.69 (0.055)	−0.012 (0.025)	−0.31	0.76
	10.0	0.62 (0.048)	0.84 (0.046)	0.19 (0.027)	6.13	<0.0001
	100.0	0.046 (0.016)	0.77 (0.038)	0.92 (0.027)	18.64	<0.0001
methyl salicylate	1.0	0.59 (0.044)	0.59 (0.041)	0.00 (0.041)	−0.064	0.95
	10.0	0.73 (0.045)	0.80 (0.048)	0.038 (0.023)	2.82	0.007
	100.0	0.40 (0.041)	0.80 (0.032)	0.40 (0.041)	9.60	<0.0001
phenylethanol	1.0	0.73 (0.038)	0.71 (0.050)	−0.07 (0.032)	−0.50	0.62
	10.0	0.72 (0.045)	0.77 (0.054)	0.007 (0.029)	1.80	0.076
	100.0	0.22 (0.034)	0.78 (0.036)	0.59 (0.040)	11.93	<0.0001

in plants of the family Pyrolaceae, Ericaceae, and Betulaceae, such as in essential oil of wintergreen (*Gaultheria procumbens* L.) and essential oil of sweet birch bark (*Betula lenta* L.) (*29*); phenylethanol in a yeast (*30*); and phenylethanol glycosides in plants (*31*, *32*).

Some components in FYJ have long been known as insect repellents. For example, camphor has been used as a repellent against many insect species (*33*). Eucalyptol has been found to be a repellent against various insects, such as American cockroaches *(Periplaneta americana* (Linnaeus)) (*34*), mosquitoes (*Aedes aegypti*) (*22*), and Colorado potato beetles (*Leptinotarsa decemlineata* (Say)) (*35*). Menthol was used to control tracheal mites, *Acarapis woodi* (Rennie) in the hives of honey bees (*36*).

Repellency of some natural products has been tested against the red imported fire ants, such as mint oil, and water suspensions of pine needle and cedar shaving (*10*, *11*). Callicarpenal and intermedeol, two terpenoids isolated from the leaves of American beautyberry (*Callicarpa americana* L., Verbenaceae) and Japanese beautyberry (*Callicarpa japonica* Thunb.), were found to be repellent against imported fire ants (*9*). Therefore, natural products may be an excellent source of repellents against fire ants. Using chemicals from natural products in insect pest management is generally considered as a safer alternative than using synthetic contact insecticides. FYJ has long been used in China as an over-the-counter pain and itch reliever, as a mosquito repellent, and as a treatment for insect bites and stings and headache caused by colds and carsickness. Based on its long history of topical application by humans, use of this product in many scenarios for fire ant prevention may also be safe to humans and other nontarget organisms.

Eucalyptol at 100 mg/kg showed significant repellency. In contrast, at 10 mg/kg, eucalyptol showed significant digging facilitation, indicating it might act as an attractant at this low concentration. This observation gives us a strong warning about using eucalyptol as a repellent against fire ants, because the opposite effect may occur after its concentration is reduced to a certain level. Since the lowest concentration tested in this study was 1.0 mg/kg, whether other compounds have a similar opposite effect at even lower concentrations merits further examination. A compound repelling ants at high concentrations but attracting them at low concentrations might not be used as a repellent unless the concentration can be kept high at all times, which may not be practical in fire ant management. The mixture may perform better than each individual component. At 100 mg/kg of FYJ in sand, a complete digging suppression occurred for all 6 colonies. Although eugenol and menthol did show complete suppression for several colonies, complete suppression for all colonies never happened for any individual compound. One possible explanation is that synergism may have taken place among those compounds. Such possible synergism among the repellent components has never been investigated for imported fire ants. The results of this study warrant future intriguing detailed study on this topic.

The success of a fire ant repellent product depends heavily on its delivery system. This study does not recommend the use of FYJ in fire ant management without modification. More knowledge is needed in order to successfully use FYJ and its active compounds in fire ant management.

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